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# Nouvelles stratégies thérapeutiques dans l'asthme sévère : inhibition de la voie de signalisation dépendante de la GTPase Rac

Dorian Hassoun

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# THESE DE DOCTORAT DE

NANTES UNIVERSITE

ECOLE DOCTORALE N° 605

*Biologie Santé*

Spécialité : Physiologie, Physiopathologie, Biologie Systémique Médicale

Par

**Dorian HASSOUN**

## **Nouvelles stratégies thérapeutiques dans l'asthme sévère**

Inhibition de la voie de signalisation dépendante de la GTPase Rac

Thèse présentée et soutenue à Nantes le 13/12/2022

Unité de recherche : L'institut du thorax research unit

Inserm UMR 1087 / CNRS UMR 6291

### **Rapporteurs avant soutenance :**

Cindy BARNIG  
Thomas TRIAN

Professeur d'université – Praticien Hospitalier, Université de Franche-Comté  
Professeur d'université, Université de Bordeaux

### **Composition du Jury :**

Président : Sophie BROUARD

Directrice de recherche, CNRS, HDR, Nantes Université

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Thomas TRIAN

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Sophie BROUARD

Directrice de recherche, CNRS, HDR, Nantes Université

Philippe BONNIAUD

Professeur d'université – Praticien Hospitalier, Université de Bourgogne

Dir. de thèse : Vincent SAUZEAU

Chargé de recherche, INSERM, HDR, Nantes Université

### **Invité(s)**

Antoine MAGNAN

Professeur d'université – Praticien Hospitalier, Université de Paris-Saclay

# Remerciements

*Un cycle se termine, un autre commence et l'aventure se poursuit.*

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## **Curriculum vitae**

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### **POSITION ACTUELLE**

Doctorant en 3<sup>ème</sup> année de thèse de sciences au sein de l'institut du thorax, équipe 3 Maladies vasculaires et pulmonaires sous la direction du Dr Vincent SAUZEAU (CR Inserm)

Collaborateur de gardes hospitalières, CHU de Nantes, service de pneumologie.

### **DIPLOMES ET CERTIFICATIONS**

- 2021 Expérimentation animale, niveau 1 – Fonction de conception ou de réalisation des procédures expérimentales – Module Rongeur/Lapin
- 2019 Diplôme d'Etudes Spécialisées de Pneumologie (mémoire soutenu le 30/03/2019)  
Instantané de la prise en charge de l'asthme sévère en 2018-Expérience du CHU de Nantes
- Diplôme d'Etat de Docteur en Médecine (thèse soutenue à Nantes le 25/10/2019)  
Mepolizumab dans l'asthme sévère éosinophilique en pratique clinique courante
- 2015 Deuxième Cycle des Etudes Médicales (DCEM), examen classant national, 624<sup>ème</sup>
- 2012 Mastère 2 recherche, Université de Nantes  
Biologie Santé Spécialité Biologie, Biotechnologie et Recherche Thérapeutique  
Formation à l'expérimentation animale de niveau 2
- 2011 Mastère 1, Université de Nantes, Biologie Santé  
UE Immunologie approfondie, Biostatistiques
- 2010 Concours de l'Ecole de l'Inserm-Liliane Bettencourt, 2<sup>ème</sup> ex-aequo
- 2009 Concours du Premier Cycle des Etudes Médicales (PCEM 1), 7<sup>ème</sup>
- 2008 Baccalauréat Général Scientifique, option Sciences de la vie et de la Terre,  
spécialité Mathématiques, Académie de Nantes, mention Très Bien

### **EXPERIENCE PROFESSIONNELLE**

#### *Activité clinique*

- 2020-2022 Collaborateur de garde, Service de pneumologie, CHU de Nantes, Pr F-X. BLANC
- 2019 Interne, Unité de transplantation thoracique, CHU de Nantes, Pr J.-C. ROUSSEL
- 2018-2019 Interne, Service des explorations fonctionnelles, CHU de Nantes, Pr Y. PEREON
- 2017-2018 Interne, Service de réanimation polyvalente, CHD de La Roche-sur-Yon, Dr J.-C. LACHERADE
- 2016-2017 Interne, Service de médecine interne, CHU de Nantes, Pr M. HAMIDOU

2015-2018 Interne, Service de pneumologie, CHU de Nantes, Pr F-X. BLANC  
Unités d'oncologie thoracique, d'hospitalisation conventionnelle, de soins intensifs de pneumologie, d'hospitalisation de jour

*Activité de recherche :*

2019-2022 Préparation d'une thèse de sciences, Ecole doctorale Biologie-Santé, domaine Biologie, Médecine, Santé.  
Sujet : Nouvelles stratégies thérapeutiques dans l'asthme sévère : inhibition de la voie de signalisation dépendante de la GTPase Rac.  
Sous la direction du Dr V. SAUZEAU, l'institut du thorax, équipe 3 Maladies vasculaires et pulmonaire.

2018 - NARACAS : Evaluation de l'expression et de l'activité de la protéine Rac1 dans les cellules musculaires lisses des bronches de patients asthmatiques

*Participation à la rédaction du protocole*

2011-2012 Inserm UMR 1087 CNRS UMR 6291, l'institut du thorax  
Equipe AVENIR « Pathologies bronchiques et allergiques », Pr A. MAGNAN

*Travaux de Mastère 1 et 2*

*Activité d'enseignement*

*Formation initiale*

Physiologie respiratoire. 2<sup>ème</sup> année de médecine (5h/an), 1<sup>ère</sup> année IFSI (3h30/an)  
Voix de recherche dans l'asthme. UE Master 1 Biologie et Pathologie du Thorax. (2h/an)  
Introduction à la recherche dans l'asthme. UE Master 1 Initiation à la recherche (1h/an)  
LCA immunologie respiratoire – exemple de l'asthme. UE Physiologie et physiopathologie des grandes fonctions (2h/an)  
Intérêt des explorations fonctionnelles dans le diagnostic et le suivi de l'asthme. DES de pneumologie, région Grand Ouest. (1h en 2020)  
Physiopathologie de l'asthme. DES d'allergologie, Rennes (1h/an)

*Formation continue*

Tolérance à l'effort chez le patient atteint de BPCO : Formation Infirmier en Pratiques Avancées (3h)

*Encadrement*

Co-encadrement d'un Travail d'Etude et de Recherche de master 1 : 1 étudiant en 2020-2021

Co-encadrement d'un stage de Master 2 Recherche : 1 étudiant en 2021-2022

**PUBLICATIONS**

**Hassoun D.**, Rose L., Blanx F.-X., Magnan A., Loirand G., Sauzeau V. Bronchial smooth muscle cell in asthma: where does it fit? *BMJ Open Respir Res.* 2022 Sep;9(1):e001351.

**Hassoun D.**, Loirand G., Sauzeau V. Un nouvel acteur physiopathologique dans l'asthme sévère : la GTPase Rac. *Rev Mal Respir.* 2022 Feb;39(2):100-103.

**Hassoun D.**, Malard O, Barbarot S, Magnan A, Colas L. Type 2 immunity-driven diseases: Towards a multidisciplinary approach. *Clin Exp Allergy.* 2021 Dec;51(12):1538-1552. doi: 10.1111/cea.14029.

Dilasser F, Rose L, **Hassoun D**, Klein M, Rousselle M, Brosseau C, Guignabert C, Taille C, Dombret MC, Di Candia L, Heddebaut N, Bouchaud G, Pretolani M, Magnan A, Loirand G, Sauzeau V. Essential role of smooth muscle Rac1 in severe asthma-associated airway remodelling. *Thorax*. 2021 Apr;76(4):326-334.

Moui A, Klein M, **Hassoun D**, Dijoux E, Cheminant MA, Magnan A, Bouchaud G. The IL-15 / sIL-15R $\alpha$  complex modulates immunity without effect on asthma features in mouse. *Respir Res* 2020; 21: 33.

Colas L, **Hassoun D**, Magnan A. Needs for Systems Approaches to Better Treat Individuals With Severe Asthma: Predicting Phenotypes and Responses to Treatments. *Front Med (Lausanne)* 2020; 7: 98.

**Hassoun D**, Moui A, Colas L, Blanc FX, Magnan A. [Update in severe asthma physiopathology and treatments]. *Rev Med Interne* 2019; 40: 508-516.

**Hassoun D**, Dirou S, Arrigoni PP, Durant C, Hamidou M, Neel A, Agard C. Radiological pleuroparenchymal fibroelastosis associated to limited cutaneous systemic sclerosis: a case report. *BMC Pulm Med* 2018; 18: 73.

Braza F, Dirou S, Forest V, Sauzeau V, **Hassoun D**, Chesne J, Cheminant-Muller MA, Sagan C, Magnan A, Lemarchand P. Mesenchymal Stem Cells Induce Suppressive Macrophages Through Phagocytosis in a Mouse Model of Asthma. *Stem Cells* 2016; 34: 1836-1845.

Bouchaud G, Braza F, Chesne J, Lair D, Chen KW, Rolland-Debord C, **Hassoun D**, Roussey-Bihouee T, Cheminant MA, Brouard S, Bodinier M, Vrtala S, Magnan A. Prevention of allergic asthma through Der p 2 peptide vaccination. *J Allergy Clin Immunol* 2015; 136: 197-200 e191.

Rolland-Debord C, Lair D, Roussey-Bihouee T, **Hassoun D**, Evrard J, Cheminant MA, Chesne J, Braza F, Mahay G, Portero V, Sagan C, Pitard B, Magnan A. Block copolymer/DNA vaccination induces a strong allergen-specific local response in a mouse model of house dust mite asthma. *PLoS One* 2014; 9: e85976.

Botturi K, Langelot M, Lair D, Pipet A, Pain M, Chesne J, **Hassoun D**, Lacoeyille Y, Cavailles A, Magnan A. Preventing asthma exacerbations: what are the targets? *Pharmacol Ther* 2011; 131: 114-129.

## **PUBLICATION DIDACTIQUE**

Forest V, Hassoun D, Leblond A.-L, Lemarchand P, Thérapie génique et cellulaire des maladies respiratoires, *Traité de pneumologie, Encyclopédie Médico-Chirurgicale (EMC)* 2013, ISSN 1155-195X, [http://dx.doi.org/10.1016/S1155-195X\(13\)61155-X](http://dx.doi.org/10.1016/S1155-195X(13)61155-X).

## **COMMUNICATIONS EN CONGRES ET ASSOCIEES**

- 22 septembre 2022 : L'année 2021 en revue. Actualités en recherche fondamentale dans l'asthme sévère.  
**Hassoun D.**  
Journées d'échanges sur l'Asthme Sévère, G2A SPLF  
Conférence invitée
- 30 juin 2022 : Rôle de la GTPase Rac dans les mécanismes inflammatoires dans l'asthme sévère.  
**Hassoun D.**  
Ecole d'été du Cluster LungO2 Innovation  
Conférence invitée
- 24 mars 2022 : Analyses bio-informatiques appliquées à la cytométrie en flux sur la plateforme Cytocell : cas pratique d'un modèle murin d'asthme allergique.  
**Hassoun D.**, Jouand N.  
Congrès Gene2Bio  
Conférence invitée

- 9 au 10 décembre 2021 : Role of Rac GTPase activity in inflammation development in severe asthma  
**Hassoun D.**, Rose L., Rousselle M., Magnan A., Blanc F.-X., Loirand G. and Sauzeau V.  
 Journées scientifiques de l'école doctorale Biologie Santé Bretagne Loire  
 Poster scientifique
- 15 au 16 octobre 2021 : Rôle de la GTPase Rac dans les mécanismes inflammatoires dans l'asthme sévère  
**Hassoun D.**, Rose L., Rousselle M., Magnan A., Blanc F.-X., Loirand G. and Sauzeau V.  
 Journées de la Recherche Respiratoire (J2R)  
 Communication orale + poster scientifique  
 Prix du meilleur Poster session Asthme/Epidémiologie
- 1<sup>er</sup> juillet 2021 : Nouvelle stratégie thérapeutique dans l'asthme sévère : inhibition de la voie de signalisation dépendante de la GTPase Rac  
**Hassoun D.**  
 Ecole d'été du cluster LungO<sup>2</sup>  
 Communication orale
- 24 au 27 janvier 2020 : 24<sup>ème</sup> Congrès de pneumologie de langue française, Nantes, France  
 Mission CPLF. Revue des maladies respiratoires actualités Vol 12 - N° 1S - juin 2020
- 12 au 16 juin 2019 : Specific ventilation pattern during hyperventilation test with deep sighing in clinical practice  
**Hassoun D.**, Verger E., Beasse A., Herve I.-A., Le Blanc C., Chambellan A.  
 3<sup>ème</sup> congrès de Physiologie et Biologie Intégrative, Montpellier, France  
 Communication orale
- 14 au 15 mars 2019 : Session Cas cliniques  
 7<sup>èmes</sup> Journées d'échanges sur l'asthme sévère, Nantes, France  
 Communication orale
- 24 au 27 janvier 2019 : Maladie des ensileurs révélée par une bronchiolite compliquée d'hémoptysie  
**Hassoun D.**, Eschapasse E., Chene A.-L., Sagan C., Defrance C., Kassam D., Blanc F.-X.  
 23<sup>ème</sup> Congrès de pneumologie de langue française, Marseille, France  
 Poster scientifique
- 30 au 31 mars 2017 : Session Cas cliniques  
 5<sup>èmes</sup> Journées d'échanges sur l'asthme sévère, Strasbourg, France  
 Communication orale
- 1<sup>er</sup> au 5 septembre 2012 : Immune modulation by mesenchymal stem cells in a mouse model of house dust mite allergic asthma  
**Hassoun D.**, Lair D., Forest V., Cheminant M.-A., Sagan C., Chesné J., Botturi-Cavaillès K., Reboulleau D., Lemarchand P., Magnan A.  
 European Respiratory Society annual congress, Vienna, Austria  
 Poster scientifique

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## Liste des abréviations

ABPA = Aspergillose BronchoPulmonaire Allergique  
Abr = Active BCR-related  
ACQ = Asthma Control Questionnaire  
ACT = Asthma Control Test  
ADEPT = Airways Disease Endotyping for Personalized Therapeutics  
AP1 = Activator Protein 1  
Arf = ADP ribosylation factor  
Arp2/3 = Actin Related Protein 2/3  
ATF2 = Activating Transcription Factor 2  
ATS = American Thoracic Society  
ATU = Autorisation Temporaire d'Utilisation

BCR = B Cell Receptor  
 $\beta$ -NAD = beta-nicotinamide adenine dinucleotide  
BPCO = BronchoPneumopathie Chronique Obstructive

cADP-r= cyclic Adenosin Di Phosphate ribose  
CaM = Calmodulin  
CD = Cluster of differentiation  
Cdc42 = Cell Division Control protein 42 homolog  
CML = Cellules Musculaires Lisses  
CO = Monoxide de carbone  
CPI-17 = protein kinase C-potentiated phosphatase inhibitor protein of 17 kDa  
CRIB = Cdc42-Rac Interactive Binding  
CRTh2 = chemoattractant receptor-homologous molecule expressed on Th2 cells  
CSH = Cellules Souches Hématopoïétiques  
CSI = CorticoStéroïde Inhalé  
CV = Capacité Vitale  
CXCL8 = C-X-C motif ligand 8  
CysLT1 = Cysteinyl Leukotriene receptor 1

DAG = DiAcylGlycerol  
DAMP = Damage-Associated Molecular Pattern  
Dbl = diffuse B cell lymphoma  
DH = Dbl Homology  
DHR = DOCK Homology Region  
Dia = Diaphanous related forms  
DEP = Débit Expiratoire de Pointe  
DOCK = Dedicator Of CytoKinesis  
DRF = Diaphanous-Related Formin

ECP = Eosinophil Cationic Protein  
EDN = Eosinophil Derived Neurotoxin  
EGF = Epithelial Growth Factor  
EGFR = EGF Receptor

ELMO = Engulfment and cell MOtility  
EMTU = Epithelial Mesenchymal Trophic Unit  
EPX = Eosinophil Peroxidase  
ERK = Extracellular Regulated Kinase  
ERS = European Respiratory Society

FeNO = Fraction exhalée du monoxyde d'azote  
FGF = Fibroblast Growth Factor

GAP = GTPase-activating Proteins  
GATA3 = GATA-binding protein 3  
GBD = GTPase-Binding Domain  
GDI = Guanine Dissociating Inhibitors  
GDP = Guanine Di-Phosphate  
GEF = Guanine Exchange Factor  
GINA = Global INitiative in Asthma  
GIT1 = G protein-coupled receptor kinase-Interactor 1  
GM-CSF = Granulocyte Macrophage Colony Stimulating Factor  
GPCR = G Protein-Coupled Receptors  
GR = Glucocorticoid Receptor  
GTP = Guanine Tri-Phosphate

HAS = Haute Autorité de Santé  
HRB = Hyper-réactivité bronchique

ICAM = Intercellular Adhesion Molecule  
IFN = Interféron  
IgE = Immunoglobuline E  
IL = Interleukine  
ILC2 = Innate Lymphoid Cell 2  
IMC = Indice de Masse Corporelle  
iNOS = iNOS = inducible NO Synthase  
IP3 = Inositol Tri Phosphate  
IQGAP1/2 = IQ motif-containing GTPase-activating protein  
ITA = Immunothérapie Allergénique

JNK = c-Jun NH2-terminal Kinase

LC-HRMS = Liquid-Chromatography High Resolution Mass Spectrometry  
LFA = Lymphocyte Function-associated Antigen  
LIMK = LIM Domain Kinase

MAPK = Mitogen-activated protein kinase  
MEK1 = MAP (Mitogen-Activated Protein) Kinase/ERK (Extracellular Signal-Regulated Kinase) Kinase 1  
MLC = Myosin Light Chain  
MLCP = Myosin Light Chain Phosphatase  
MLCK = Myosin Light Chain Kinase  
MLK = Mixed Lineage Kinase

NADPH = Nicotinamide adénine dinucléotide phosphate  
NANC = Non Adrénérique Non Cholinergique  
NFAT = Nuclear factor of activated T-cells  
NLR = NOD-like receptor  
NO = Monoxyde d'azote

ORL = Oto-Rhino-Laryngologique

PAF = Platelet Activating Factor  
PAK = Protease-Activated protein Kinase  
PAMP = Pathogen-Associated Molecular Pattern  
PAR = Protease-Activated Receptor  
PBD = P21-Binding Domain  
PDGF = Platelet-Derived-Growth-Factor  
PH = Pleckstrin Homology  
Pi = Phosphate inorganique  
PI3 = Phosphatidylinositol-3  
PI3K = Phosphatidylinositol-3 Kinase  
PIP2 = phosphatidylinositol-4,5-bisphosphate  
PKC = Protein Kinase C  
PLC = PhosphoLipase C  
POR = Partner of Rac  
PRR = Pattern Recognition Receptor

Rab = Ras-related in brain  
Rac = Ras-related C3 botulinum toxin substrate  
Raf = Rapidly Accelerated Fibrosarcoma  
Ran = Ras-related nuclear protein  
RhoA = Rho family small GTPase A  
ROCK = Rho Kinase  
ROR $\alpha$  = RAR-related Orphan Receptor alpha

SARP = Severe Asthma Research Program  
SCF = Stem Cell Factor  
SH = SRC-homology domain  
SM22 $\alpha$  = Smooth Muscle protein 22 alpha  
SP2A = Société Pédiatrique de Pneumologie et d'Allergologie  
SPLF = Société de Pneumologie de Langue Française  
SR = Sarcoplasmic Reticulum  
SRF = Serum Response Factor  
STAT3 = Signal Transducer and Activator of Transcription 3  
SWAP-70 = Switch-associated protein 70

TAK = TGF  $\beta$ -activated kinase  
TCF = T Cell factor/lymphoid enhancer Factor  
TEM = Transition Epithélio-Mésenchymateuse  
TGF = Transforming Growth Factor  
TIAM = T-cell lymphoma invasion and metastasis-inducing protein 1

TLR = Toll-Like Receptors  
TMA = Tests Multi-Allergéniques  
TNF = Tumor Necrosis Factor  
TRIO = Triple Functional Domain Protein  
TSLC-1 = Tumor Suppressor in Lung Cancer-1  
TSLP = Thymic Stromal Lymphopoïetin

U-BIOPRED = Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes

VCAM = Vascular Cell Adhesion Molecule  
VEGF = Vascular Endothelial Growth Factor  
VEMS = Volume Expiré Maximal en une Seconde

WASP = Wiskott Aldrich Syndrom Protein  
WAVE = WASP family Verprolin-homologous

## **I. Introduction**

### **A. Etat de l'art de l'asthme en 2022**

#### **1. Diagnostic de l'asthme et de l'asthme sévère**

L'asthme est une **pathologie chronique des voies aériennes** se manifestant par des épisodes de dyspnée sifflante, de blocage expiratoire, de toux ou d'oppression thoracique associés à une limitation variable des débits aériens dans un contexte d'inflammation bronchique chronique (1).

Actuellement, il n'existe pas **d'étalon-or diagnostique** pour l'asthme. Les recommandations émises par les différentes sociétés savantes (SPLF, ERS, ATS), s'accordent sur l'importance d'évaluer la probabilité clinique de l'asthme à partir de critères cliniques, fonctionnels respiratoires et inflammatoires (2-5) (Tableau 1).

**Chez le nourrisson et l'enfant en bas âge (< 36 mois)**, le diagnostic se pose sur l'association de symptômes respiratoires évocateurs récurrents (toux, sifflements etc.) (6). Les épisodes infectieux respiratoires, les malformations bronchopulmonaires et les inhalations de corps étrangers constituent les principaux diagnostics différentiels à évoquer. L'un des enjeux du diagnostic et de la prise en charge de l'asthme à cet âge est de prévenir la persistance de la maladie durant l'enfance.

**Chez l'enfant de plus de 36 mois**, la recherche de signes fonctionnels respiratoires compatibles s'accompagne de la mise en évidence d'un trouble ventilatoire obstructif variable par spirométrie forcée dès que possible (après 5 ans) (3, 7). Par ailleurs, la réalisation d'un bilan allergologique est recommandée de manière systématique au vu de la fréquence élevée des formes allergiques (environ 60%) (8, 9).

**A l'adolescence et à l'âge adulte**, la réalisation d'une spirométrie forcée recherchant un trouble ventilatoire obstructif et d'un bilan allergologique (prick-tests et dosage des IgE spécifiques) sont recommandés pour étayer la probabilité clinique (2, 5). La mesure de la FeNO, la recherche d'une hyperéosinophilie, le dosage d'IgE totales ou la réalisation d'expectorations induites, sont limités à des enquêtes diagnostiques spécifiques (ABPA) ou des contextes particuliers (asthme sévère).

I - A - 1 Diagnostic de l'asthme et de l'asthme sévère

Âge	Critères cliniques	Explorations fonctionnelles respiratoires	Autres examens complémentaires	Recommandations	Réf.
<b>Enfants de moins de 36 mois</b>	Toux et sifflements récurrents ( $\geq 3$ ) à prédominance nocturne Terrain atopique personnel ou familial	Aucune	Radiographie thoracique de face (diagnostics différentiels) Bilan allergologique (prick-tests cutanés et TMA)	Française HAS et SP2A (2009)	(6)
<b>Enfants de moins de 6 ans</b>	Episodes récurrents de toux, de sifflements et de dyspnée à prédominance nocturne Facteurs déclenchants : effort, pleurs, rires, expositions allergéniques, infections virales. Terrain atopique personnel ou familial	Spirométrie forcée (> 5 ans, fonction de la compréhension)	Bilan allergologique (prick-tests cutanés et IgE spécifiques) +/- radiographie thoracique de face (diagnostics différentiels)	Internationale GINA (2022)	(7)
<b>Enfants de plus de 6 ans</b>	Episodes récurrents de toux, de sifflements et de dyspnée	Spirométrie forcée avec test de réponse aux bronchodilatateurs Mesure de la FeNO Tests de provocation directs ou indirects si spirométrie forcée insuffisante Variabilité du DEP complémentaire mais insuffisante seule	A visée pré-thérapeutique : bilan allergologique (pricks-tests cutanés et IgE spécifiques)	Européenne ERS (2021)	(3)
<b>Adolescents (&gt; 12 ans) et adultes</b>	Episodes récurrents de toux, de sifflements et de dyspnée à prédominance nocturne Facteurs déclenchants : effort, pleurs, rires, expositions allergéniques, infections virales. Terrain atopique personnel ou familial	Spirométrie forcée avec test de réponse aux bronchodilatateurs Tests de provocation directs ou indirects si spirométrie forcée insuffisante Variabilité du DEP complémentaire mais insuffisante seule	Bilan allergologique (prick-tests cutanés et IgE spécifiques) Eosinophilie sanguine réservée au bilan d'asthme sévère	Française SPLF (2021) SPLF et SP2A (2022)	(2, 5)

Tableau 1 Recommandations diagnostiques de l'asthme en fonction de l'âge

Abréviations : DEP = Débit Expiratoire de Pointe, ERS = European Respiratory Society, FeNO = Fraction exhalée du monoxyde d'azote, GINA = Global Initiative in Asthma, HAS = Haute Autorité de Santé, SP2A = Société Pédiatrique de Pneumologie et d'Allergologie, SPLF = Société de Pneumologie de Langue Française, TMA = Tests Multi-Allergéniques.

Quel que soit l'âge, la recherche **d'une variabilité** des symptômes respiratoires (toux, dyspnée, sifflements) et de l'obstruction bronchique est essentielle.

**Sur le plan clinique**, cette variabilité est mise en évidence à l'interrogatoire et à l'examen physique par la fluctuation des symptômes ressentis et des signes physiques au cours du suivi. A domicile, une variation spontanée significative du DEP diurne au-delà de 20% sur une durée de 15 jours constitue un argument en faveur d'un asthme (10).

**Sur le plan des explorations fonctionnelles respiratoires**, la mise en évidence d'une obstruction bronchique significative s'effectue par spirométrie forcée (11). Elle se définit par un ratio VEMS/CV maximale inférieur à la limite inférieure de la norme (2). Lorsqu'une obstruction significative est absente ou invariable spontanément, des tests de réponse aux bronchodilatateurs et/ou de provocation peuvent être réalisés. Le test de réponse aux bronchodilatateurs est jugé significatif en cas d'élévation significative (+200mL et +12%) du VEMS ou de la CVF au décours d'une inhalation de béta-2 mimétique à courte durée d'action (12). A l'inverse, les tests de provocation ont pour objectif de mettre en évidence une HRB se définissant par une réponse bronchoconstrictrice disproportionnée. Ils peuvent être soit direct (exemple : test à la méthacholine) soit indirect (épreuve d'effort ou test d'hyperventilation isocapnique) (13, 14). Leur recours est guidé par la probabilité pré-test de l'asthme. En effet, les tests directs sont plus sensibles mais moins spécifiques que les tests indirects (15, 16).

Une problématique de **diagnostics à défaut et à tort de l'asthme** existe (17). Dans une population de sujets adultes (18-69 ans), 4.6% des individus présentent un trouble ventilatoire obstructif dont seulement 45% sont symptomatiques. Or, seulement 35.6% bénéficient d'un diagnostic : 29.6% présentent un asthme, 8.1% une bronchopneumopathie obstructive et/ou un emphysème et/ou une bronchite chronique et 0.1% des dilatations des bronches (18). Des observations concordantes existent chez l'enfant avec une fréquence d'asthme non diagnostiquée estimée à 42% (19). Parallèlement, 30 à 35% des diagnostics d'asthme portés au sein d'une population adulte sont en fait erronés (17, 20).

Au-delà du diagnostic de l'asthme et de son traitement (développé dans la section « Prise en charge de l'asthme en France » p28), **deux situations spécifiques** se dégagent : *l'asthme sévère* d'une part et *l'asthme difficile à traiter* d'autre part.

**L'asthme sévère** bénéficie d'une définition reconnue internationalement. Elle s'applique à tout asthme confirmé et correctement traité (adhésion au traitement, observance thérapeutique et prise en charge des comorbidités) (a) nécessitant de fortes doses de corticoïdes inhalés associés à une autre classe thérapeutique ou l'utilisation d'une corticothérapie systémique pour contrôler l'asthme (GINA 4 et 5) ou (b) demeurant non contrôlé malgré ces traitements ou (c) s'aggravant malgré un tel traitement (21).

**L'asthme difficile** correspond aux situations pour lesquelles un traitement lourd (GINA 4 et 5) est en place mais avec une observance thérapeutique non optimale et/ou des comorbidités/facteurs favorisants insuffisamment pris en charge. La problématique principale posée par cette catégorie est d'optimiser la prise en charge afin d'identifier les patients présentant un authentique asthme sévère.

## 2. Données épidémiologiques mondiales et françaises

**A l'échelle mondiale**, la prévalence de l'asthme est estimée à 3 416 cas pour 100 000 habitants (normalisé sur l'âge) soit environ 262 millions d'individus (Global Burden of disease 1990-2019) (22). La *morbi-mortalité* de la maladie reste élevée malgré les progrès thérapeutiques, évaluée à 273.6 années de vie ajustée sur l'incapacité (Disability-Adjusted Life Years) pour 100 000 habitants. En comparaison, celle de la BronchoPneumopathie Obstructive est estimée à 926 années de vie / 100 000 habitants (23). On estime à 461 000 le nombre de patients qui décède d'asthme chaque année. Si les taux de prévalence, de morbi-mortalité et de mortalité ont globalement diminué sur les 30 dernières années (-24, -51.3 et -42.5% respectivement), il existe cependant des disparités régionales (22). Par ailleurs, on note une morbi-mortalité d'autant plus importante que le niveau socio-économique est bas (24). Chez l'enfant et l'adolescent, la fréquence de l'asthme reste stable depuis 1990 à 9 et 11% respectivement (Global Asthma Network Phase I, 2021) (25).



**L'asthme difficile et l'asthme sévère** représentent environ 10 et 4% de la population de patients asthmatiques adultes (26). Les patients asthmatiques sévères souffrent d'une morbidité plus importante que les autres patients avec notamment des exacerbations plus fréquentes et plus graves (27). Il en découle un coût financier plus important avec un recours plus fréquent aux soins (médicaments, hospitalisations en soins intensifs ou réanimation etc...) (28). On estime actuellement que 60% des dépenses de santé dans l'asthme sont liées aux asthmatiques sévères (29).

**En France**, la prévalence de l'asthme chez l'adulte est évaluée entre 6 et 8% de la population française avec une fréquence plus élevée chez les personnes ayant une situation socio-économique défavorable (données déclaratives) (30, 31). Chez l'enfant, la prévalence de l'asthme était de 11% en 2012 et inversement corrélée avec le niveau socio-économique des familles (32). L'asthme sévère concerne quant à lui 18 personnes pour 10 000 habitants soit 3 à 4 % de la population asthmatique adulte française (33, 34). La mortalité annuelle imputée à l'asthme demeure stable depuis une vingtaine d'années aux alentours de 1 000 décès. Son impact est par ailleurs concordant avec les données internationales sur le plan des conséquences tant personnelles (qualité de vie, activités de la vie quotidienne) que sociétales (coût de la maladie) (33, 35).

**Au total**, l'asthme est une pathologie fréquente responsable d'une morbi-mortalité significative, notamment dans sa forme sévère, représentant un enjeu de santé publique majeur.

### **3. Les phénotypes d'asthme**

L'étendue de la définition diagnostique de l'asthme entraîne **une grande hétérogénéité** des présentations cliniques rencontrées en pratique clinique. Afin de constituer des groupes homogènes, des *études de cluster* ont été réalisées sur des cohortes de patients asthmatiques adultes modérés et sévères de grande envergure (ADEPT, U-BIOPRED, SARP) (36, 37). Ces études partiellement non-biaisées se basent sur des modèles mathématiques regroupant les patients selon un ensemble déterminé de variables cliniques et paracliniques.

Récemment, **une étude française de grande envergure** (n=1502) s'est attachée à décrire les différents phénotypes identifiables dans une population de patients asthmatiques sévères suivis au sein des hôpitaux généraux (38). Cinq groupes ont pu être différenciés après une analyse de cluster (classification hiérarchique suivie d'une méthode par k-means) : (1) un groupe de patient asthmatique allergique de début précoce, (2) un groupe spécifique d'asthme associé à l'obésité, (3) un groupe de patients asthmatiques de début tardif avec une obstruction bronchique sévère, (4) un groupe de patients asthmatiques éosinophiliques et (5) un groupe de patients asthmatiques présentant une sensibilisation à l'aspirine. Ces différents phénotypes retrouvés dans la population française sont globalement comparables avec ceux retrouvés dans les cohortes internationales (Tableau 2).

**Chez l'enfant**, deux présentations phénotypiques sont classiquement distinguées : les sifflements intermittents, déclenchés par des épisodes viraux d'une part et les formes récurrentes persistant après 6 ans et associées à l'asthme (39). Une hétérogénéité existe au sein du groupe des asthmes allergiques de l'enfant en fonction du nombre et du type de sensibilisations. Ainsi, dans une cohorte française d'enfants asthmatiques allergiques (6-12 ans) se différencient un groupe d'asthme léger à modéré associé à une mono-sensibilisation aux acariens, un groupe avec allergies multiples associées à un asthme sévère, un groupe avec des sensibilisations multiples avec un asthme léger et un groupe présentant une sensibilisation au pollen souffrant d'exacerbations sévères (40). Des cas particuliers se constituent dans les formes sévères de la maladie tels que l'asthme allergique de début précoce avec sensibilisations multiples, l'asthme de début tardif (puberté) associé aux atteintes ORL éosinophiliques et les formes liées à l'obésité, associées au sexe féminin.

### I - A - 3 Les phénotypes d'asthme

Caractéristiques phénotypiques	Modalités	SARP (36)	ADEPT (41)	U-BIOPRED (42)	FASE-CPHG (38)
		Asthme modéré à sévère N= 726 Royaume-Uni et Etats-Unis	Asthme modéré à sévère N= 156 Amérique du Nord et Europe	Asthme modéré à sévère N=266 Europe	Asthme sévère N=1502 France
Age de début de l'asthme	Enfance, précoce	Cluster 1, 2 et 4	Phénotype A1	Cluster T1, T3 et T4	<b>Cluster 1</b>
	Adulte, tardive	Cluster 3 et 5		Cluster T2	<b>Cluster 2, 3, 4 et 5</b>
Fonction respiratoire	Préservée	Cluster 1, 2	Phénotype A1, A2	Cluster T1 et T4	<b>Cluster 1, 4 et 5</b>
	Altérée	Cluster 3, 4 et 5	Phénotype A3 et A4	<b>Cluster T2, T3</b>	<b>Cluster 2 et 3</b>
Atopie	Présente, prédominante	Cluster 1, 2 et 4	Phénotype A2	Cluster T1, T3 et T4	<b>Cluster 1, 2 et 5</b>
	Absente ou minoritaire	Cluster 3 et 5	Phénotype A3 et A4	<b>Cluster T2</b>	<b>Cluster 3 et 4</b>
Inflammation	Eosinophilique (type 2) ou mixte	NA	Phénotype A2 et A4	Cluster T2, T3	<b>Cluster 4</b>
	Non éosinophilique (non type 2)	NA	Phénotype A1, A3	Cluster T1 et T4	
Morphotype	Normal	Cluster 1, 2	NA	Cluster T1, T3	<b>Cluster 1, 3, 4 et 5</b>
	Obésité	Cluster 3	NA	Cluster T2 et T4	<b>Cluster 2</b>
Sévérité Réponse au traitement	Léger à modéré Bonne réponse	Cluster 1, 2 et 3	Phénotype A1 et A2	Cluster T1	NA
	<b>Sévère Réponse incomplète</b>	Cluster 3, 4 et 5	<b>Phénotype A4</b>	<b>Cluster T2, T3 et T4</b>	NA

Tableau 2 Association entre les caractéristiques phénotypiques et les principaux clusters identifiés dans les études cliniques internationales (européenne et américaine) et française menées chez l'adulte.

Abréviations : ADEPT = Airways Disease Endotyping for Personalized Therapeutics, FASE-CPHG = France Asthme SEvère – Collège des Pneumologues des Hôpitaux Généraux, SARP = Severe Asthma Research Program, U-BIOPRED = Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes.

### I - A - 3 Les phénotypes d'asthme

Au total, près d'une **centaine d'études de cluster** menées sur des cohortes de patients asthmatiques adultes sont recensés (43). La synthèse de ces études est complexe du fait de l'hétérogénéité des populations de patients asthmatiques inclus (sévérité de la maladie, traitement, âge etc.). Le choix de la méthode mathématique utilisée (clusterisation hiérarchique, clusterisation par K-means, clusterisation en 2 étapes etc.) ainsi que les variables explicatives choisies biaisent la constitution des groupes phénotypiques. Malgré ces limites, certaines caractéristiques phénotypiques sont plus fréquemment décrites (Figure 1).

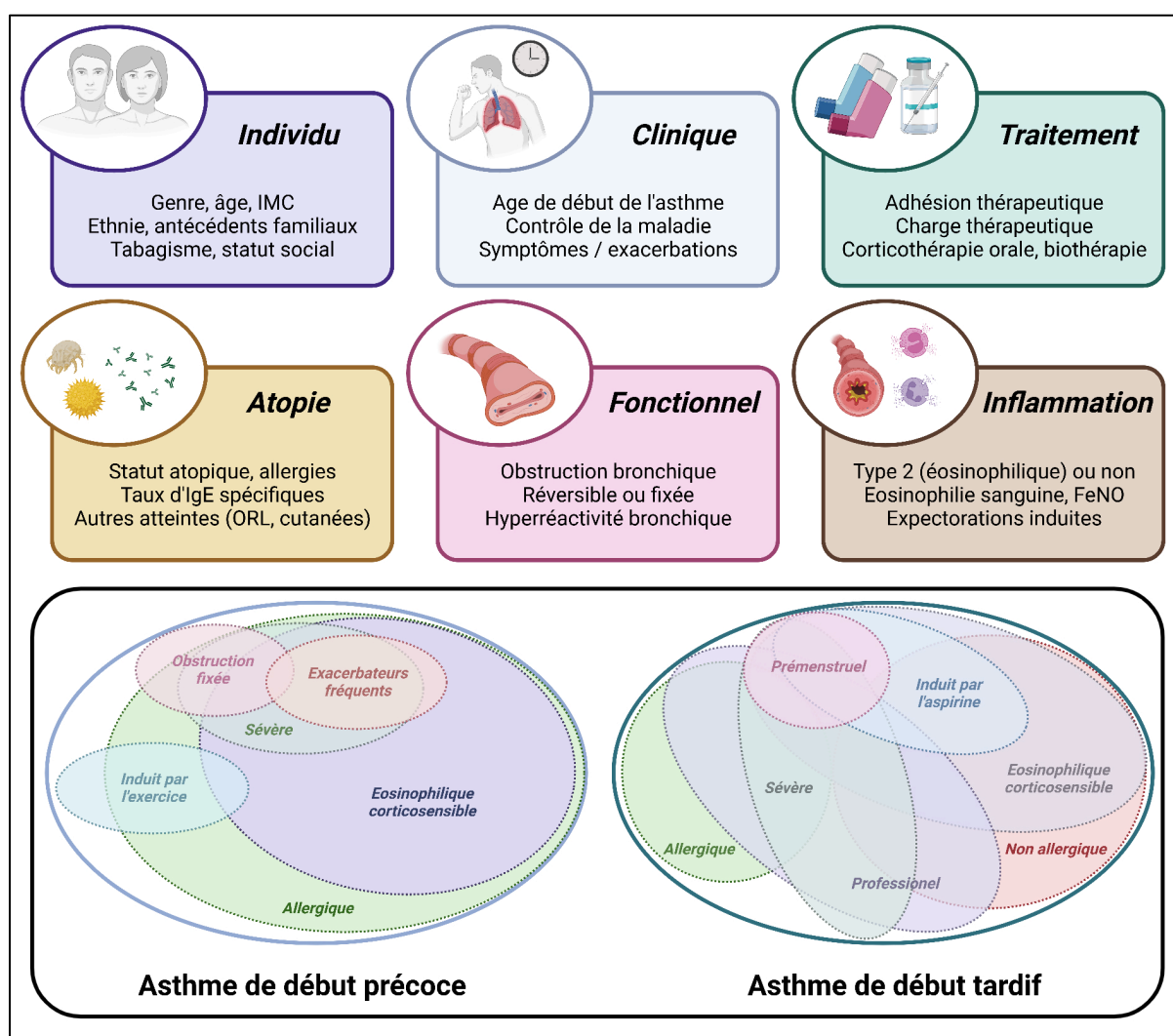


Figure 1 Phénotypes d'asthmes et caractéristiques cliniques, biologiques et fonctionnels. Adapté de (44, 45).

Les zones de recouvrement représentent les associations phénotypiques observées dans les populations de sujets asthmatiques de début précoce et tardif.

Abréviations : FeNO = Fraction Exhalée du Monoxide d'azote, IgE = Immunoglobuline E, IMC = Indice de Masse Corporelle, ORL = Oto-Rhino-Laryngologique.

**L'intérêt pratique** de la description et de la reconnaissance de ces différents phénotypes est encore discuté. Il a été montré que la stabilité de ces phénotypes cliniques est variable chez l'adulte à court et moyen termes (1-3 ans) d'une étude à une autre (41, 46, 47). Ces variations dépendent des biomarqueurs inflammatoires versatiles mais aussi des modifications de traitement (intensification et décroissance). Des constatations proches ont été faites concernant les types inflammatoires dans de petites études (<100 patients) menées chez l'enfant, tant à l'état stable qu'en exacerbation (48, 49).

**Sur le plan pronostic**, le phénotypage par clustering d'une cohorte de patients asthmatiques sévères français ne distingue pas de différence significative des taux d'exacerbation à moyen terme (3 ans) entre les groupes (50). En revanche, à long terme (20 ans), il a été démontré que les clusters phénotypiques sont associés à des évolutions cliniques différentes (51). Ainsi, le phénotype allergique avec hyperréactivité bronchique et le phénotype non allergique présentent une symptomatologie plus importante associée à une intensité thérapeutique élevée perdurant au cours du suivi.

La mise en évidence de caractéristiques cliniques et biologiques propres regroupant les patients asthmatiques interroge sur de potentiels **mécanismes physiopathologiques spécifiques sous-jacents**.

#### 4. Physiopathologie de l'asthme

Le développement et le maintien de l'asthme reposent sur **trois mécanismes physiopathologiques généraux** : *l'hyper-réactivité bronchique, le remodelage bronchique et l'inflammation bronchique* (Figure 2).

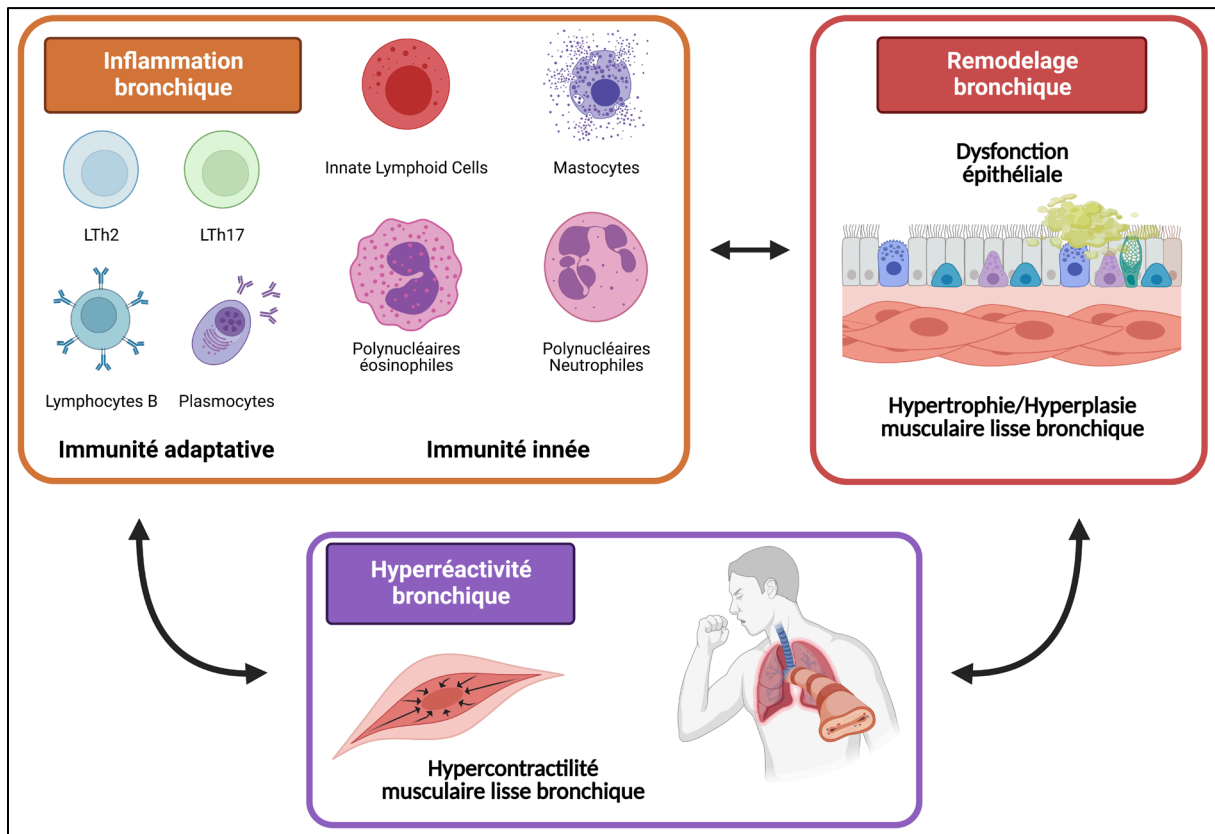


Figure 2 Mécanismes physiopathologiques de l'asthme

##### a) L'hyper-réactivité bronchique

**L'HRB** se définit par une réponse anormalement importante des voies aériennes en réponse à des stimuli bénins ou pathologiques. Elle se manifeste par un excès de bronchoconstriction dépendant de la contraction des CML. Cette bronchoconstriction anormale est favorisée par l'environnement épithélial et inflammatoire local.

**Les CML** bronchiques dérivent de précurseurs mésenchymateux et se développent en parallèle des bourgeons épithéliaux lors de l'embryogénèse (52). Leur développement est régulé par des stimuli mécaniques (tension) mais aussi cytokiniques épithéliaux. Présentes de la trachée jusqu'aux bronchioles, les CML constituent un composant de la paroi bronchique conservé au fil de l'évolution (53). En

dehors du développement, le rôle physiologique de l'appareil musculaire lisse bronchique demeure néanmoins controversé (54). En revanche, son rôle dans les mécanismes de bronchoconstriction au cours de l'asthme sont bien décrits et relèvent à la fois de facteurs extrinsèques que de facteurs intrinsèques (Annexes, p 176) (55).

- **Régulation extrinsèque de la contraction musculaire lisse bronchique**

Le système nerveux autonome régule la bronchoconstriction via des afférences nerveuses sensibles bronchiques, une intégration au niveau du tronc cérébral et des efférences nerveuses vers l'appareil musculaire lisse bronchique (56). Sur le plan anatomique et fonctionnel, 3 voies efférentes sont décrites : le système cholinergique, le système adrénergique et le système NANC (Tableau 3) (57-59).

Neurotransmetteur	Système	Effet	Référence
Acétylcholine	Cholinergique	Contraction	(60)
Adrénaline	Adrenergique	Relaxation	(61)
Noradrénaline	Adrenergique	Relaxation	(61)
Substance P	NANC	Contraction	(62)
Neurokinine A	NANC	Contraction	(63)
Neurokinine B	NANC	Contraction	(64)
Neurokinine P	NANC	Contraction	(64)
Neurokinine $\gamma$	NANC	Contraction	(65)
Peptide calcitonin gene-related peptide	NANC	Contraction	(65)
Vasoactive intestinal peptide	NANC	Relaxation	(66)
Peptide histidine isoleucine	NANC	Relaxation	(67)
Peptide histidine methionine	NANC	Relaxation	(65)

Tableau 3 Principaux neurotransmetteurs impliqués dans le tonus des CML

Abréviation : NANC = Non-Adrénargique Non-Cholinergique.

**Le tonus cholinergique** est le principal modulateur physiologique du tonus bronchique via la stimulation des récepteurs muscariniques M2 et M3 fortement exprimés par les CML bronchiques. Ces récepteurs sont respectivement associés au protéines G de type G $\alpha$ i et G $\alpha$ q dont la stimulation entraîne l'activation de la contraction musculaire lisse bronchique (Régulation intrinsèque de la contraction, p 13).

**Le tonus adrénergique** a pour principal fonction de contrebalancer les effets pro-contractiles du système cholinergique. La liaison de l'adrénaline ou de la noradrénaline sur les récepteurs  $\beta$ 2 adrénergique présents sur les CML induit leur relaxation.

**Le système nerveux non adrénergique non cholinergique**, quant à lui, joue un rôle de modulation du tonus musculaire lisse bronchique avec une activité pro-contractile ou relaxante en fonction des neurotransmetteurs impliqués.

**Au cours de l'asthme**, des altérations du système nerveux autonome sont suspectées (Figure 3) (68).

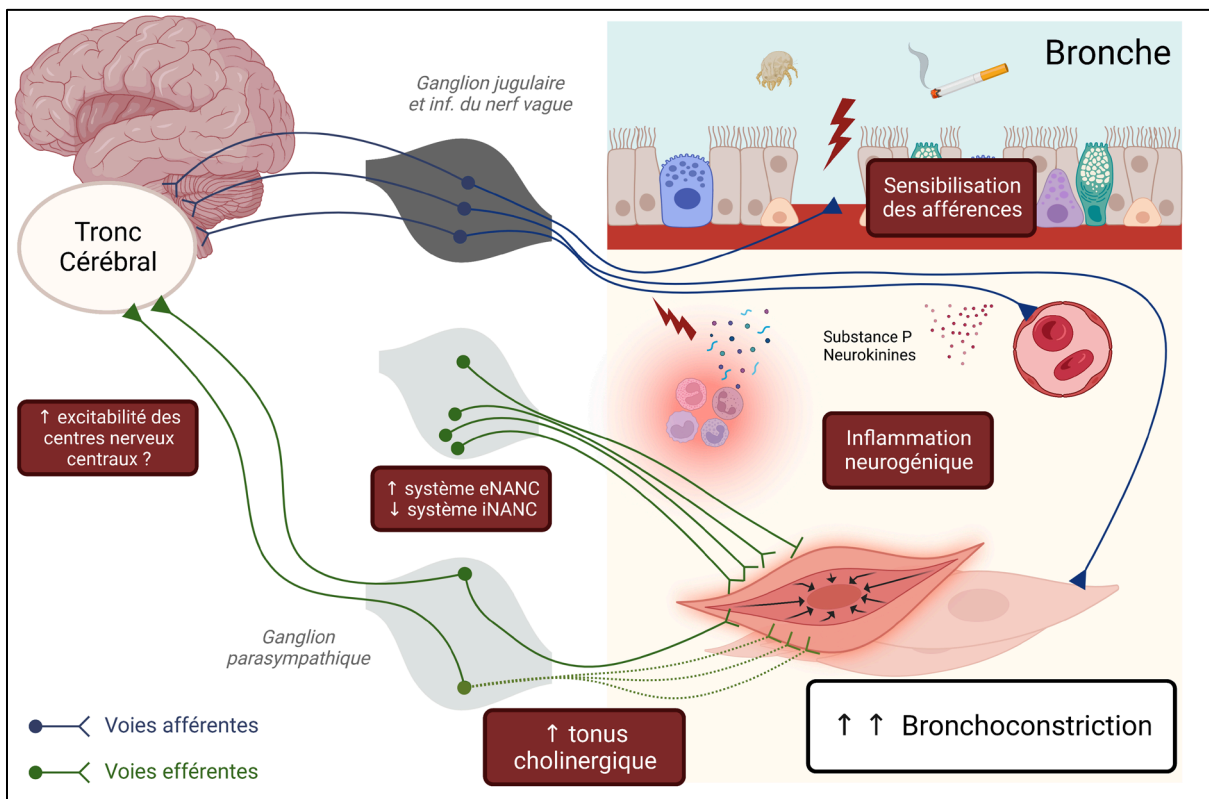


Figure 3 Dysfonction de la régulation du système nerveux autonome au cours de l'asthme. Adapté de (68).

Abréviation : Non-Adrénergique Non Cholinergique



Tout d'abord, une sensibilité accrue **des afférences nerveuses** secondaire aux atteintes épithéliales et aux médiateurs inflammatoires locaux est observée au cours de l'asthme (69). Inversement, des données montrent une action pro-inflammatoire des terminaisons nerveuses sensibles par libération de substance P ou de neurokinines : c'est l'inflammation neurogénique (70).

Concernant **le système nerveux central**, il a été observé dans un modèle simiesque d'asthme allergique aux acariens (n=3) une augmentation de l'excitabilité des neurones du noyau du tractus solitaire impliqués dans l'intégration des afférences sensibles des voies aériennes (71). Cependant, il n'existe pas actuellement de données directes prouvant une plasticité nerveuse des centres du tronc cérébral au cours de l'asthme chez l'Homme.

Concernant **les efférences nerveuses**, on note dans le cadre de l'asthme une augmentation du tonus cholinergique pro-contractile liée à une hyperexcitabilité neuronale d'une part et une hyperdensité locale d'autre part (72). Parallèlement, les l'induction d'un asthme chez la souris stimule les voies efférentes cholinergiques et dévie le NANC vers une réponse pro-contractile (73).

**Au total**, l'ensemble de ces altérations nerveuses participent à l'augmentation de la stimulation bronchoconstrictrice au cours de l'asthme.

- **Régulation intrinsèque de la contraction musculaire lisse bronchique**

Au cours de l'asthme, les CML présentent **une hypercontractilité** facilitant la survenue et le maintien de la bronchoconstriction. Il a été démontré que les CML bronchiques de patients asthmatiques présentent une augmentation de leur capacité maximale de contraction ainsi que de leur vitesse de raccourcissement en comparaison avec des sujets sains (74). Parallèlement, une contraction supérieure des CML bronchiques de patients asthmatiques a été observée en réponse à l'histamine comparée à des volontaires sains (75).

**Sur le plan fonctionnel**, la contraction des CML est dépendante de l'état de phosphorylation de la chaîne légère de myosine de 20kDa (MLC20). Le niveau de phosphorylation de la MLC20 est régulé par deux enzymes : la MLCK et la MLCP. Deux voies de signalisation distinctes influent sur les activités de ces 2 enzymes : **la voie du Ca<sup>2+</sup> et la voie de sensibilisation au Ca<sup>2+</sup>** (Figure 4).

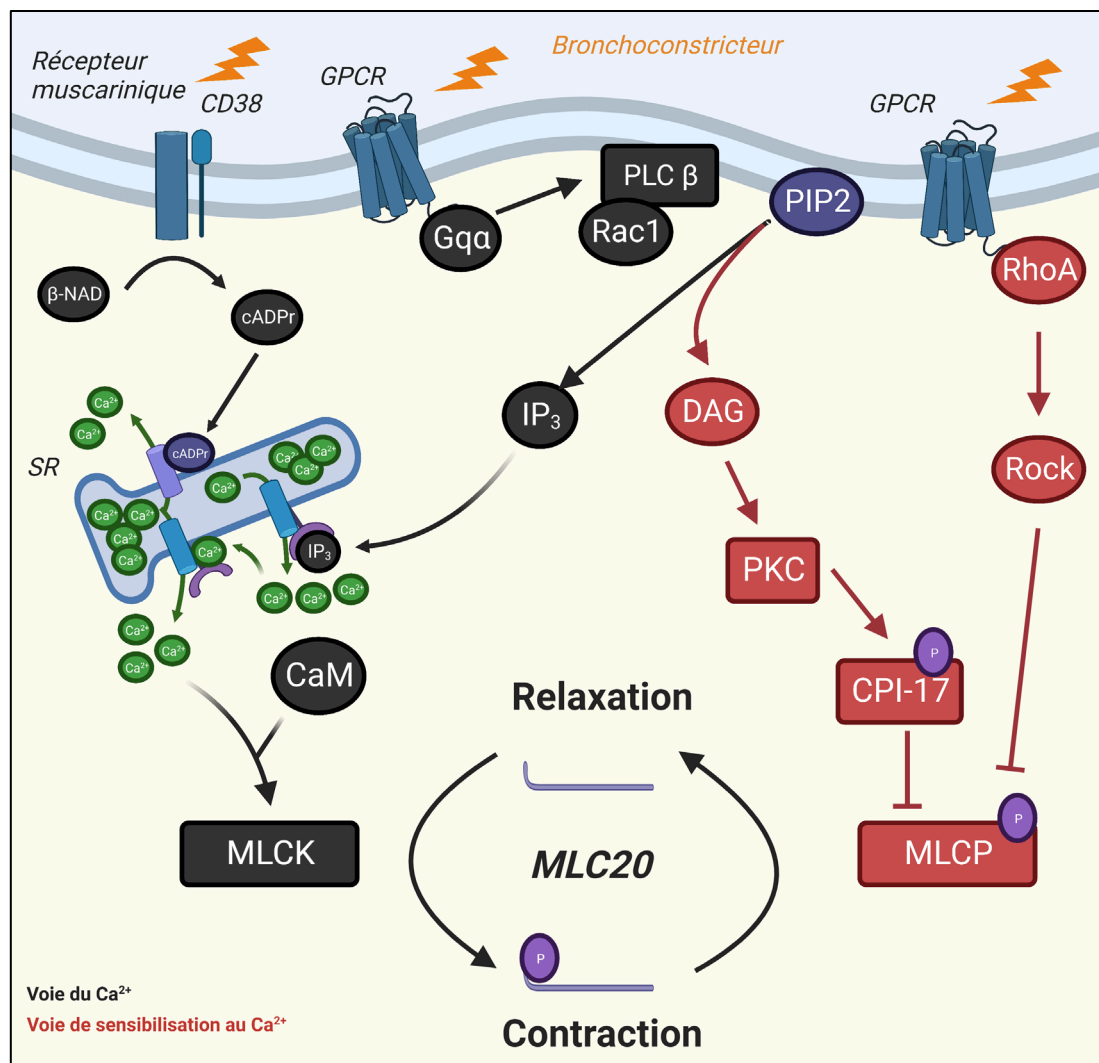


Figure 4 Régulation intracellulaire de la contraction des CML. D'après (55).

Abréviations : β-NAD = beta-nicotinamide adenine dinucleotide, cADP-r= cyclic Adenosin Di Phosphate ribose, CaM = Calmodulin, CD = Cluster of differentiation, CPI-17 = protein kinase C-potentiated phosphatase inhibitor protein of 17 kDa, DAG = DiAcylGlycerol, GPCR = G Protein-Coupled Receptors, IP3 = Inositol Tri Phosphate, MLC = Myosin Light Chain, MLCK = Myosin Light Chain Kinase, MLCP = Myosin Light Chain Phosphatase, PKC = Protein Kinase C, PLC = PhosphoLipase C, PIP2 = phosphatidylinositol-4,5-bisphosphate, Rac1 = Rac family small GTPase 1, ROCK = Rho Kinase, RhoA = Rho family small GTPase A, SR = Sarcoplasmic Reticulum.

La stimulation des récepteurs aux protéines G à 7 segments transmembranaires et des récepteurs muscariniques par des composés bronchoconstricteurs active la PLC et la production d'IP3 mais aussi la production de cADPr à partir de β-NAD (76-78). Ces seconds messagers se fixent à leurs récepteurs sarcoplasmiques déclenchant le relargage des stocks Ca<sup>2+</sup>. L'élévation du Ca<sup>2+</sup> intracellulaire active la calmoduline qui stimule l'activité de la MLCK. La phosphorylation de la MLC par la MLCK initie les cycles de contraction actine/myosine : **c'est la voie du Ca<sup>2+</sup>**.

Cette voie de signalisation est **dysfonctionnelle dans les CML** au cours de l'asthme. En effet, la MLCK est surexprimée dans les CML des patients asthmatiques (74, 79). Par ailleurs, les molécules pro-inflammatoires (IL-1 $\beta$ , IL-13, TNF $\alpha$ ) augmentent l'expression du CD38 par les CML chez les patients asthmatiques provoquant un relargage accrue du Ca<sup>2+</sup> sarcoplasmique (voie de la cADPr) (77, 80).

Parallèlement, les bronchoconstricteurs activent la GTPase RhoA qui stimule son effecteur ROCK. Cette sérine/thréonine kinase phosphoryle la MLCP inhibant son activité (81). Parallèlement, la PKC s'active en réponse à l'élévation du DAG produit par la PLC. La phosphorylation de CPI-17 par la PKC entraîne sa fixation sur la sous-unité catalytique de la MLCP qui inhibe son activité phosphatase. L'activation de ces voies de signalisation maintient des niveaux élevés de phosphorylation de la MLC20 indépendamment des variations des concentrations intracellulaires de Ca<sup>2+</sup> : c'est **la voie de sensibilisation calcique**.

La stimulation de cette voie au cours de l'asthme participe à **l'hypercontractilité des CML**. Tout d'abord, il a été montré que l'expression et l'activité de la CPI-17 s'élèvent dans les CML dans les modèles d'asthme chez le rat (82). Par ailleurs, l'expression de RhoA dans les CML augmente en réponse à l'inflammation (IL-17, IL-13) dans des modèles animaux d'asthme (83-85). L'inhibition de la ROCK par le composé Y-27632 prévient l'apparition de l'hyper-réactivité bronchique chez le cochon d'inde (86).

#### b) Le remodelage bronchique

Le remodelage bronchique se définit **par l'ensemble des altérations structurelles et fonctionnelles suivantes** : la dysfonction épithéliale, l'épaississement de la lame basale, l'œdème bronchique et l'hypertrophie/hyperplasie des CML (Figure 5) (87, 88). Le remodelage bronchique peut apparaître précocement dans l'asthme avec des lésions observables dès l'enfance (89, 90). La sévérité de l'atteinte histologique (muscle lisse bronchique et épaississement de la lame basale) est corrélée avec celle de la maladie (91). Chez l'enfant comme chez l'adulte, les lésions de remodelage bronchique sont associées à la persistance de l'obstruction bronchique au cours du temps (92, 93). Il est à noter que l'importance relative de ces différentes composantes de remodelage diffère d'une population de patients asthmatiques à une autre suggérant des mécanismes physiopathologiques sous-jacents hétérogènes (94, 95).

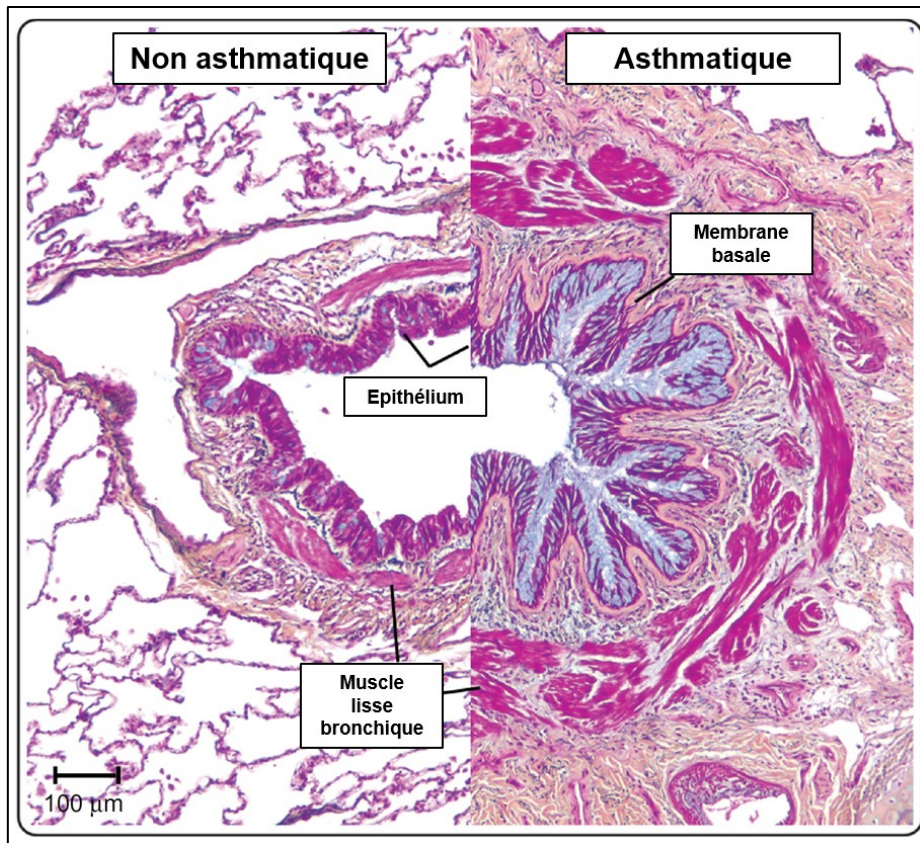


Figure 5 Le remodelage bronchique au cours de l'asthme (Adaptée de (96)).

Sections de bronches colorées au pentachrome de Movat.

- **La dysfonction épithéliale**

**A l'état physiologique**, l'épithélium bronchique joue un rôle de barrière vis-à-vis de l'environnement extérieur. Il intervient aussi dans la régulation des réponses immunes développées à son encontre (balance tolérance/inflammation). L'épithélium respiratoire est pseudostratifié et composé de cellules columnaires ciliées, de cellules à mucus (ou cellules à gobelet) ainsi que de cellules indifférenciées impliquées dans son auto-renouvellement. L'appareil mucociliaire participe à l'élimination des corps étrangers et à la protection anti-infectieuse. Les complexes de jonction intercellulaire et d'interaction avec la lame basale assurent l'étanchéité de la barrière épithéliale.

**Au cours de l'asthme**, l'appareil mucociliaire est altéré avec une réduction de la proportion de cellules ciliées d'une part et une augmentation du nombre de cellules à mucus d'autre part (Figure 6) (97). De plus, l'efficacité du battement ciliaire est réduite et la surproduction de MUC5AC au détriment de MUC5AB augmente la viscosité du mucus (98, 99). Parallèlement, les ruptures des structures d'adhésion

intercellulaire aboutissent à la mise à nu de la membrane basale dans les cas les plus sévères (100). Ces anomalies sont causées à la fois par des éléments extérieurs (infectieux, allergéniques) mais aussi par les processus inflammatoires sous-épithéliaux.

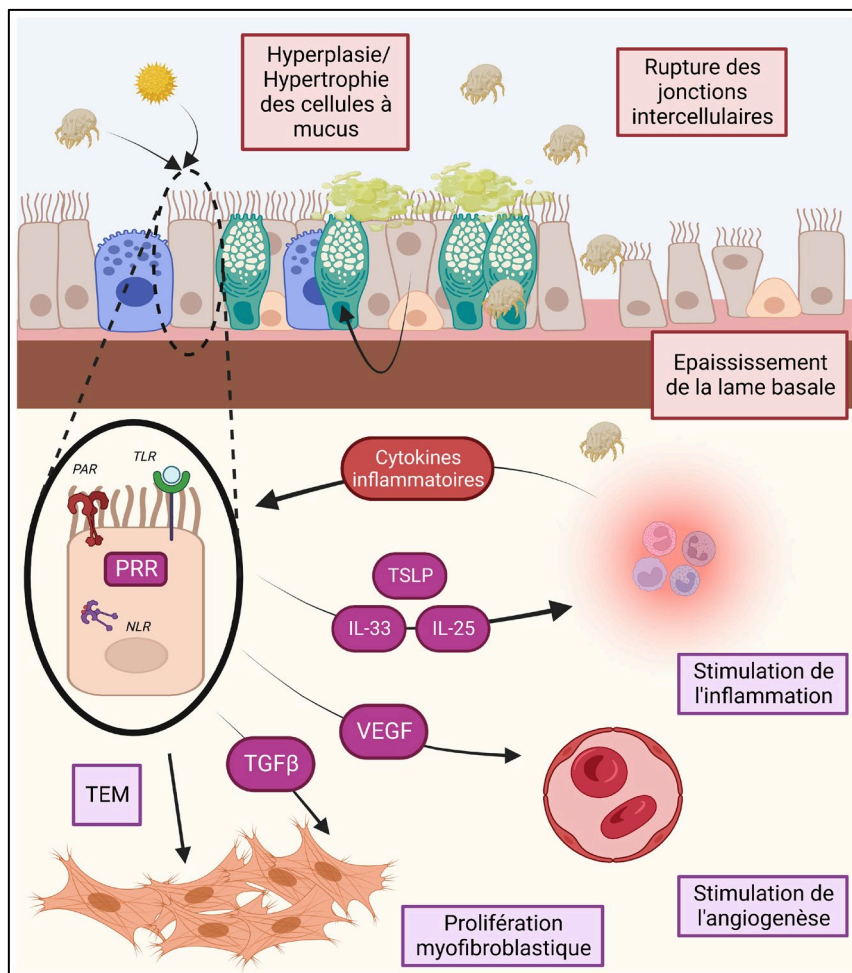


Figure 6 La dysfonction épithéliale au cours de l'asthme.

Les cellules épithéliales bronchiques jouent un rôle actif dans **l'initiation et le maintien de l'inflammation bronchique** au cours de l'asthme. L'expression des PRR leur permet de répondre aux DAMPs et aux PAMPs environnementaux. Les cellules épithéliales expriment ainsi des TLR impliqués dans la reconnaissance allergénique (acariens, chats etc.), des NLR stimulant les réponses anti-microbiennes et des PAR sensibles aux enzymes associées aux allergènes (101-103). Les cellules épithéliales activées par ces signaux de danger relarguent des composés pro-inflammatoires tels que la TSLP, l'IL-33 et l'IL-25. Ces **alarmines** jouent un rôle majeur dans l'initiation des réponses inflammatoires bronchiques, notamment éosinophiliques (cf. endotype de type 2) (104).

**La TSLP** est une cytokine de la famille de l'IL-7 qui stimule la réponse inflammatoire allergique en activant les cellules dendritiques (105). Son expression est augmentée au sein de l'épithélium bronchique de patients asthmatiques en comparaison avec des sujets sains (106). De plus, les cytokines inflammatoires de type 2 majorent la production de TSLP par les cellules épithéliales ce qui participe à l'auto-entretien de la réponse inflammatoire (107).

**L'IL-25 (ou IL-17E)**, quant à elle, est produite au niveau de la muqueuse bronchique en réponse à l'exposition de l'épithélium à des allergènes (108). Sa production par l'épithélium bronchique est corrélée à l'intensité de l'inflammation éosinophilique locale (infiltration bronchique, expectoration induite) et générale (éosinophilie sanguine) chez les patients asthmatiques (109).

**L'IL-33**, affiliée à l'IL-1, est exprimée à la fois par les cellules épithéliales et les CML bronchiques (110). Les signaux de danger locaux entraînent le relargage des formes nucléaire séquestrées (111, 112). Au cours de l'asthme, l'IL-33 participe au développement des réponses immunes de type 2 (éosinophilique) ainsi qu'à l'hyperréactivité bronchique (113, 114).

Outre son implication dans les mécanismes inflammatoires, l'épithélium bronchique intervient dans les **modifications structurelles de la paroi bronchique** observées au cours de l'asthme (115). L'unité trophique épithélio-mésenchymateuse (EMTU) associant les cellules épithéliales et les fibroblastes sous-épithéliaux régule les mécanismes de réparation de l'épithélium bronchique. En cas de perte de l'intégrité épithéliale, une réaction inflammatoire contrôlée s'initie. Les progéniteurs locaux (cellules basales épithéliales) et recrutés (cellules souches dérivés de la moelle osseuse) migrent puis prolifèrent recouvrant ainsi la zone dénudée (116). Parallèlement, les cellules fibroblastiques se différencient en myofibroblastes et produisent une matrice fibrosante soutenant la réparation épithéliale. Dans un contexte physiologique, la différenciation épithéliale des cellules progénitrices et l'apoptose des myofibroblastes restaurent l'intégrité et la fonction de l'épithélium.

**Au cours de l'asthme**, une rupture des équilibres lésion/inflammation/réparation est observée. Des anomalies de la voie de l'EGF, associée à la prolifération cellulaire épithéliale bronchique, ont été mises en évidence chez les patients asthmatiques. En effet, l'expression de l'EGFR est augmentée dans les cellules épithéliales bronchiques

au cours de l'asthme (117, 118). Par ailleurs, la production de TGF $\beta$  par les cellules structurales (épithéliales, myofibroblastiques, CML) favorise la prolifération des myofibroblastes et la modification de la matrice extracellulaire (119). Il est à noter que le TGF $\beta$  favorise la transition épithélio-mésenchymateuse des cellules épithéliales bronchiques de patients asthmatiques *in vitro* (120). Néanmoins, la pertinence de ce phénomène reste débattue *in vivo* (121).

- **La dysfonction musculaire lisse bronchique**

**A l'état physiologique**, les CMLs présentent un faible taux de prolifération et un phénotype contractile caractérisé par une expression importante de smooth muscle (sm)- $\alpha$ -actine, de sm  $\gamma$ -actine, de chaîne lourde de sm myosine, de calponine, de h-caldesmone, de SM22 $\alpha$ , de smootheline et de metavinculine (122).

**L'hyperplasie et l'hypertrophie** de l'appareil musculaire lisse bronchique observées au cours de l'asthme relèvent de différents mécanismes (123).

Dans un environnement inflammatoire, les CMLs évoluent vers **un phénotype proliférant et migratoire**. En effet, la stimulation de CML *in vitro* par un ensemble de facteurs de croissance et de cytokines augmentés dans l'asthme entraîne un switch vers un phénotype proliférant (Tableau 4) (124).

**Les alarmines épithéliales** (TSLP et IL-25) sont décrites pour stimuler l'expression de protéines inflammatoires et de molécules de la MEC par les CML (88, 125, 126). Par ailleurs, les stimulations mécaniques (compression/relaxation) et les agressions physiques de l'épithélium entraînent le relargage de molécules pro-inflammatoires (IL-6, IL-8 et monocyte chemoattractant protein-1) et d'enzymes stimulant la prolifération des CML (127, 128). Il est à noter que les cytokines inflammatoires ont un effet synergique sur les changements phénotypiques des CML. En effet, alors que l'IL-13 seule a des effets anti-proliférants sur les CML, elle induit l'expression par les CML du récepteur CysLT1 les sensibilisant aux leucotriènes ce qui majore à terme leur prolifération (129, 130). Enfin, les CML stimulées en condition inflammatoire produisent des molécules pro-inflammatoires ou pro-prolifération (PDGF, FGF, IL-1 $\beta$ , TGF- $\beta$ , IL-5, IL-6, IL-8 et IL-17) ce qui amplifie ces changements phénotypiques (57).

I - A - 4 Physiopathologie de l'asthme

Facteurs	Source cellulaire	Effets	Réf
<b>Facteurs de croissance</b>			
<b>PDGF</b>	Plaquettes, monocytes/macrophages, CML, cellules épithéliales	Pro-prolifération	(131-134)
<b>FGF</b>	Fibroblastes, monocytes/macrophages, CML	Pro-prolifération	(134-137)
<b>EGF</b>	Cellules épithéliales, plaquettes	Pro-prolifération	(138)
<b>Cytokines</b>			
<b>TGF-<math>\beta</math></b>	CML, lymphocytes T, cellules épithéliales	Pro-prolifération	(136, 139-141)
<b>TNF-<math>\alpha</math></b>	CML, cellules épithéliales, lymphocytes T, monocytes/macrophages	Pro-prolifération, anti-prolifération	(134, 142-144)
<b>IL-1<math>\beta</math></b>	Lymphocytes T, monocytes/macrophages, CML, cellules épithéliales	Pro-prolifération	(145, 146)
<b>IL-6</b>	Lymphocytes T, monocytes/macrophages, CML, cellules épithéliales	Pro-prolifération	(143, 146)
<b>IFN-<math>\gamma</math></b>	Lymphocytes T, cellules NK	Pro-prolifération	(147)
<b>IL-4</b>	Lymphocytes T, mastocytes	Pro-prolifération	(148, 149)
<b>Médiateurs inflammatoires</b>			
<b>Histamine</b>	Mastocytes, basophiles	Pro-prolifération	(150, 151)
<b>Thromboxane A2</b>	Mastocytes, monocytes/macrophages	Pro-prolifération	(152, 153)
<b>Sphingosine 1-phosphate</b>	Plaquettes, plasma	Pro-prolifération	(154)
<b>Enzymes et divers</b>			
<b>Tryptase</b>	Mastocytes	Pro-prolifération	(151, 155)
<b>Thrombine</b>	Plasma	Pro-prolifération	(156)
<b>Elastase</b>	Neutrophiles	Pro-prolifération	(157)
<b>ROS</b>	Monocytes-macrophages, neutrophiles, éosinophiles, mastocytes	Pro-prolifération	(158)

*Tableau 4 Cytokines et molécules influant sur la prolifération des cellules musculaires lisses dans l'asthme*

*Abréviations : EGF = Epithelial Growth Factor, FGF = Fibroblast Growth Factor, IFN = Interféron, IL = Interleukine, PDGF = Platelet-Derived-Growth-Factor, ROS = Reactive oxygen species, TGF = Transforming Growth Factor, TNF = Tumor Necrosis Factor.*



**La migration de CML et de leur progéniteur** en réponse à des agents mitotiques comme le PDGF, le VEGF, le TGF-  $\beta$  et l'IL-1 $\beta$  participe à l'hyperplasie musculaire lisse bronchique. La MAPK P38 ainsi que PI3K et ERK sont sollicitées lors de ces phénomènes migratoires. L'inhibition de ces voies de signalisation réduit les capacités motrices des CML liées à leur impact sur le cytosquelette d'actine (159-161).

**La composition de la MEC** modifie le phénotype et les fonctions des CMLs (162). La laminine, composante de la MEC, réduit les capacités proliférantes des CMLs et majore leur expression de protéines associées à la contraction telles que la sm- $\alpha$ -actine et la chaîne lourde de la myosine (163). Or, au cours de l'asthme sévère, les CML sécrètent des métalloprotéinases participant à la dégradation de cette MEC (164). Par ailleurs, la fibronectine, dont la synthèse est accrue au cours de l'asthme, promeut la prolifération des CML et diminue leurs propriétés contractiles (163, 165).

Indépendamment de la prolifération, les CMLs activées présentent des capacités accrues **d'interaction avec les cellules immunitaires**. En condition inflammatoire, l'expression par les CML de protéines membranaires d'interaction telles que VCAM-1, ICAM-1, CD44 et LFA-1 augmente (166). Par ailleurs, les mastocytes sont sensibles aux agents chimiotactiques ou stimulant comme le SCF, le TGF- $\beta$ 1 ou le TSLC-1, pouvant être produits par les CML (167). L'expression membranaire de CD44 et de CD51 par les CML stimule parallèlement l'adhésion de ces cellules (168). Ces interactions privilégiées des mastocytes avec les CML favorisent leur dégranulation ce qui majore en retour la contraction et la prolifération des CML (169). D'autres populations cellulaires inflammatoires comme les lymphocytes T et les éosinophiles ont été décrits comme interagissant avec les CML au cours de l'asthme (170).

c) Les endotypes inflammatoires

L'amélioration de la compréhension des mécanismes immunologiques et moléculaires impliqué dans l'asthme a permis de séparer 2 principaux endotypes inflammatoires : éosinophilique ou T2 et non éosinophilique ou non T2 (171, 172).

- **L'endotype de type 2**

L'inflammation de type 2 joue un rôle majeur dans **les réponses immunes en lien avec l'environnement**, notamment en réponse aux infections helminthiques ou aux venins (173, 174). Sur le versant pathologique, elle constitue le principal mécanisme par lequel les allergies se développent et se maintiennent chez un individu (175). Ainsi, l'inflammation de type 2 est associée à un nombre important de pathologies liées à l'environnement tel que l'asthme, la dermatite atopique et la rhinosinusite chronique avec des implications pathologiques et thérapeutiques communes (Annexes, p 161) (176).

La réponse inflammatoire de type 2 se caractérise par la production de **trois cytokines majeures** : l'IL-4, l'IL-5 et l'IL-13 (Figure 7). Bien qu'elles puissent être produites par de nombreux types cellulaires, les lymphocytes Th2 et les ILC-2 constituent les principales sources cellulaires impliquées dans l'initiation et la pérennisation de la réponse. Ces populations cellulaires sont sensibles aux productions cytokiniques provenant de l'épithélium bronchique (alarmines).

**La réponse adaptative Th2** se développe en réponse à l'IL-4 qui entraîne l'activation du facteur de transcription GATA3 puis l'expression des cytokines de type 2. Celle-ci intervient en réponse à la présentation antigénique par les cellules dendritiques localisées au niveau de la paroi bronchique et migrant vers les ganglions lymphatiques (177, 178). D'autres cellules présentatrices d'antigènes professionnelles sont en mesure d'initier une telle réponse. Les lymphocytes B contribuent notamment à l'expansion et la polarisation des lymphocytes Th2 dans des conditions de faibles concentrations antigéniques (179). Les macrophages de type M2, en nombre supérieur dans les lavages bronchoalvéolaires de patients asthmatiques, réduisent la capacité des cellules dendritiques à induire une réponse Th1 *in vitro* (180). Leur rôle exact dans l'initiation et ou le maintien de la réponse Th2 *in vivo* reste néanmoins discuté (181, 182).

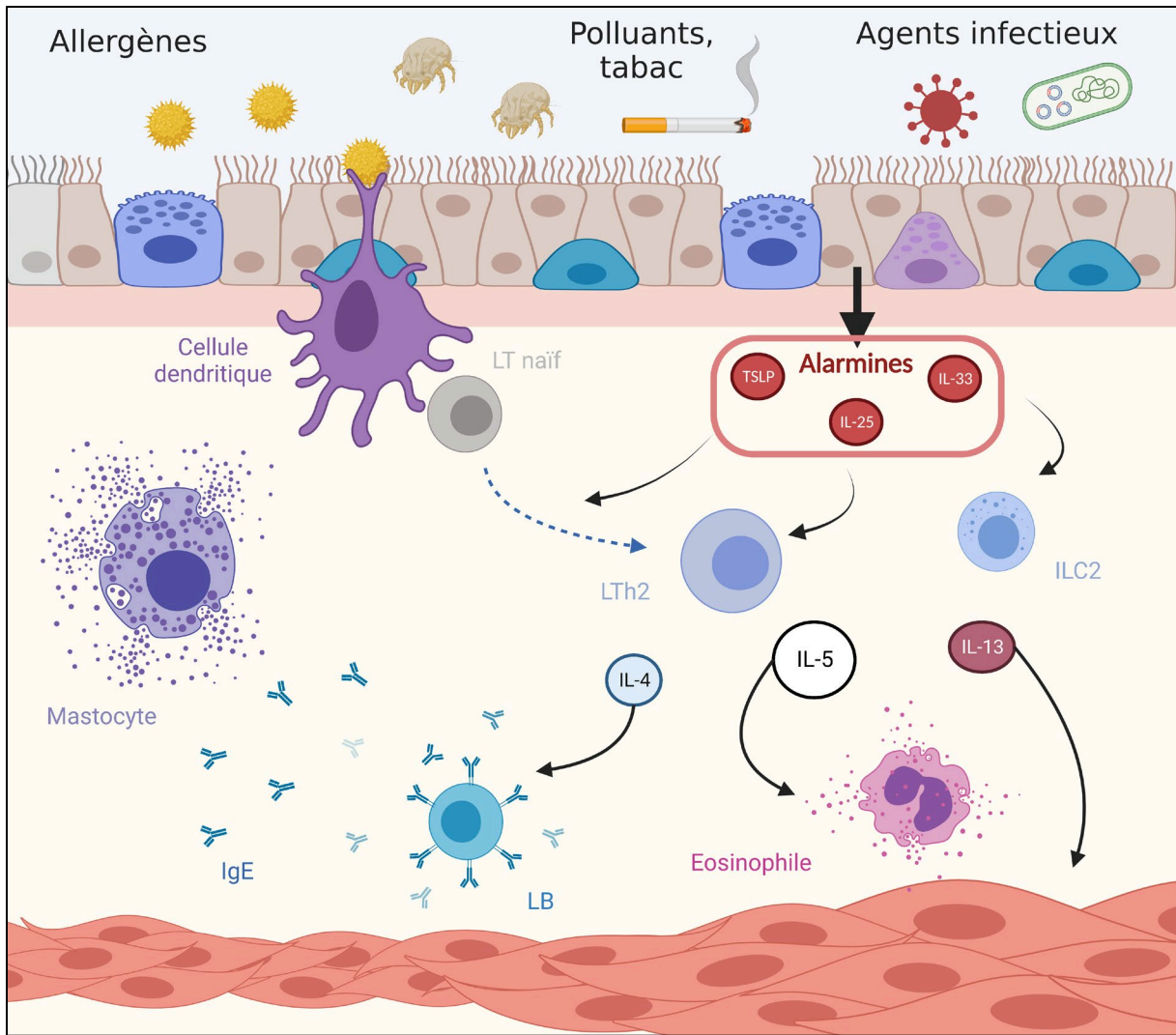


Figure 7 L'endotype T2 dans l'asthme

**Les ILC2** sont des cellules de l'immunité innée se développant sous l'impulsion de l'IL-7 et dont la polarisation est influencée par les alarmines épithéliales (183, 184). L'expression et l'activation des facteurs de transcription GATA3 et ROR $\alpha$  induit la production d'IL-5, d'IL-9 et d'IL-13 (185, 186). Un nombre supérieur d'ILC2 est observé dans les voies aériennes des patients asthmatiques sévères éosinophiliques en comparaison avec les patients asthmatiques modérés et les sujets sains (187). L'exposition allergénique chez des sujets humains entraîne l'activation locale des ILC2 (188, 189). Indépendante d'une reconnaissance spécifique d'antigène, l'activation des ILC2 joue aussi un rôle important dans les asthmes éosinophiliques non allergiques. Enfin, une étude récente a montré que les ILC2 stimulées avec de la TSLP présente une résistance accrue aux corticostéroïdes et pourrait être impliquées dans la sévérité de la maladie (190).

Les cytokines de type 2 produisent des effets différents mais complémentaires au niveau cellulaire et tissulaire.

**L'IL-4** initie ses effets via 2 types de récepteurs membranaires hétérodimériques : le récepteur de type I, spécifique de l'IL-4, constitué de l'IL-4R $\alpha$  et de la sous-unité  $\gamma_c$  et le récepteur de type II, fixant l'IL-4 et l'IL-13, constitué de l'IL-4R $\alpha$  et de la sous-unité IL-13R $\alpha$ 1 (191). L'IL-4 induit le switch isotypique des lymphocytes B vers la production d'IgE (192). Les IgE spécifiques ainsi produites se fixent sur leurs récepteurs exprimés par les mastocytes tissulaires. Lors d'une nouvelle exposition à l'antigène, le recrutement des IgE entraîne la dégranulation cellulaire et le relargage *in situ* de molécules pro-inflammatoires telles que l'histamine, la prostaglandine E2 et la tryptase (193). Il s'agit du mécanisme impliqué dans la réponse précoce observée dans le cadre de l'asthme allergique. Parallèlement, l'IL-4 stimule l'expression de protéines d'ancrage membranaire (VCAM1 et LFA-1) par les cellules endothéliales ce qui favorise le recrutement des cellules effectrices (194).

**L'IL-13**, quant à elle, favorise le remodelage bronchique. Elle agit sur l'épithélium bronchique en stimulant la métaplasie des cellules à mucus (195). Elle diminue par ailleurs les jonctions serrées au sein de l'épithélium bronchiques aggravant sa dysfonction (196). Enfin, elle induit la production par l'épithélium bronchique de l'iNOS, majorant la production locale de monoxyde d'azote (197). Concernant les CML, L'IL-13 est associée au développement de l'hyper-réactivité bronchique. Ainsi, l'absence d'IL-13 chez la souris réduit drastiquement la réponse des CML à la méthacholine (198).

**L'IL-5** est la principale cytokine impliquée dans l'inflammation éosinophilique bronchique observée dans l'asthme de type 2. Elle stimule la production de polynucléaires éosinophiles en polarisant les progéniteurs myéloïdes (199, 200). Associée à l'éotaxine, l'IL-5 augmente le recrutement local des éosinophiles par chimiotactisme et participe à leur activation (201, 202). Enfin, cette cytokine réduit l'apoptose des éosinophiles tissulaires et augmente leur résistance aux corticostéroïdes (203, 204). *In situ*, les polynucléaires éosinophiles activés relarguent leurs granules riches en protéines et enzymes cytotoxiques, en cytokines (notamment de type 2) ainsi qu'en médiateurs lipidiques. Ceux-ci intensifient la réponse inflammatoire, altèrent l'épithélium bronchique et modifient le phénotype des CML bronchiques participant au remodelage bronchique (205-208).

Au cours de l'asthme, **la balance entre les réponses inflammatoires de type 2 et la tolérance immune** est altérée. Le relargage de cytokines de type 2 par des cellules effectrices telles que les polynucléaires éosinophiles et basophiles ainsi que les mastocytes participe à l'amplification et l'auto-entretien de l'inflammation. Parallèlement, l'efficacité des réponses tolérogènes est réduite au cours de l'asthme. Il est observé une diminution de la quantité et de l'activité des lymphocytes T régulateurs producteurs d'IL-10 chez les patients asthmatiques sévères (209, 210). Des constatations concordantes ont été faites concernant les lymphocytes B régulateurs producteurs d'IL-10 (211-213).

Sur le plan clinique, **les critères diagnostiques** d'asthme éosinophilique ont évolué parallèlement aux progrès thérapeutiques (biothérapies de l'asthme sévère). Une définition consensuelle de l'asthme sévère éosinophilique est proposée par l'ERS et se base sur l'association de critères majeurs et mineurs (214). L'élévation significative des polynucléaires éosinophiles recueillis sur une expectoration induite (>3%) constitue l'étalon-or historique. Du fait de son accessibilité restreinte aux centres experts, le recours à l'éosinophilie sanguine et à la fraction exhalée du monoxyde d'azote est désormais recommandé par les sociétés savantes américaines et européennes (ATS et ERS) (215). L'hyperéosinophilie sanguine ( $\geq 0.5\text{G/L}$ ) est retrouvée chez environ 10% des patients asthmatiques adultes alors que 50% des patients asthmatiques sévères présentent une élévation des éosinophiles sur l'expectoration (216, 217). Néanmoins, les corrélations entre l'éosinophilie sanguine, la FeNO et l'infiltration éosinophilique bronchique sont imparfaites (218, 219).

L'un des archétypes de l'asthme éosinophilique est **l'asthme allergique**. Celui-ci se développe principalement durant l'enfance et l'adolescence. Ces sujets présentent ou ont présenté d'autres pathologies du spectre allergique telles que la dermatite atopique, l'allergie alimentaire et la rhinoconjonctivite allergique constituant la « marche atopique ». L'enquête allergologique ciblée constitue une étape diagnostique cruciale dans le cadre de la prise en charge de l'asthme (2). Parmi les **asthmes éosinophiliques non allergiques**, certaines formes spécifiques se distinguent. On peut citer notamment l'asthme associé à la polypose naso-sinusienne et aggravé par les anti-inflammatoires non stéroïdiens, représentant environ 7,2% des patients asthmatiques (220). Ces formes sont plus sévères avec une altération de la qualité de vie, notamment par les troubles ORL (anosmie, obstruction nasale etc...)

(221). Des données suggèrent que la source des cytokines de type 2 dans ces phénotypes pourrait être rattachée aux ILC2 (187).

- ***L'endotype non type 2***

**Les endotypes non type 2** sont retrouvés chez près de la moitié des patients asthmatiques légers à modérés (222). Des analyses transcriptomiques menées sur des brossages épithéliaux de patients asthmatiques légers à modérés retrouvent aussi une répartition équilibrée entre les signatures T2 et non T2 (223). Sur le plan inflammatoire, les endotypes non T2 englobent les asthmes pauci-granulocytaires d'une part et les asthmes neutrophilique d'autre part.

**Les asthmes pauci-granulocytaires** correspondent à des formes peu ou pas inflammatoires mais néanmoins associées aux atteintes structurelles (remodelage bronchique) et à l'hyper-réactivité bronchique caractéristiques de l'asthme. Ils présentent des altérations moins marquées de la fonction respiratoire et de la qualité de vie quotidienne bien que des formes sévères puissent exister (224, 225). La physiopathologie des asthmes pauci-granulocytaires reste peu connue malgré sa fréquence élevée (40-45%). Les modèles animaux et les études transcriptomiques et protéomiques menées chez l'Homme (U-BioPred, ADEPT) ont montré des altérations du contrôle neurologique, du phénotype des CML et dans une moindre mesure de la production de radicaux libres (226).

**Les asthmes neutrophiliques**, associée ou non avec des éosinophiles, sont associés à des formes plus sévères d'asthme notamment sur le plan des exacerbations et de l'altération de la fonction respiratoire (227, 228). Les enzymes et cytokines libérées par les polynucléaires neutrophiles sont liées à certaines caractéristiques du remodelage bronchique, telle que l'hyperplasie des CML, ainsi qu'à l'hyper-réactivité bronchique (229-232). Les patients asthmatiques présentant une inflammation neutrophilique répondent peu ou pas aux corticostéroïdes (233). Cette infiltration neutrophilique est secondaire au développement de réponses adaptatives et innées spécifiques (Figure 8).

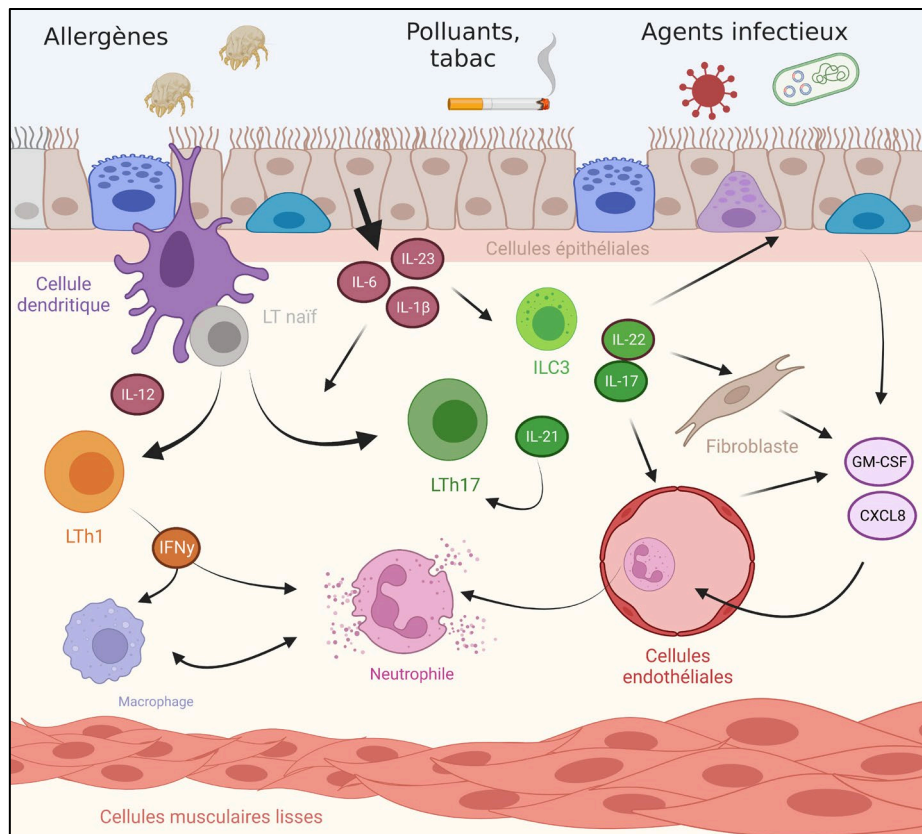


Figure 8 L'endotype neutrophilique dans l'asthme

**Les lymphocytes Th17** se développe en présence d'IL-1 $\beta$  ou d'IL-6 et d'IL-23. Ces cytokines induisent l'activation de la voie STAT3 puis l'expression des facteurs de transcription ROR $\gamma$ t et la production d'IL-17 et d'IL-22 (234). Il est à noter que les lymphocytes Th17 présentent une certaine plasticité avec la description de populations mixtes Th2/Th17 et Th1/Th17 en fonction de l'environnement inflammatoire (234, 235). **Les ILC3** constituent le pendant inné des lymphocytes Th17 se développant sous l'impulsion de l'IL-1 $\beta$  et d'IL23 et produisant elles aussi de l'IL17 et de l'IL-22 (183, 236). Il a été observé chez la souris obèse que l'inflammation neutrophilique et le développement de l'HRB est liée à l'expansion d'ILC3 dans un modèle (237). Parallèlement, une équipe a montré au cours de l'asthme neutrophilique que les macrophages présentent une augmentation de l'expression de gènes impliqués dans réponses anti-bactériennes, le chimiotactisme neutrophilique et les mécanismes de phagocytose/efferocytose (238). Ces observations plaident en faveur de mécanismes multiples impliqués dans l'infiltration neutrophilique bronchique au cours de certaines formes d'asthme.

**L'IL-17**, principale cytokine impliquée dans ces formes neutrophiliques, favorise l'infiltration tissulaire des polynucléaires neutrophiles en entraînant la production de chimiokines et facteurs de croissance (CXCL8, GM-CSF) par les cellules épithéliales et endothéliales ainsi que par les fibroblastes (239). L'augmentation de la production d'IL-17 a été confirmée au cours de l'asthme sévère et corrélée à l'augmentation de production de cytokines et chimiokines pro-neutrophiliques par les cellules épithéliales bronchiques (cultures primaires) (240). Outre la promotion de l'inflammation neutrophilique, l'IL-17 A agit directement sur le muscle lisse bronchique majorant sa contractilité ce qui aggrave l'HRB (84, 241-244). Enfin, l'activation des réponses de type 17 est corrélée au développement de mécanismes de cortico-résistance tels que l'augmentation de l'expression du GR $\beta$  (dominant négatif) par les monocytes ou de MEK1 par les lymphocytes T CD4<sup>+</sup> antagonisant les effets du GR $\alpha$  (245).

**La reconnaissance de l'inflammation neutrophilique** est néanmoins complexe du fait de l'absence de biomarqueur fiable. En effet, la neutrophilie sanguine et l'expectoration induite sont faiblement corrélées à l'infiltration tissulaire des voies aériennes et sujettes à de nombreux facteurs confondants (246). Des données conflictuelles existent par ailleurs concernant le lien entre corticostéroïdes inhalées et infiltration neutrophilique des voies aériennes questionnant l'existence d'un asthme neutrophilique (247, 248). Par ailleurs, des analyses transcriptomiques montrent que les signatures d'expression liées aux inflammations de type 2, neutrophiliques et pauci-granulocytaires ne sont pas stables au cours du temps (249). La possibilité pour un même individu d'expérimenter divers types inflammatoires au cours de l'histoire naturelle de sa maladie est actuellement en cours d'exploration.

## 5. *Prise en charge de l'asthme en France*

La prise en charge de l'asthme se définit dans 2 contextes différents mais complémentaires : celle de l'asthme aigu ou en exacerbation et celle de l'asthme chronique (prise en charge de fond). Celle-ci s'articule dans un cas comme dans l'autre autour de classes thérapeutiques anti-inflammatoires et bronchodilatatrices.



**Le principal objectif** du traitement de l'asthme est d'obtenir **le contrôle de la maladie** avec le minimum de traitement nécessaire. L'obtention d'un contrôle optimal est en effet corrélée à une amélioration de la morbi-mortalité chez le patient asthmatique quel que soit le degré de sévérité (250, 251). Ce contrôle se définit selon les modalités décrites ci-dessous (Tableau 5).

<i>Critères</i>	<i>Asthme contrôlé</i>	<i>Asthme partiellement contrôlé</i>	<i>Asthme non contrôlé</i>
<i>Symptômes diurnes</i>	Aucun à 2 maximum par semaine	Plus de 2 fois par semaine	
<i>Symptômes ou réveils nocturnes</i>	Aucun	Au moins une fois par semaine	
<i>Fréquence d'utilisation du traitement de secours</i>	Aucun à 2 maximum par semaine	Plus de 2 fois par semaine	Au moins 3 critères d'asthme partiellement contrôlé
<i>Retentissement sur les activités</i>	Aucun	Au moins une fois*	
<i>Exacerbations</i>	Aucune	Au moins une exacerbation*	
<i>VEMS ou DEP</i>	Normaux	<80% de la valeur théorique ou de la meilleure valeur personnelle	

*Tableau 5 Critères de contrôle de l'asthme. D'après (252).*

*\* depuis la dernière consultation médicale*

*Abréviations : DEP = Débit Expiratoire de Pointe, VEMS = Volume Expiré Maximal en 1 Seconde*

Le praticien dispose d'outils d'évaluation quantitative de ce contrôle parmi lesquels se trouvent le score de l'ACQ et de l'ACT (253, 254). Ces deux questionnaires se différencient par la période de rappel des symptômes, 1 semaine pour l'ACQ et 4 semaines pour l'ACT. Ces 2 scores sont utilisés en pratique clinique courante et en recherche (255, 256).

**La stratégie globale** de prise en charge de l'asthme chronique repose sur la répétition de cycle d'évaluation/traitement visant à maintenir un contrôle optimal de la pathologie. La prise en charge des facteurs déclenchants (exposition allergénique, pollution, expositions professionnelles) et/ou aggravants (reflux gastro-œsophagien, apnées du sommeil, facteurs psychologiques etc.) y est systématiquement associée. Les différents paliers de prise en charge sont présentés ci-dessous (Tableau 6).

Prise en charge globale	Education thérapeutique, contrôle de l'environnement, traitement des comorbidités				
Paliers	1	2	3	4	5
Traitement de fond		CSI doses faible OU CSI/formotérol à la demande	CSI dose faible + BDLA	CSI dose moyenne + BDLA	CSI dose forte + BDLA Si non contrôlé, ajout de tiotropium Ajout biothérapie (anti-IgE, anti-IL-5/IL-5R, anti-IL4R $\alpha$ ) Thermoplastie bronchique
Autres traitements de fond	CSI dose faible	LTRA	CSI doses moyennes OU CSI doses faibles + LTRA OU CSI dose faible + théophylline	CSI dose forte OU CSI dose forte + LTRA OU CSI dose forte + théophylline	CSO faible dose au long cours
Traitement de secours	BDCA à la demande				
		CSI-formotérol à la demande si traitement de fond par CSI-formotérol			
Stratégies associées		ITA si allergie aux acariens			

Tableau 6 Stratégie thérapeutique globale et détail des paliers de traitement de fond.

Abréviations : BDCA = BronchoDilatateur de Courte Durée d'Action, BDLA = Bronchodilatateurs de Longue Durée d'Action, CSI = Corticostéroïdes inhalés, LTRA = antagonistes des récepteurs aux leucotriènes.

#### a) Traitements conventionnels : corticostéroïdes et bronchodilatateurs

**Les corticostéroïdes** constituent la principale classe thérapeutique utilisée pour contrôler la maladie asthmatique. Leur développement sous forme inhalée dans le courant des années 1980 a considérablement amélioré le pronostic de la pathologie réduisant la mortalité et améliorant la qualité de vie des patients asthmatiques (257).

**Le mécanisme d'action** des corticostéroïdes dans l'asthme a fait l'objet d'un intérêt particulier depuis leur apparition dans l'arsenal thérapeutique. L'activation du récepteur aux glucocorticoïdes inhibe les voies AP1 et NF $\kappa$ B réduisant la production de cytokines et de chimiokines pro-inflammatoires par les lymphocytes T et les cellules épithéliales (258). L'infiltration et la survie tissulaire des cellules effectrices telles que les éosinophiles et les mastocytes sont ainsi significativement réduites (259).

Enfin, les glucocorticoïdes diminuent directement la survie des cellules effectrices à l'exception des polynucléaires neutrophiles (259, 260).

Même si les corticostéroïdes inhalés cible préférentiellement les effecteurs inflammatoires, ils influent aussi sur **la contractilité** et à minima sur **la prolifération** des CML bronchiques. Les glucocorticoïdes réduisent l'expression de la SM22 $\alpha$  et de la MLCK des CML bronchiques en réponse au TGF $\beta$  ce qui diminue leur contractilité (261). Par ailleurs, ils diminuent la phosphorylation de CPI-17 entraînant une réduction de la phosphorylation de la MLC et par voie de conséquence de l'HRB (262). Concernant le remodelage, le ciclésionide a un effet bénéfique sur le phénotype des CML bronchiques ainsi que sur l'hyperplasie des cellules à mucus (modèle d'asthme chez le rat) (263). Enfin, de petites études cliniques ont démontré que de fortes doses de corticostéroïdes améliorent l'hypervascularisation de la muqueuse bronchique et l'épaississement de la lame basale (264, 265). Néanmoins, l'existence d'un remodelage bronchique chez des patients traités par de fortes doses de corticoïdes pose la question de mécanismes d'échappement.

**Les bronchodilatateurs** de courte et de longue durée d'action comportent principalement les béta-2 mimétiques et les anticholinergiques. Ceux-ci agissent sur les CML en provoquant une réponse bronchodilatatrice et en limitant le stimulus bronchoconstricteur respectivement. Ces réponses découlent de l'inhibition des voies de signalisation impliquées dans la bronchoconstriction développée précédemment (L'hyper-réactivité bronchique, p 10). Les progrès thérapeutiques réalisés sur les 30 dernières années, concernant ces classes thérapeutiques, ont principalement consisté en l'amélioration du délai et de la durée d'action des molécules ainsi que les dispositifs d'inhalation.

#### b) Les biothérapies anti-inflammatoires

Les biothérapies utilisées dans le traitement de l'asthme sévère chez l'Homme, ciblent principalement les voies impliquées dans l'endotype de type 2 (Tableau 7).

Phénotype Endotype	Molécule	Cible	Critères d'inclusion principal	Effets thérapeutiques	Ref
Allergique	<b>Omalizumab</b> Anticorps monoclonal humanisé	IgE	Asthme sévère sous haute dose de CSI Prick-test positif à un aéroallergène IgE sériques totales comprises entre 30 et 700UI/mL	Réduction du taux d'exacerbation	(266, 267)
	<b>Mepolizumab</b> Anticorps monoclonal humanisé		II-5	Asthme sévère sous haute dose de CSI Eosinophilie sanguine $\geq 150/\text{mm}^3$ à l'inclusion ou $\geq 300/\text{mm}^3$ au cours de l'année précédente. + Traitement d'entretien par corticostéroïdes systémiques (5-35mg de prednisone ou équivalent).	Réduction du taux d'exacerbation Réduction des doses de corticoïdes oraux
Eosinophilique	<b>Reslizumab</b> Anticorps monoclonal humanisé	II-5	Asthme insuffisamment contrôlé malgré un CSI à dose au moins moyenne. Eosinophilie sanguine $\geq 400/\text{mm}^3$	Réduction du taux d'exacerbation	(270)
Eosinophilique	<b>Benralizumab</b> Anticorps monoclonal humanisé	Récepteur de l'IL-5	Asthme sévère avec des CSI à haute dose. Eosinophilie sanguine $\geq 300/\text{mm}^3$ .	Réduction du taux d'exacerbation Réduction des doses de corticoïdes oraux	(271-273)
Inflammation de type 2	<b>Dupilumab</b> Anticorps monoclonal entièrement humain	Récepteur $\alpha$ de l'IL-4 (commun avec IL-13)	Asthme non contrôlé malgré des doses moyennes à élevées de CSI + deux médicaments de contrôle.	Réduction du taux d'exacerbation Réduction des doses de corticoïdes oraux	(274, 275)
Eosinophilique	<b>Tezepelumab</b> Anticorps monoclonal entièrement humain	TSLP	Asthme non contrôlé malgré des doses moyennes ou élevées de CSI	Réduction du taux d'exacerbation	(276)

Tableau 7 Biothérapies ayant fait la preuve de leur efficacité dans l'asthme sévère chez l'Homme (études de phase III)

**L'omalizumab**, anticorps monoclonal humanisé recombinant anti IgE, bloque la fixation des IgE sur leurs récepteurs par la formation de complexes immuns. Les études cliniques en vie réelle confirment son efficacité avec une diminution du taux

d'exacerbation, une amélioration de la qualité de vie et une capacité de réduction de la corticothérapie sous traitement (277, 278). Il a été montré que l'éosinophilie sanguine pré-thérapeutique ne détermine pas l'efficacité du traitement chez les patients asthmatiques sévères atopiques (279). La recherche de biomarqueur de prédiction de réponse à l'omalizumab reste donc active.

Les biothérapies ciblant la voie de l'IL-5, **le mepolizumab, le reslizumab** et le **benralizumab** ont toutes fait la preuve de leur efficacité chez le patient asthmatique sévère éosinophilique. Une méta-analyse indirecte a mis en évidence une réduction moyenne du taux annuel d'exacerbations de 43% avec une bonne tolérance clinique, sans réelle amélioration cliniquement significative du contrôle de l'asthme (ACQ-5) ou de la fonction respiratoire (VEMS) (280).

La voie de l'IL-4/IL-13 constitue une alternative pour limiter l'inflammation de type 2. Celle-ci est ciblée par **le dupilumab**, anticorps anti-IL4-R $\alpha$ . Les études de phase 3 démontrent chez les patients traités une réduction de 60 à 80% des exacerbations associée à une amélioration importante du VEMS (environ 400 mL), sans que le taux d'éosinophiles ne semble affecter la réponse (281). Tout comme les anti-IL5, le dupilumab permet de réduire la posologie de corticoïdes oraux d'entretien (70.1% vs 41.9% groupe placebo) (275). Des données françaises ont confirmé la pertinence de son utilisation en pratique clinique courante (ATU) (282). Il est à noter que les biothérapies ciblant directement l'IL-13 (tralokinumab et lebrikizumab) n'ont pas réussi à démontrer une efficacité significative malgré l'utilisation de biomarqueurs (FeNO, périostine) (283, 284).

**Les alarmines** font actuellement l'objet de plusieurs essais cliniques de phase 2 et de phase 3. Récemment, **le tezepelumab**, un anticorps anti-TSLP, a démontré une réduction significative du taux annualisé d'exacerbation dans l'asthme (276). Cette amélioration est observée indépendamment de l'éosinophilie sanguine ( $\geq$  ou  $<300/\text{mm}^3$ ). En parallèle, un anticorps ciblant l'IL-33, REGN3500, est actuellement testé dans la dermatite atopique et l'asthme en monothérapie ou en association avec le dupilumab (NCT03736967, NCT03112577, NCT03736967). Actuellement, aucun anticorps anti-IL-25 n'est actuellement testé en clinique.

Des cibles potentielles **indépendante des cytokines de type 2** sont actuellement explorées. C'est le cas du CRTh2 dont l'activation par la prostaglandine 2 favorise la chimiotaxie et l'activation des lymphocytes Th2 et des éosinophiles. Dans une étude de preuve de concept (asthme modéré à sévère), l'AZD1981, un antagoniste oral de la CRTh2, n'a pas montré d'augmentation significative du débit expiratoire de pointe (critère d'évaluation principal) mais des tendances vers une amélioration du score de contrôle de la maladie (285). D'autres données provenant de patients asthmatiques atopiques montrent une tendance à l'augmentation du VEMS sous traitement (286). Néanmoins, le fevipirant, un autre antagoniste oral de la CRTh2, n'a pas permis d'obtenir une amélioration significative du taux d'exacerbation dans l'asthme sévère (287).

Il n'existe pas actuellement de biothérapie disponible pour le traitement de l'asthme sévère non éosinophilique, notamment neutrophilique. **Le brodalumab**, un anticorps ciblant l'IL-17, n'avait pas réussi à faire la preuve d'une efficacité significative dans une population d'asthmatique sévère (288). L'absence de biomarqueur explique potentiellement l'échec par ciblage non optimal de la population incluse.

c) La thermoplastie bronchique

**La thermoplastie bronchique** est un traitement endoscopique interventionnel s'adressant aux patients asthmatiques sévères exacerbateurs fréquents inéligibles aux biothérapies ou aux essais thérapeutiques. La technique consiste en l'application d'une sonde thermique au contact des bronches pour réduire l'épaisseur de la paroi et limiter l'activité contractile des CML. Un total de 3 séances espacées d'au moins 3 semaines est nécessaire pour un traitement complet.

Dans l'essai clinique AIR-2, la thermoplastie bronchique améliore de manière significative **la qualité de vie des patients** avec une tendance en faveur d'un meilleur contrôle de l'asthme (289, 290). Une bonne tolérance à long terme de la thermoplastie bronchique a été confirmée après 5 ans de suivi (291). En ce qui concerne le remodelage bronchique, la thermoplastie bronchique diminue légèrement l'épaisseur de la paroi des voies aériennes et le piégeage aérien, 2 ans après l'intervention dans une petite série de cas prospectifs (n=11) (292). Néanmoins, aucune amélioration significative de la lumière des voies aériennes n'a été observée. Enfin, la thermoplastie

bronchique n'améliore pas la fonction des petites voies aériennes telle qu'évaluée par oscillométrie à impulsion 6 mois après la procédure (n=43) (293).

d) L'immunothérapie allergénique

**L'ITA** vise à induire chez un individu donné une tolérance à un allergène spécifique. Dans l'asthme, l'indication de l'ITA est restreinte aux asthmes associés à une rhino-conjonctivite allergique aux acariens. Sa principale contre-indication est l'absence de contrôle de la maladie et/ou l'existence d'une obstruction bronchique significative (VEMS < 70 %). Sur le plan immunologique, l'ITA se base sur le concept de réorientation de la réponse lymphocytaire B et T vers des profils régulateurs.

Dans le cadre de **l'asthme allergique aux acariens**, une méta-analyse montre que l'ITA améliore les symptômes respiratoires et réduit l'utilisation des traitements de secours (294, 295). À visée préventive, des études montrent que la réalisation d'une ITA dans le cadre de la rhinoconjonctivite chez l'enfant et l'adolescent réduirait l'incidence future de l'asthme évaluée sur les seuls symptômes (296). Des observations complémentaires ont été faites sur la diminution du recours ultérieur à des traitements d'asthme chez les patients traités par ITA pour une rhino-conjonctivite aux pollens. Dans l'asthme sévère, des études sont nécessaires pour évaluer l'intérêt de l'ITA chez les patients traités par omalizumab dans l'optique d'améliorer le contrôle ou de sevrer la biothérapie.

e) Perspectives d'amélioration

A l'ère de **la médecine personnalisée**, le concept de traits traitables se développe dans l'asthme, sévère ou non (297). Ceux-ci désignent des caractéristiques pouvant être identifiées, accessibles à un traitement ciblé et dont la prise en charge améliore significativement le contrôle et le retentissement de la maladie.

Parmi **les traits traitables** proposés se trouvent : la contraction musculaire lisse bronchique et l'hyper-réactivité bronchique, l'œdème de la muqueuse bronchique, l'hypersécrétion de mucus, l'atteinte des petites voies aériennes, l'inflammation de type 2, la toux réflexe d'hyperréactivité (neurogène) et les facteurs infectieux (297). Au-delà des facteurs bronchiques locaux, des caractéristiques extra-pulmonaires ont aussi été associées à l'histoire naturelle de la maladie et à son contrôle (298). C'est le cas notamment de l'obésité, du degré d'activité physique et des troubles anxieux et

dépressifs qui sont associés à des présentations plus sévères d'asthme (299). Sur le plan pratique, une étude récente randomisée et contrôlée sur un petit effectif (n=55) a montré qu'une prise en charge orientée sur les traits traitables améliore significativement la qualité de vie des patients ainsi que le contrôle de l'asthme en comparaison avec une prise en charge standard (300). Des études supplémentaires sont néanmoins nécessaires pour améliorer le niveau de preuve et évaluer la faisabilité (pratique, socio-économique) d'une telle stratégie.

A l'heure actuelle, les **traits traitables** correspondent principalement à des caractéristiques évaluables à l'échelon de l'individu, du tissu ou de la cellule. Des études ciblant la recherche non biaisée de nouvelles voies physiopathologiques par analyses génomiques, transcriptomiques et protéiques réalisées sur des prélèvements systémiques et locaux ont pu être menées sur des cohortes de grandes envergures (U-BIOPRED, ADEPT, SARP etc.). Celles-ci ont pu confirmer l'intérêt des voies connues (type 2) et découvrir de nouvelles voies prometteuses (voie de l'IL-6 et inflammasome) dont l'intérêt devra être confirmé (Annexes, p 137) (301).

L'exploration des **mécanismes moléculaires** sous-tendant les voies physiopathologiques impliquées dans l'asthme sévère pourrait aboutir au développement de nouvelles stratégies thérapeutiques (Figure 9).

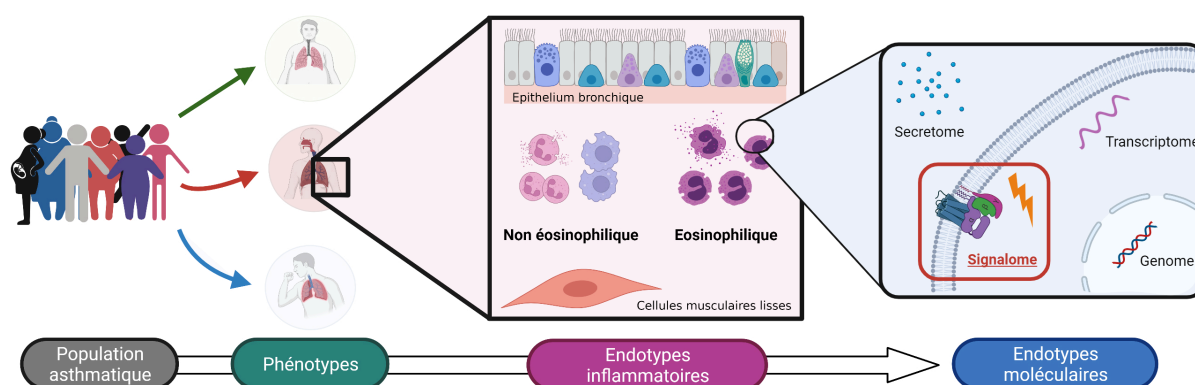


Figure 9 Les traits traitables dans l'asthme, à la recherche de nouvelles voies d'intérêt. Issu de (302).



## **B. La GTPase Rac**

### **1. La famille des Rho-GTPase**

La superfamille des protéines Ras comprend 5 familles de protéines : les familles Ras, Arf, Ran, Rab et Rho. La première description des **GTPase monomériques de la famille Rho** chez l'Homme date de 1985 avec la découverte de RhoA, RhoB et RhoC (303). Avec les avancées des techniques et outils de séquençage génomique un ensemble de 20 protéines homologues de cette famille a été décrit. Celles-ci se répartissent en 8 sous-familles : RhoA, Rac, Cdc42, RhoUV, RhoBTB, RhoDF, RhoH et Rnd (304). Spécifiques des cellules eucaryotes, elles sont singulièrement conservées chez les vertébrés.

#### a) Structure des RhoGTPases

**Les GTPases de la famille Rho** présentent une homologie de séquence élevée estimée à 80-90% avec l'existence de domaines fortement conservés (Figure 10A). Sur le plan structural, les GTPase de la famille Rho sont des protéines monomériques (20kDa) constituées de 5 feuillets  $\beta$  et de 6 hélices  $\alpha$  (Figure 10B) (305). Sa structure fonctionnelle comporte un ensemble de 4 domaines fonctionnels : le domaine G, les régions switch I/II, la région d'insertion et la boîte CAAX.

**Le domaine G** est constitué d'un ensemble de 5 séquences conservées d'acides-aminés qui forme la poche nucléotidique interagissant avec le GDP/GTP (Figure 10A et C). Celle-ci se réalise d'une part par le biais d'un ion  $Mg^{2+}$  fixée par une thréonine de la région G2 et d'autre part par les interactions avec les groupements phosphate du nucléotide par la région G1 (ou boucle P).

**Les régions switch I et II** sont impliqués dans les interactions distinctes entre le GTP et le GDP. La reconnaissance du GTP se réalise par le biais d'une thréonine du switch I et d'une glycine du switch II interagissant avec le 3<sup>ème</sup> groupement phosphate du GTP. En l'absence de GTP, un relâchement de la structure tridimensionnelle de la GTPase survient : c'est le mécanisme de « ressort de charge » (cf. Figure 10D) (306). Ces changements conformationnelles modifient l'affinité des RhoGTPases avec ses partenaires protéiques.

I - B - 1 La famille des Rho-GTPase

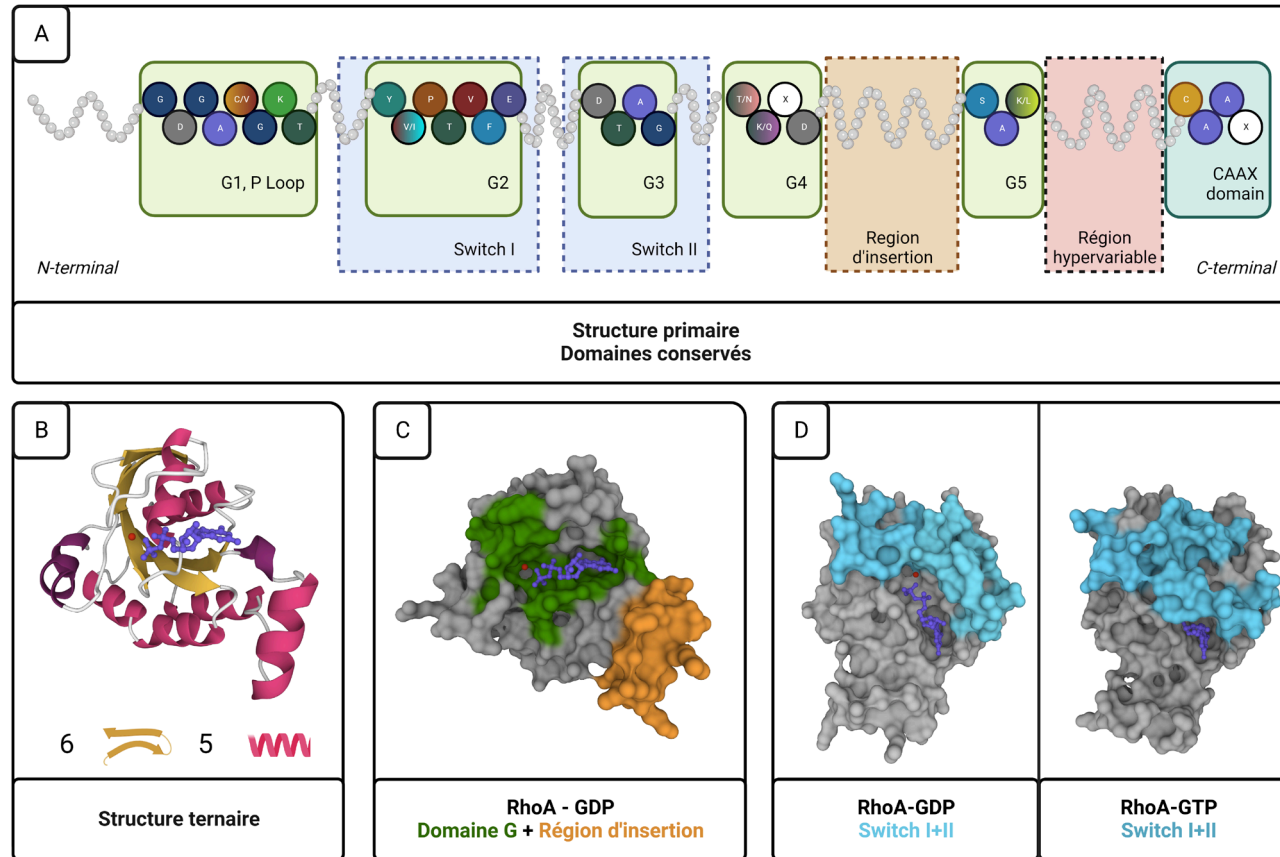


Figure 10 Relation structure-fonction des Rho-GTPases.

A) Structure primaire avec mise en avant des séquences conservées. B) Structure ternaire de RhoA fixant du GDP (violet) avec le  $Mg^{2+}$  associé (rouge). C) Surface moléculaire de RhoA avec mise en évidence de la poche nucléotidique et de la région d'insertion. D) Surface moléculaire avec représentation des modifications conformationnelles des zones switch en fonction de la fixation du GDP/GTP (307, 308).

Abréviations utilisées : GDP = Guanine di-phosphate, GTP = Guanine tri-phosphate, RhoA = Rho homologous A,.

**La région d'insertion** est une zone d'interaction des RhoGTPases avec leurs activateurs et leurs effecteurs. Elle est présente dans toutes les RhoGTPases mais sa composition et sa conformation varient entre ses différents membres ce qui participe à la sélectivité des partenaires protéiques.

**La boîte CAAX** localisée à l'extrémité C-terminale peut fixer une séquence lipidique par geranylgeranylation ou palmitoylation ou farnesylation en fonction des membres. L'ajout de ce motif hydrophobe permet l'ancrage des Rho-GTPases au sein des bicouches lipidiques membranaires contrôlant sa localisation cellulaire.

b) Cycle d'activation des RhoGTPases

**Sur le plan fonctionnel**, les Rho-GTPases sont des interrupteurs moléculaires oscillant entre une forme active et une forme inactive (Figure 11). La forme active se localise au niveau membranaire et lie le GTP alors que sa forme inactive liée au GDP est cytosolique.

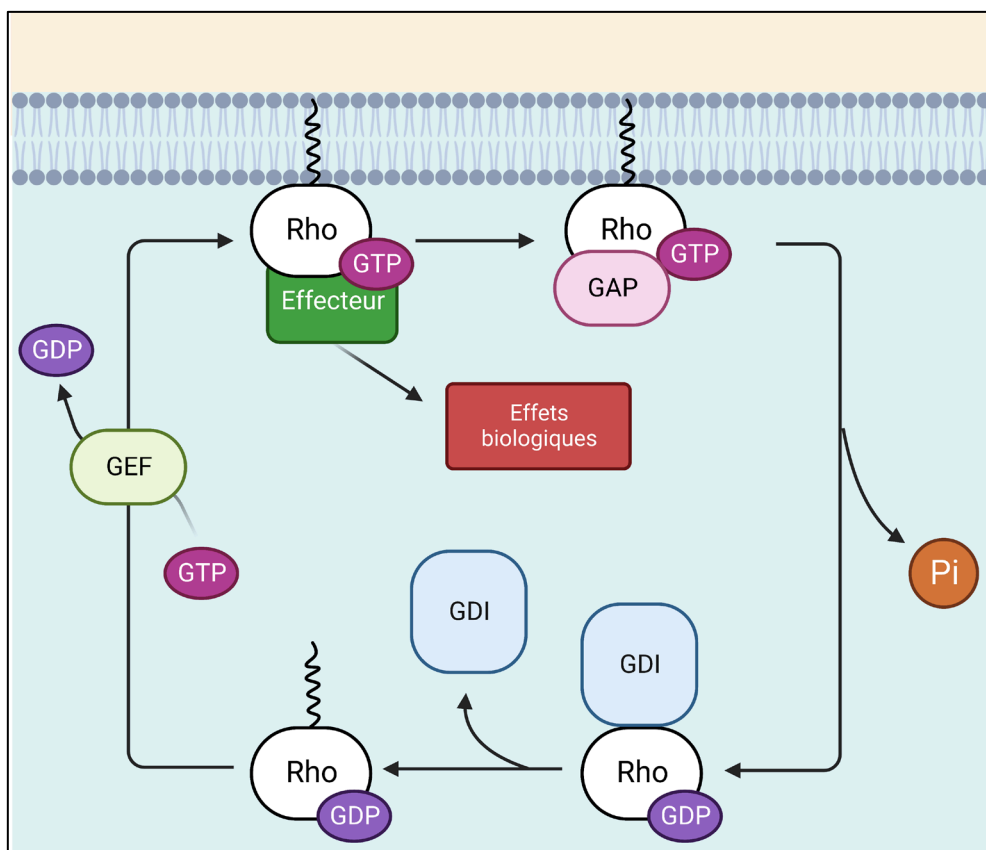


Figure 11 Cycle d'activation/inactivation des RhoGTPases. Adapté de (309).

Abréviations : GAP = GTPase Activating Protein, GDI = Guanine Dissociating Inhibitor, GEF = Guanine Exchange Factor, Rho = Ras homologous.

Les RhoGTPases possèdent une faible activité intrinsèque d'hydrolyse du GTP et d'échange GDP/GTP. Le cycle GDP/GTP est régulée par trois familles de partenaires protéiques : les GEF, les GAP et les GDI (310). **Les GEF** interagissent avec les formes liant le GDP et catalysent l'échange du GDP par du GTP permettant l'activation des RhoGTPases (311). **Les GAP** se lient avec les formes Rho-GTP et stimulent l'activité GTPasique entraînant l'inactivation des Rho-GTPase (312). Enfin, **les GDI** interagissent avec la boîte CAAX et stabilisent les RhoGTPases sous leur forme cytosolique liée au GDP (313). L'activation des Rho GTPase est aussi modulée par des modifications post-traductionnelles directes telles que la phosphorylation, l'ubiquitylation et la sumoylation (314).

### c) Fonctions cellulaires des RhoGTPases

Lorsqu'elles sont **activées**, les Rho-GTPase modulent le cytosquelette, la polarité cellulaire, le trafic vésiculaire, la transcription génique et les activités enzymatiques par le biais de leurs effecteurs (315, 316).

**La migration cellulaire** nécessite une réorganisation du cytosquelette d'actine dépendante de l'activation des RhoGTPases (Figure 12) (317). RhoA contrôle la contraction actine/myosine tel que décrit précédemment (Régulation intrinsèque de la contraction p13). Sur le front de migration de la cellule, Cdc42 activée provoque par le biais des DRF la polymérisation des filaments d'actine en faisceaux parallèles nécessaire à la formation des **filipodes** (318). Parallèlement, les GTPases Cdc42 et Rac1 activent les WASPs et les WAVES aboutissant au recrutement des complexes Arp2/3 (319, 320). Ces complexes stimulent la polymérisation des filaments d'actine en mailles aboutissant aux **lamellipodes** (321). L'inactivation de la cofiline par les effecteurs de Rac tel que la PAK participe au maintien de filaments polymérisés au pôle antérieur de la cellule lors de la migration en limitant leur désassemblage.

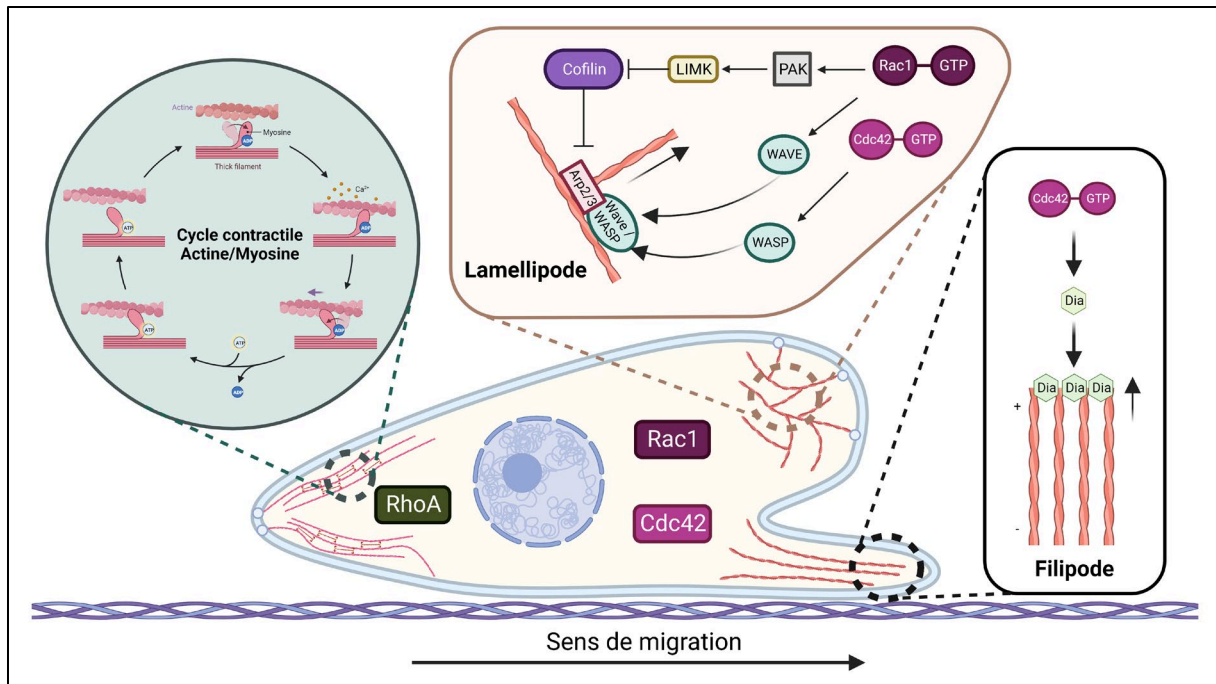


Figure 12 Rôles principaux des RhoGTPases dans la régulation du cytosquelette lors de la migration cellulaire.

Abréviations : Cdc42 = Cell division control protein 42 homolog, Dia = Diaphanous-related formins, LIMK = LIM domain Kinase, PAK = Protease-activated protein Kinase, Rac1 = Ras-related C3 botulinum toxin substrate 1, RhoA = Ras Homologous A, WASP = Wiskott-Aldrich Syndrom Protein, WAVE = WASP family Verprolin-homologous.

Outre leurs effets sur le cytosquelette d'actine, les RhoGTPases participent à la régulation de **la transcription génique** (Figure 13). Elles interagissent avec les différents membres des MAPK impliqués dans la transduction du signal de la membrane plasmique jusqu'au noyau via l'activation de facteurs de transcription (322). Elles interviennent ainsi dans des voies de signalisation majeures telles que les voies ERK, JNK ou TAK.

Enfin, les RhoGTPases influencent **les activités enzymatiques** impliquées dans le métabolisme des lipides membranaires telles que la PI3K ou la PLC dont les produits de dégradation (PIP2, DAG) sont impliqués dans les cascades de signalisation (cf. contraction des CML). Elles régulent aussi la production de radicaux libres par les cellules immunitaires par le biais du complexe NADPH oxydase (323).

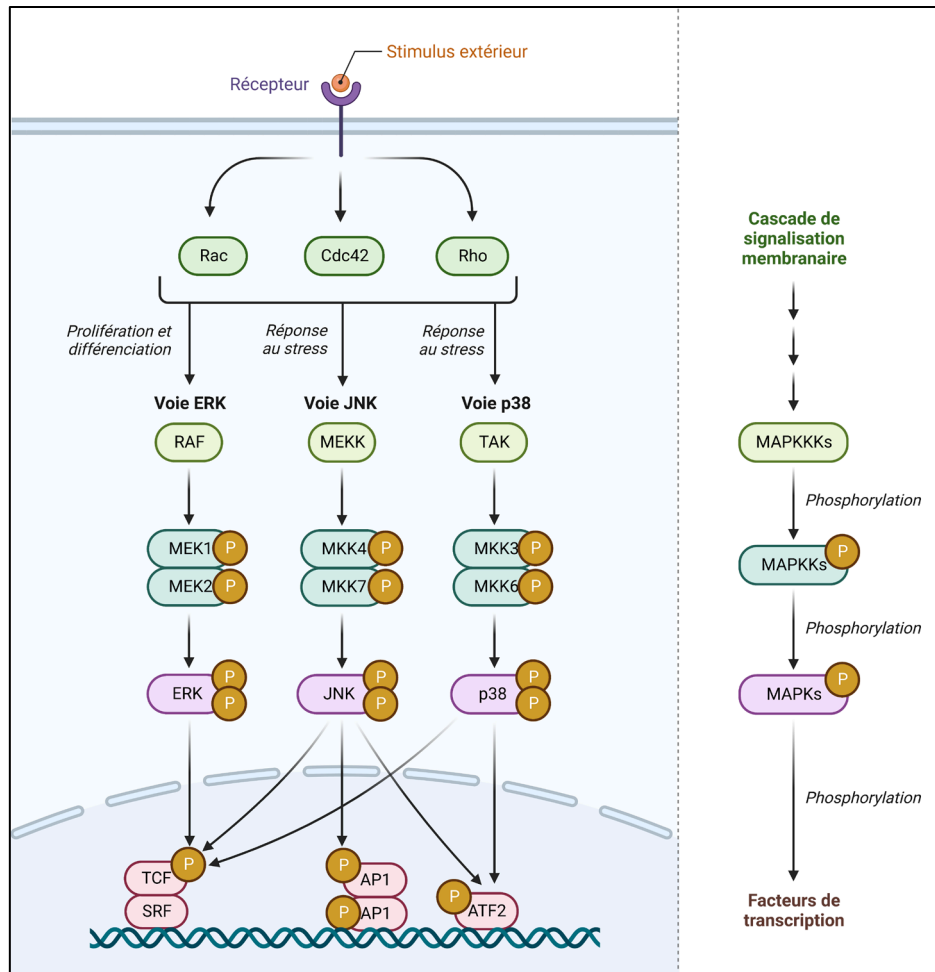


Figure 13 Régulation de la transcription médiée par les RhoGTPases : exemple des MAPK.

Abréviations : AP-1 = Activator Protein 1, ATF2 = Activating Transcription Factor 2, Cdc42 = Cell division control protein 42 homolog, ERK = Extracellular Regulated Kinase, JNK = c-Jun NH2-terminal Kinase, MEK = MAP Kinase/ERK Kinase, Rac = Ras-related C3 botulinum toxin substrate, RAF = Rapidly Accelerated Fibrosarcoma, Rho = Ras homologous, SRF = Serum Response Factor, TAK = TGF  $\beta$ -activated kinase, TCF = T-cell factor/lymphoid enhancer factor.

Ces fonctions dépendantes des RhoGTPases nécessite **une régulation spatio-temporelle** (324). C'est le cas de la **migration cellulaire** qui implique une coordination temporelle (activation séquentielle) et spatiale (front de migration) du recrutement et de l'activation des RhoGTPases (325). De même, lors d'une phagocytose induite par des immunoglobulines, on observe une activation de Cdc42 et de Rac1 au niveau du phagosome lors de la phase d'extension, tandis qu'à la phase de clôture, l'activation de Rac1 augmente et celle de Rac2 apparait (326). Enfin, Cdc42 et RhoA interviennent séquentiellement lors des transports vésiculaires et de l'exocytose par des mécanismes dépendants et indépendants du cytosquelette d'actine (327).

## 2. La GTPase Rac

### a) Structure et fonction des Rac-GTPases

**La GTPase Rac** (Ras-related C3 botulinum toxin substrate) a été découverte chez l'Homme en 1989 (328). Trois isoformes sont décrites chez l'Homme dont les gènes *rac1*, *rac2* et *rac3* sont localisés respectivement au niveau des chromosomes 7 (7p22), 22 (22q13.1) et 17 (17q25.3).

**Sur le plan structurel**, les Rac-GTPases sont des protéines comprenant de 191 à 192 acides aminés répartis en 6 feuillets bêta et 8 hélices alpha pour un poids moléculaire d'environ 21kDa (Figure 14). Les homologies de séquence sont élevées (>80%) avec une conservation des séquences constituant le domaine G, spécifique des GTPases (cf. partie B.1.a). Si la boîte CAAX diffère entre les différents membres, toutes sont la cible d'une modification post-traductionnelle de type géranylgeranylation.

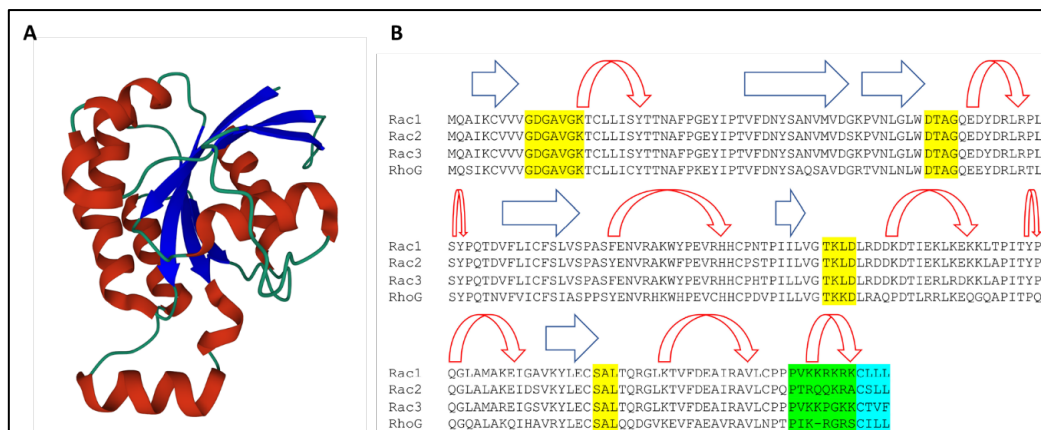


Figure 14 Structure et séquences des Rac-GTPases

A) Structure tridimensionnelle de Rac1 (329), en rouge les hélices  $\alpha$  et en bleu les feuillets  $\beta$  (329). B) Séquences d'acides aminés des Rac-GTPases (issues de (330)), en jaune les domaines G, en vert la séquence hypervariable et en bleu la boîte CAAX. Les flèches en rouge représentent les hélices  $\alpha$  et celles en bleu les feuillets  $\beta$ .

L'isoforme **Rac1** est exprimée de manière ubiquitaire (Figure 15). L'isoforme **Rac2**, initialement décrite comme exprimée dans les lymphocytes T activés, est spécifiquement retrouvée au sein des cellules hématopoïétiques (331). **Rac3** est, quant à elle, majoritairement exprimée au sein des tissus cérébraux (332). Enfin, **RhoG**, affiliée aux Rac-GTPases, possède un profil d'expression relativement ubiquitaire à l'instar de Rac1.

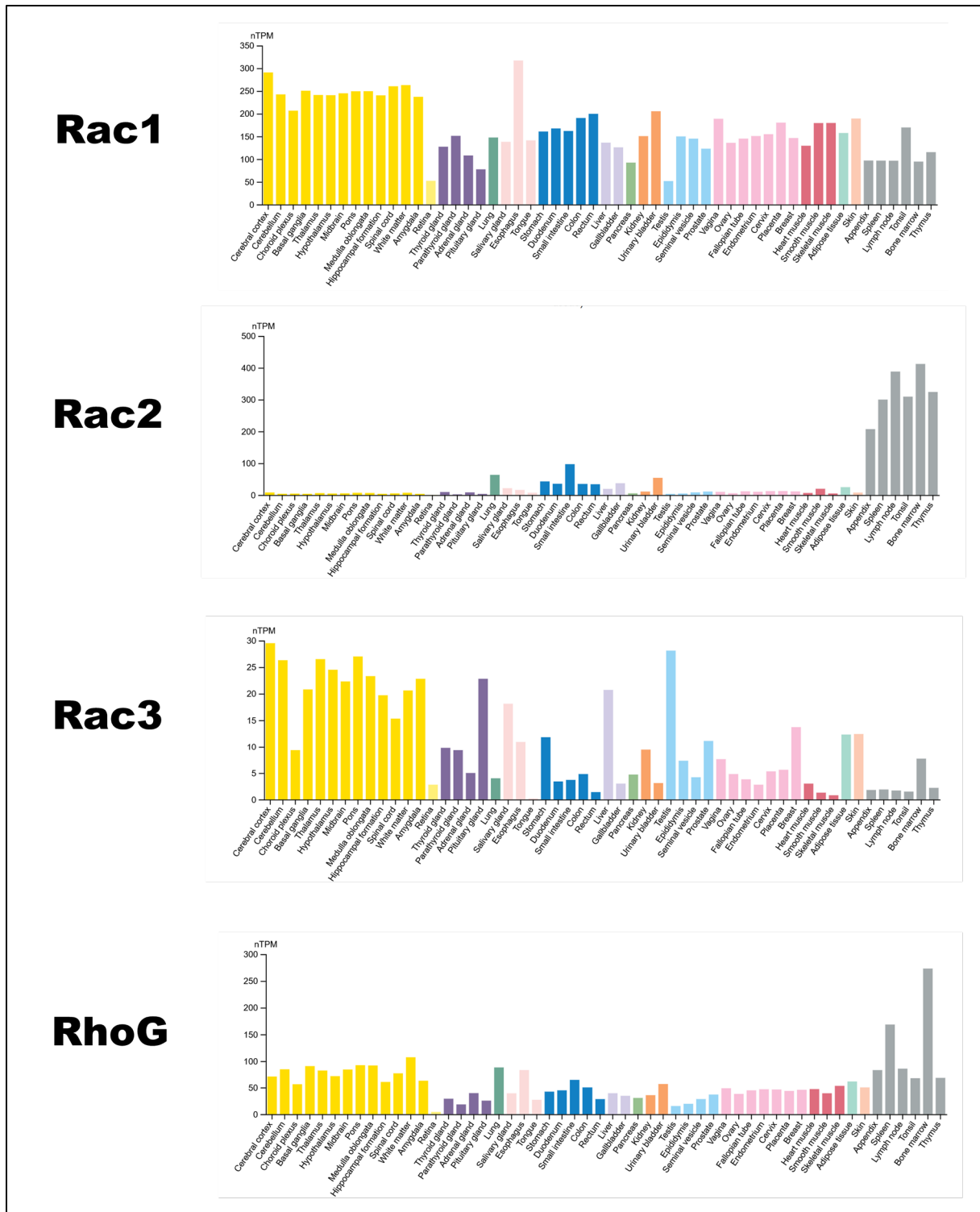


Figure 15 Profil d'expression tissulaire des Rac-GTPases.

Données extraites du projet « The Human Protein Atlas » ([proteinatlas.org](http://proteinatlas.org)) (333-335)



**Les modèles de mutagenèse dirigée** chez l'animal ont précisé les rôles respectifs des différentes Rac-GTPases. Tandis que les KO constitutionnels de Rac1 sont léthaux à des phases précoces du développement, ceux de Rac2 entraînent des altérations du développement des réponses immunes notamment neutrophiliques (336, 337). L'extinction globale de l'expression de Rac3 a pour conséquence l'apparition de trouble du comportement (hyper-réactivité) chez la souris à l'âge adulte (338). La délétion constitutionnelle de RhoG chez la souris n'entraîne pas de décès prématuré, ni de trouble majeur du développement bien qu'une altération légère du système immunitaire soit observée (augmentation des réponses lymphocytaires T et B) (339).

Tout comme les autres membres de la famille des Rho-GTPases, les Rac-GTPases interagissent avec les mêmes **familles de partenaires protéiques** : les GEF, les GAP et les GDI.

b) Les GEF de Rac

Près de 80 GEF interagissant avec les RhoGTPases ont été identifiés. Ceux-ci sont classés en 2 grandes familles en fonction de leur domaine actif : les « diffuse B cell lymphoma (Dbl) Homology (DH)/Pleckstrin Homology (PH) » d'une part et les Dedicator of Cytokinesis (DOCK) d'autre part.

- **Les GEF de la famille Dbl**

Vingt-trois membres de la famille des GEF de type Dbl ont été décrits comme interagissant avec les Rac-GTPases (340). Les domaines DH et PH conditionnent la localisation cellulaire et les fonctions de cette famille de GEF. **Le domaine DH** est impliqué dans la reconnaissance de la GTPase ainsi que dans l'échange nucléotidique. Trois régions conservées (CR1 à CR3) interagissent avec la GTPase tandis que la structure tridimensionnelle globale conditionne la sélectivité de celle-ci. Localisé à la partie C-terminale du GEF, **le domaine PH** interagit avec les phospholipides permettant la localisation membranaire du GEF. De plus, il participe à l'autoinhibition du GEF par encombrement stérique (310).

- **Les GEF de type DOCK**

Les GEFS de **la famille DOCK** interagissent spécifiquement avec Rac et Cdc42 et comptent un total de 11 membres. Les DOCK 1 à 5 présentent une spécificité plus importante pour Rac grâce à leur interaction privilégiée avec les protéines d'adaptation ELMO (341). Sur le plan structurel, elles présentent 2 domaines fonctionnels : le DHR1 et le DHR2. Le DHR1 permet l'interaction privilégiée du GEF de type DOCK avec les phospholipides, conditionnant sa localisation membranaire. Le DHR2, quant à lui, catalyse l'échange nucléotidique.

**DOCK2** est la protéine la mieux caractérisée de cette famille et participe à la régulation des réponses immunes (342). Elle intervient dans les mécanismes de chimiotactisme des lymphocytes vers les ganglions lymphatiques, dans la transduction du signal lors de la synapse immunologique (engagement du TCR) ainsi que dans la formation du complexe NADPH oxidase au sein des polynucléaires neutrophiles (343-345).

- **Les autres GEF**

Certains GEF n'appartenant ni à la famille Dbl ni DOCK ont été décrits comme pouvant interagir avec les RacGTPases. C'est le cas de SWAP-70 qui présente un domaine PH ainsi qu'un domaine CC qui lui permettent de lier le second messager PIP3 et d'activer Rac (346). Celui-ci est par ailleurs impliqué dans les mécanismes de phagocytose Rac-dépendante des cellules dendritiques (347).

c) Les GAP de Rac

**Les GAPs** catalysent l'hydrolyse du GTP en GDP inactivant les RacGTPases. Parmi les 66 RhoGAP découverts chez l'Homme, 35 ont été décrites comme interagissant avec Rac *in vitro* dont 15 confirmées *in vivo* (348). Sur le plan fonctionnel, le domaine GAP permet l'insertion d'une arginine au niveau du site catalytique de la GTPase favorisant l'entrée de la molécule d'eau nécessaire à l'hydrolyse (349). D'autres domaines fonctionnels, tels que les domaines PH, SH2-3 et DH régulent les interactions avec les partenaires protéiques ainsi que la localisation cytosolique des GAP (350).

d) Les GDI de Rac

**Un total de 3 GDI** ont été décrit pour la famille des Rho-GTPase : GDI1 (ou RHOGDI $\alpha$ ) d'expression ubiquitaire, GDI2 (ou GDI $\beta$ ) d'expression hématopoïétique et GDI3 (ou GDI $\gamma$ ) exprimé au niveau cérébral, pulmonaire et pancréatique (351-353). Seuls les GDI1 et 2 ont été décrit comme interagissant avec les isoformes de Rac.

Les GDI **réduisent l'activation de Rac** selon différentes modalités (354). En se liant à Rac, ils masquent le résidu isoprenyl C-terminal nécessaire à son adressage membranaire. Ils inhibent par ailleurs les interactions de Rac avec ses GEF ce qui limite son activation. La libération des Rac-GTPase de leur GDI survient après interaction avec les phospholipides membranaires, après phosphorylation diminuant leur affinité pour Rac et par l'action des GDI dissociating factor (355).

e) Les effecteurs de Rac

**Une quarantaine d'effecteurs** de Rac sont connus dont les principaux sont décrits dans le **Tableau 8** (356).

**Les effecteurs de Rac** régulent le cytosquelette d'actine, la production de radicaux libres (NADPH oxydase) et la transcription génique par le biais des voies dépendantes des MAPK (Figure 16).

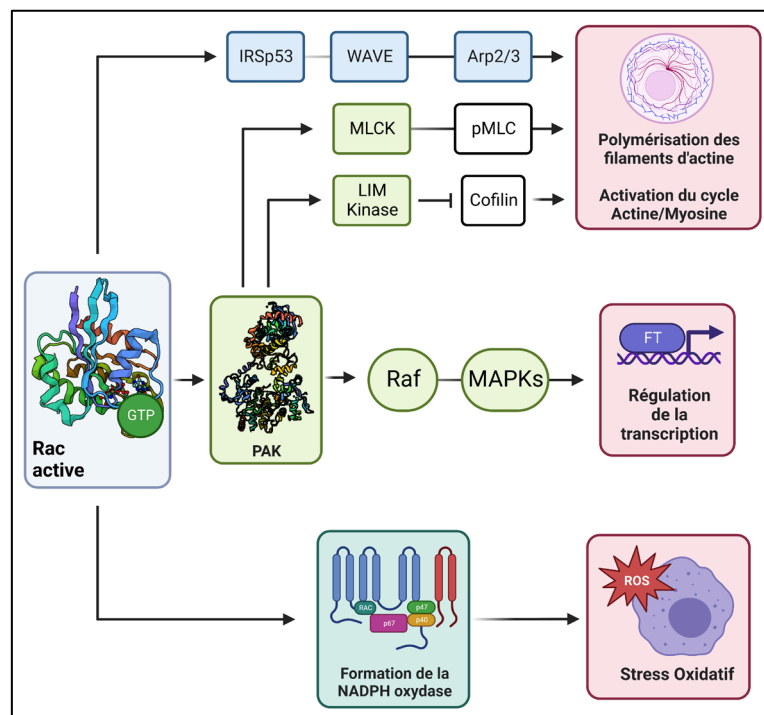


Figure 16 Principaux effecteurs de Rac et fonctions cellulaires impliquées.

Effecteurs	Isoforme affectée	Fonction	Effets	Références
<b>DAG Kinase</b>	Rac1	Kinase lipidique	Niveaux d'acide phosphatidique	(357)
<b>IQGAP1/2</b>	Rac1	Structuration	Régulation du cytosquelette d'actine Contacts intercellulaires	(358, 359)
<b>MEKK1/4</b>	Rac1	Sérine / thréonine kinase	Activation de la voie JNK	(360)
<b>MLK 2/3</b>	Rac1	Sérine / thréonine kinase	Activation de la voie JNK	(361, 362)
<b>P67<sup>phox</sup></b>	Rac1	Structuration	Génération d'espèces réactives de l'oxygène (NADPH Oxidase)	(363)
<b>P70 S6 kinase</b>	Rac1	Sérine / thréonine kinase	Régulation traductionnelle	(364)
<b>PAK1/2/3</b>	Rac1	Sérine / thréonine kinase	Activation de la voie JNK Stabilisation des filaments d'actine	(365)
<b>PI3 kinase</b>	Rac1	Kinase lipidique	Niveaux de PIP3	(366)
<b>PLC<math>\beta</math>2</b>	Rac1	Lipase	Niveaux d'IP3 et de DAG	(367)
<b>POR1</b>	Rac1	Structuration	Organisation de l'actine	(360)
<b>WAVE</b>	Rac1	Structuration	Organisation de l'actine	(368)
<b>LFA-1</b>	Rac2	Récepteur de surface cellulaire	Adhésion des lymphocytes B	(369)
<b>GIT1</b>	Rac3	ARF GAP et structuration	Régulation de l'adhésion cellulaire et différenciation	(370)

Tableau 8 Fonctions et effets des principaux effecteurs de Rac. Adapté de (371).

*Abréviations : DAG = DiAcyGlycerol, GIT1 = G protein-coupled receptor kinase-Interactor 1, IQGAP1/2 = IQ motif-containing GTPase-activating protein, LFA-1 = Lymphocyte Function-associated Antigen, MEKK = MAP/Erk Kinase Kinase, MLK = Mixed Lineage Kinase, PAK = Protease-Activated protein Kinase, PI3 = Phosphatidylinositol-3, PLC = PhosphoLipase C, POR = Partner of Rac, WAVE = WASP family Verprolin-homologous.*

**La famille des protéines PAK**, principaux effecteurs de Rac, comporte un ensemble de 6 isoformes. Celles-ci interagissent avec Rac par le biais d'un motif PBD (365, 372). Leur activité sérine-thréonine kinase est auto-inhibée par un domaine lié au domaine PBD (373). L'interaction entre la GTPase Rac et PAK entraîne un changement conformationnel avec rupture de l'interaction entre le domaine inhibiteur et le domaine catalytique (Figure 17). La protéine PAK activée phosphoryle la MCLK et la LIM kinase modulant le cytosquelette d'actine (374, 375). Parallèlement, la voie Rac-Pak activent les voies JNK et ERK stimulant la prolifération cellulaire (376, 377).

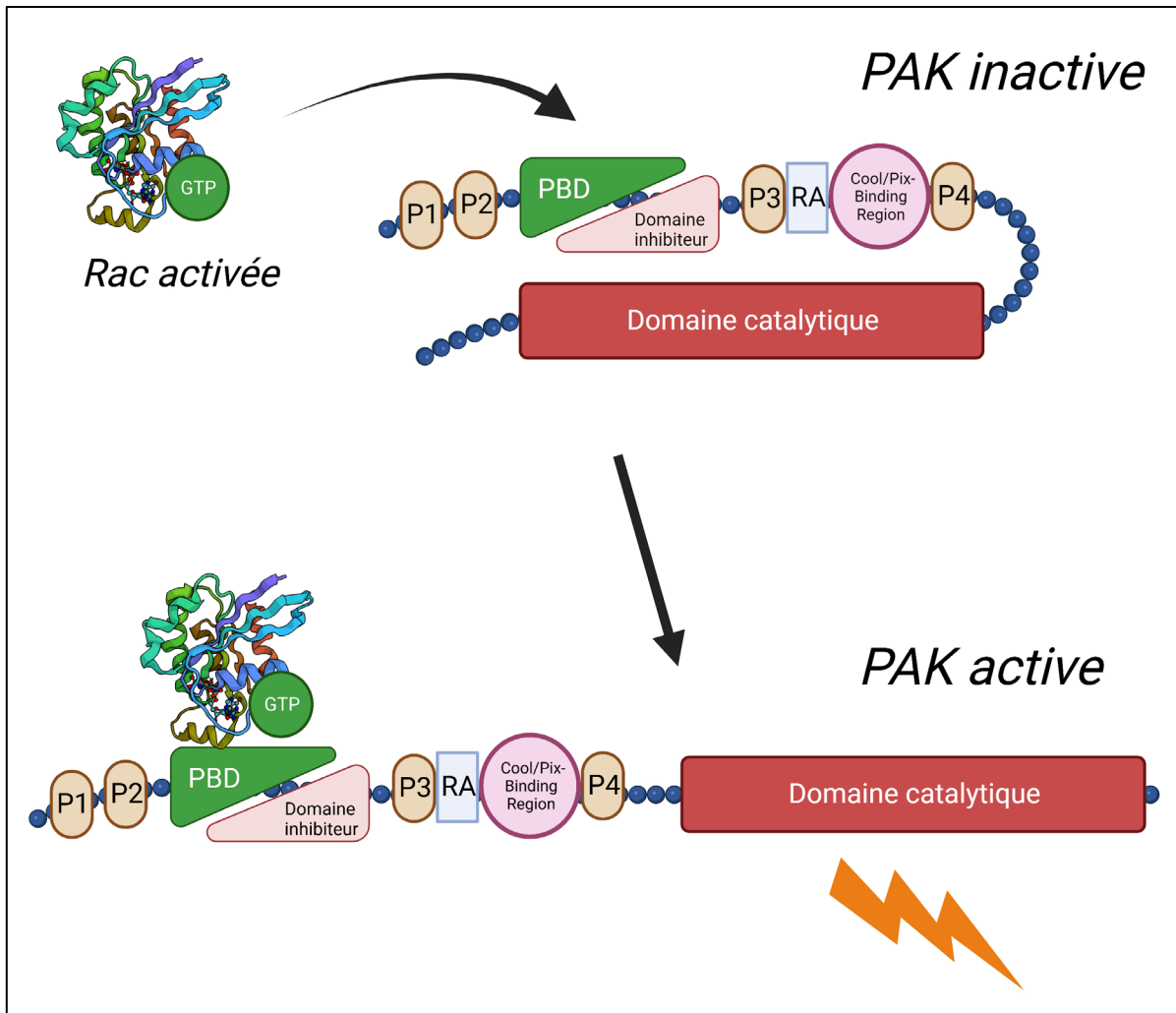


Figure 17 Mécanisme d'activation de PAK par Rac-GTP. Adapté de (365).

*P* = motif PXXP, PBD = p21-Binding Domain, RA = Région acide.

Rac est par ailleurs un des composants essentiels de **la NADPH oxydase** impliquée dans la respiration cellulaire. Elle est aussi liée aux fonctions immunes des macrophages et des polynucléaires neutrophiles (Figure 18) (378, 379). Les cytokines inflammatoires telles que le TNF $\alpha$  ou l'IL-1 $\beta$  entraîne l'activation de Rac et l'assemblage de la NADPH oxydase (380). Lorsqu'activée, Rac recrute la sous-unité p67<sup>phox</sup> permettant son association avec la sous-unité p47<sup>phox</sup> et le cytochrome (323). Rac2 est la principale isoforme impliquée dans la fonction de la NADPH oxydase de type 2 exprimée par les polynucléaires neutrophiles (381, 382). Des mutations limitant l'activation de Rac2 (dominant négatif) ont été décrites chez l'Homme et associées à des déficits immunitaires fonctionnels neutrophiliques (383-385).

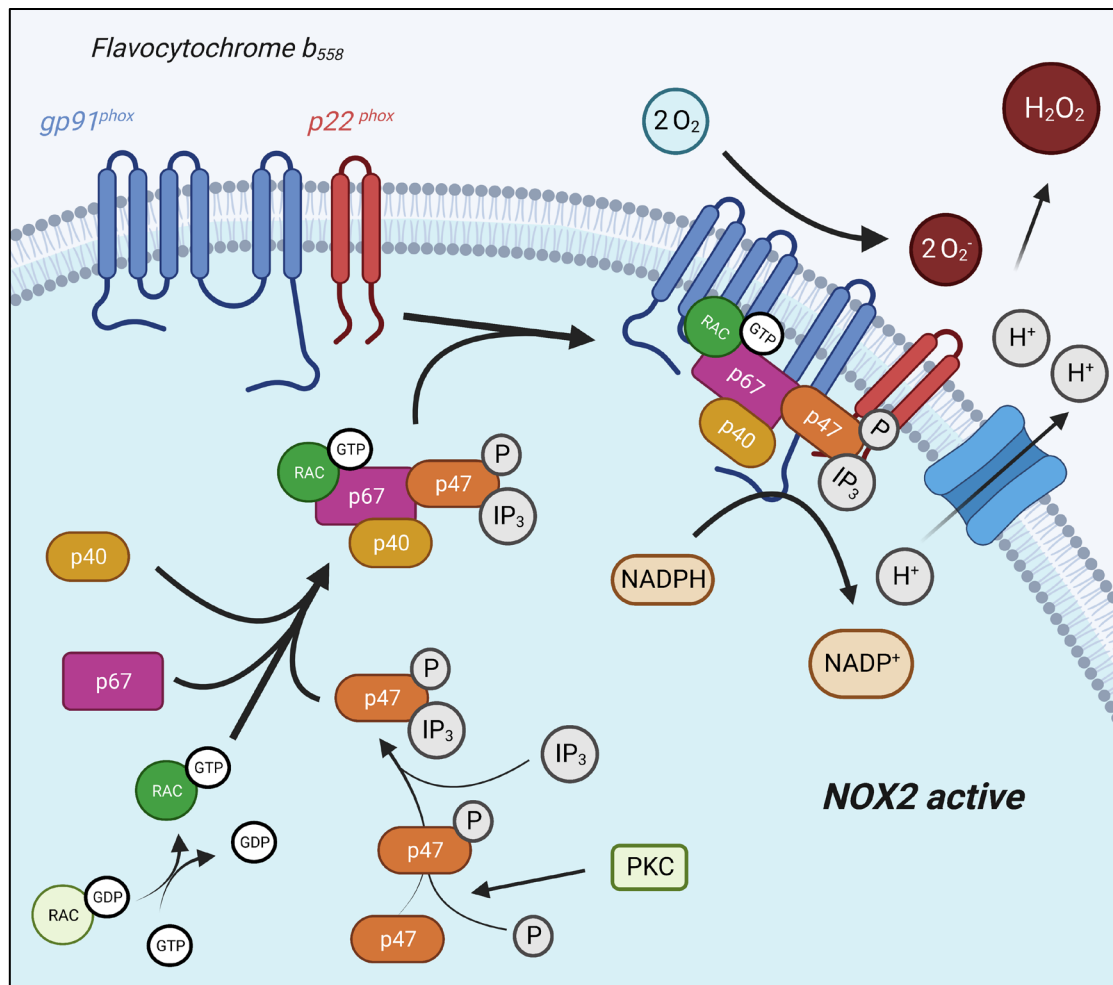


Figure 18 Rac GTPase et formation du complexe NADPH oxidase (NOX2 neutrophilique)

Abréviations : gp = glycoprotein, IP<sub>3</sub> = Inositol-tri-phosphate, NADPH = Nicotinamide adénine dinucléotide phosphate, Rac = Ras-related C3 botulinum toxin substrate.

### 3. Implication de la GTPase Rac dans l'asthme

L'implication de l'expression et de l'activité de la GTPase Rac au cours de la **physiopathologie de l'asthme** a fait l'objet d'études précliniques. Celles-ci se sont attachées à évaluer son rôle dans les mécanismes liés à l'hyper-réactivité bronchique et au remodelage (Figure 19).

Concernant les mécanismes d'**hyper-réactivité bronchique**, mon équipe d'accueil a démontré que Rac1 est suractivée au sein des CML bronchiques de patients asthmatiques (386). Une augmentation de l'expression de Rac et de 2 de ses GEF, TIAM et TRIO, par les CML est observée chez la souris dans le cadre d'un modèle d'asthme à l'ovalbumine (387). Sur le plan fonctionnel, Rac1 s'active dans les CML bronchiques humaines en réponse à une stimulation par la méthacholine (386).

### I - B - 3 Implication de la GTPase Rac dans l'asthme

Son association avec le domaine homologue de la pleckstrine de la PLC  $\beta_2$  potentialise la production d'IP<sub>3</sub> nécessaire à la contraction des CML. L'inhibition pharmacologique de Rac réduit les capacités contractiles des CML bronchiques. Cette implication de Rac a été confirmée par une autre équipe avec des anneaux trachéaux de rat stimulés au carbachol et à l'endothéline (388). *In vivo*, l'inhibition pharmacologique de l'activation de Rac améliore l'hyper-réactivité bronchique induite chez la souris (386).

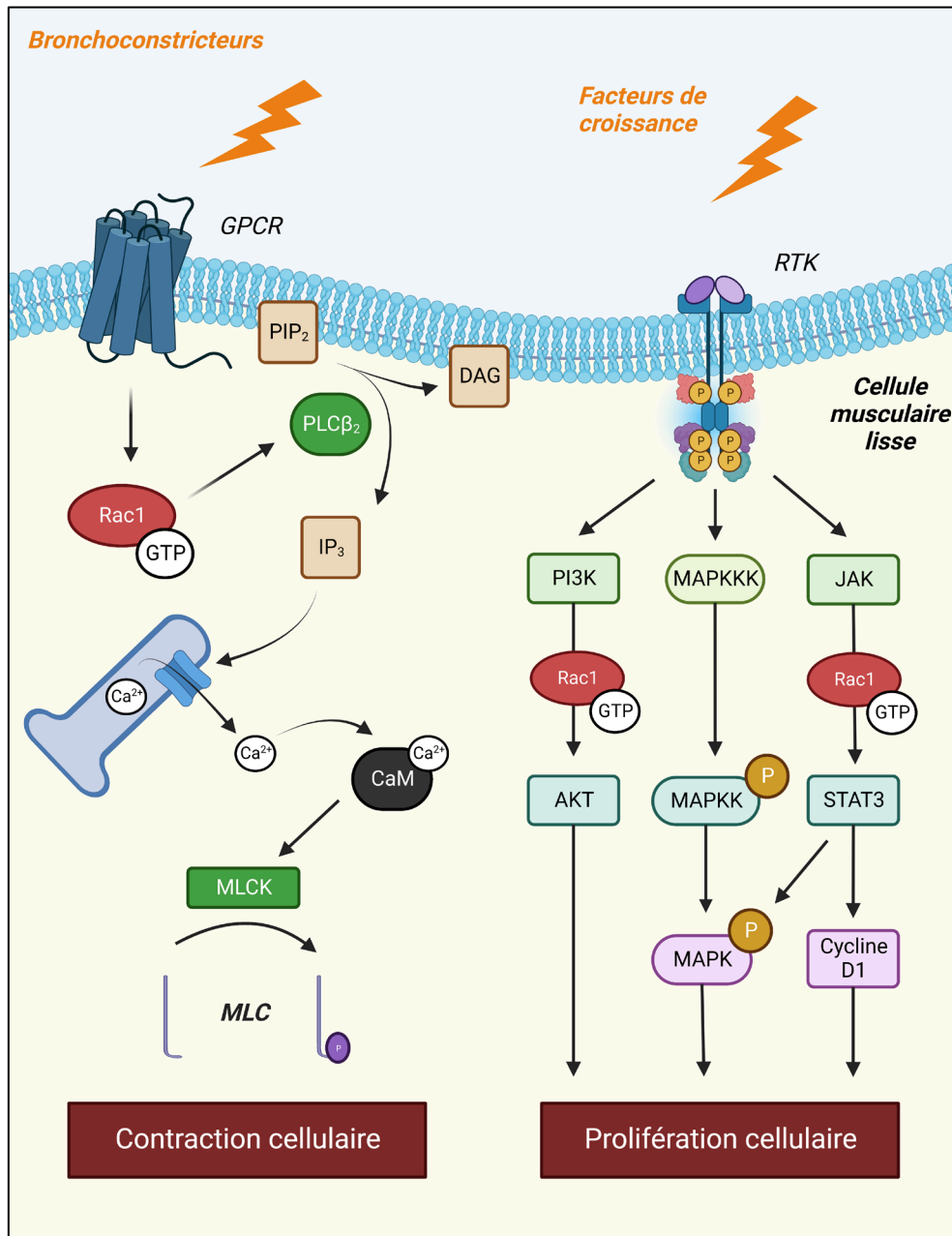


Figure 19 Rac1, contraction musculaire lisse bronchique et prolifération cellulaire.

Rac participe aussi **au remodelage bronchique**. Il a été mis en évidence une suractivation de Rac au sein des zones d'hyperplasies musculaires lisses bronchiques chez les patients asthmatiques en comparaison avec des sujets sains (389). Sur le plan mécanistique, la stimulation de CML bronchiques humaines en culture primaire avec du FGF ou du PDGF active la voie Rac1-STAT3 qui augmente leur prolifération (389). La production d'espèces réactives de l'oxygène à laquelle participe la NADPH oxydase et dépendante de Rac1 stimule la mitogenèse des CML par le biais de la cycline D1 (390).

Concernant l'impact de l'activation de Rac sur **les mécanismes inflammatoires** dans le cadre spécifique de l'asthme, peu de données sont actuellement disponibles.

Une étude publiée en 2013 a démontré que Rac est nécessaire dans les mécanismes de clairance cellulaire des corps apoptotiques et l'initiation d'une réponse immune tolérogène par les **cellules épithéliales bronchiques** en réponse à des acariens (391). Néanmoins, l'activation de Rac pourrait avoir des effets différents sur l'initiation de la réponse immune en fonction de la population ciblée. En effet, mon équipe d'accueil a démontré que la nébulisation d'un inhibiteur de Rac (NSC23766) chez la souris **réduit l'intensité de l'inflammation bronchique** éosinophilique et neutrophilique (386). Parallèlement, une équipe a démontré que la délétion d'abr, une GAP de Rac, majorait l'inflammation produite dans un modèle murin d'asthme aux extraits de cafards plaçant pour impact négatif d'une suractivation de Rac (392). Une hypothèse serait que Rac puisse jouer un rôle direct dans l'activité des cellules immunitaires au cours de l'inflammation bronchique.

Des études ont décrit les rôles des Rac-GTPases dans les cellules immunitaires dans **des conditions physiologiques** (Tableau 9) (393). Il a ainsi été montré que Rac était impliquée dans le maintien des cellules souches hématopoïétiques au sein de la moelle ainsi que dans la production des lymphocytes T et B matures (394-396). Sur le plan fonctionnel, les RacGTPases régulent par le biais du cytosquelette d'actine la migration des lymphocytes, des neutrophiles et des mastocytes en réponse aux gradients chimiokines (397-399). Elles sont aussi nécessaires à la phagocytose ainsi qu'à la production de ROS par les polynucléaires neutrophiles et éosinophiles via l'assemblage de la NADPH oxydase (381, 383, 400, 401).



### I - B - 3 Implication de la GTPase Rac dans l'asthme

Populations cellulaires	Production médullaire et survie	Fonctions effectrices	Références
<b>CSH et progéniteurs</b>	Un dominant négatif de Rac2 augmente l'apoptose des CSH. La délétion combinée de Rac1 et Rac2 réduit la production des progéniteurs lymphoïdes communs.	Rac1 est impliquée dans le chimiotactisme médullaire et la greffe des CSH. L'absence d'expression de Rac1 et Rac2 réduit la rétention des CSH dans la moelle.	(394, 395, 402-404)
<b>Lymphocytes T</b>	La délétion de Rac1 (CSH) réduit légèrement la production de lymphocytes T. La délétion combinée de Rac1 (CSH) et Rac2 bloque le développement des LT à un stade pré-TCR.	La délétion combinée de Rac1 et Rac2 réduisent les capacités prolifératives, l'adhésives et migratrices des lymphocytes T. Rac1 est activée lors de la polarisation lymphocytaire Th17. Rac1 et Rac2 favorisent le regroupement des TCR lors de la synapse immunologique et la transduction du signal (MAPK et PI3K).	(244, 344, 395, 405-408)
<b>Lymphocytes B</b>	La délétion combinée de Rac1 et Rac2 entraîne un blocage aux stades spléniques (T1, T2) du développement.	Rac1 et Rac2 sont activées après l'engagement du BCR (voie de la PI3K) entraînant la différenciation plasmocytaire. La délétion de Rac2 limite les capacités chimiotactiques des lymphocytes B.	(369, 396, 409-411)
<b>Polynucléaires neutrophiles</b>	La délétion de Rac1 dans les CSH ou de Rac2 augmentent la quantité de neutrophiles circulants.	La délétion de Rac1 (CSH) ou Rac2 diminue les capacités de migration et de chimiotactisme. La délétion de Rac2 réduit la production de ROS.	(336, 383, 397, 412-414)
<b>Macrophages</b>	Absence d'impact sur la production Inconnu sur la survie	La délétion de Rac2 réduit l'activité de la NADPH oxydase La délétion de Rac2 ou un dominant négatif de Rac1 inhibe la phagocytose médiée par anticorps. Rac2 est activée au cours de la polarisation M2 (cancer, fibrose pulmonaire).	(400, 415-417)
<b>Polynucléaires éosinophiles</b>	Inconnu	Rac1 et Rac2 sont impliqués dans la production de ROS par la NADPH oxydase. La délétion de Rac2 réduit les capacités de dégranulation.	(401, 415, 418)
<b>Mastocytes</b>	Rac2 promeut la survie mastocytaire en réponse aux cytokines.	Rac2 régule la transcription des gènes des protéases mastocytaires. La délétion ou l'inhibition combinée de Rac1 et Rac2 réduit le chimiotactisme induit par l'histamine. La délétion combinée de Rac1 et Rac2 réduit les capacités de dégranulation.	(398, 419-421)
<b>Cellules dendritiques</b>	Inconnu	La délétion combinée de Rac1 et Rac2 réduit l'efficacité de la synapse immunologique. La voie DOCK2-Rac1 est essentielle à la production d'IFN de type 1 par les cellules plasmacytoïdes.	(399, 422)

Tableau 9 Implication des Rac-GTPase dans la production et les fonctions des cellules de l'immunité (Adapté de (393))

## C. Problématique et objectifs de thèse

Les isoformes Rac1 et Rac2 soit décrites comme influant sur la production et les fonctions effectrices des cellules immunitaires. Néanmoins, peu de données sont disponibles dans le cadre de maladie inflammatoire telle que l'asthme. La réduction des infiltrats inflammatoires en présence d'un inhibiteur de Rac chez la souris nous amène à penser que Rac interviendrait directement dans l'inflammation bronchique observée dans l'asthme (Figure 20).

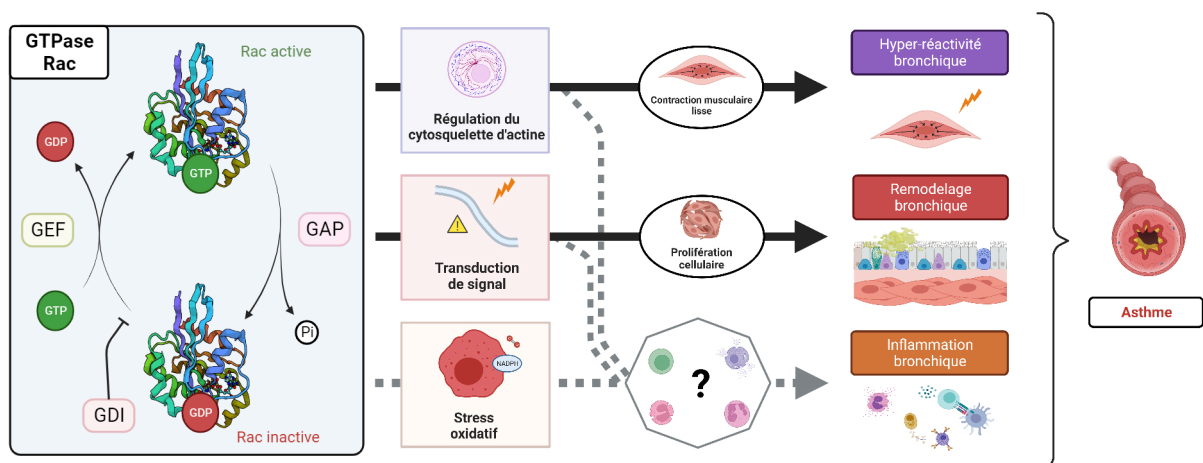


Figure 20 Implication physiopathologique de la GTPase Rac dans l'asthme.

**Nous avons émis l'hypothèse** que Rac serait exprimée et activée au sein de certaines populations inflammatoires et modulerait leurs activités effectrices au cours de l'asthme.

**L'objectif principal de ma thèse** a été tout d'abord de confirmer l'activation de Rac au sein des cellules inflammatoires puis d'identifier les populations concernées. Ensuite, mon objectif a été d'identifier les processus biologiques dépendants de Rac et d'en identifier les mécanismes.

## **II. Article original**

Le manuscrit de l'article original présenté dans les pages suivantes est en préparation et s'appuie notamment sur l'étude NARACAS (NCT03325088). Cette étude est actuellement en cours d'inclusion et les données présentées ci-après sont issues de l'analyse intermédiaire (dernière mise à jour le 28/09/2022). Les données seront actualisées lors de la clôture de l'étude.

# **Rac GTPase plays an important role in eosinophils in severe asthma.**

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**Descriptor subject:** Severe asthma, Rac, Eosinophils, Differentiation, Degranulation

**Total word count:** 4314 words

**References:** 42

## **A. Abstract**

**Introduction:** Asthma is a bronchial disease characterized by bronchial hyperresponsiveness, tissue remodeling and chronic inflammation. Our work previously demonstrated that inhibiting Rac1, a small GTPase of Rho family, in a murine severe asthma model lowered the infiltration of immune cells within the lungs. However, precise mechanisms involving Rac activation in immune processes during asthma remains unknown. Our objective was to identify inflammatory cells displaying a Rac activation in bronchial asthma and highlight affected pathways.

**Materials and methods:** Active Rac-GTP was quantified by immune fluorescence in bronchial biopsies from severe asthmatic patients. Conversely, Rac-GTP signal was measured in identified inflammatory cells by flow cytometry in a murine severe allergic asthma model. We evaluated the effects of inhaled Rac inhibitor on the bronchial and lung cytokine environment, reflect of inflammatory cells activity. Rac activation was assessed by flow cytometry and western blot during *in vitro* differentiation of eosinophils from bone marrow cells. Finally, we evaluated the effects of Rac inhibition on eosinophils degranulation induced by platelet activating factor.

**Results:** Intense Rac-GTP signal was observed in epithelial cells, smooth muscle cells and infiltrating cells in bronchial biopsies from severe asthmatic patients. Macrophages and eosinophils displayed increased Rac-GTP signal in asthmatic mice compared with control. Inhaled inhibition of Rac did not significantly modify inflammatory cytokine environment in a murine severe asthma model. However, bone marrow derived eosinophils presented an activation of Rac while differentiating associated with significantly impaired degranulation abilities when treated with Rac inhibitor.

**Conclusion:** Rac activation plays an important role in the inflammatory mechanisms present in asthma, particularly those dependent on eosinophils. The discovery of Rac activators involved in the oxidative power of eosinophils and their degranulation could lead to the development of new specific innovative treatments in eosinophilic severe asthma.

**Keywords:** Severe asthma, Rac, Eosinophils, Differentiation, Degranulation

**Abstract word count:** 289 words

**Abbreviations:**

AHR = Airway Hyperresponsiveness

CD = Cluster of Differentiation

DMSO = Dimethyl Sulfoxide

FcR = Fragment Crystallizable Receptor

FMO = Fluorescence Minus One

G-CSF = Granulocyte Colony- Stimulating Factor

GM-CSF = Granulocyte-Macrophage Colony Stimulating Factor

HDM = House Dust Mite

IFN = Interferon

KC = Keratinocyte-derived Chemokine

IL = Interleukine

MCP = Monocyte Chemoattractant Protein

MIP = Macrophage Inflammatory Protein

MFI = Median of Fluorescence Intensity

PAF = Platelet Activating Factor

PBS = Phosphate Buffered Saline

RANTES = Regulated upon Activation, Normal T cell Expressed and Secreted

RT = Room Temperature

TSLP = Thymic Stromal Lymphopoietin

## ***B. Introduction***

Asthma is a chronic airway inflammatory disease affecting 260 million adults worldwide and responsible of 460 000 deaths per year (1). Conventional therapies, based on corticosteroids and bronchodilators, reduced the burden of disease in most patients. Though, around 4% of asthmatic patients, worsen, remain uncontrolled or need high dose treatment to keep their asthma controlled, defining severe asthma (2, 3). Those patients suffer greater morbidity with higher exacerbation rate and more frequent hospitalization due to asthma (4). Moreover, a significant part of asthma total cost, around 60%, is due to severe asthma care (5). It is then an important unanswered public health issue.

Important pathophysiological breakthrough has been made considering inflammatory processes involved in asthma. Better comprehension of key pathological pathways controlled by immunoglobulin E, interleukine (IL) 4, IL-13, IL-5 and thymic stromal lymphopietin (TSLP) gave rise to specific biotherapies (6). Biotherapies targeting those pathways significantly reduce the severe exacerbation rate and improve disease control in selected severe asthmatic patients (7-9). Tough, available data demonstrate that they are only suspensive (10). Moreover, except from recently developed tezepelumab (anti-TSLP), available biotherapies are restricted to the type 2 eosinophilic asthma leaving non type 2 asthma orphan of innovative therapies. Finally, airway hyperresponsiveness (AHR) and bronchial remodeling are not directly targeted by available treatments. New innovative strategies need to be developed to fill these gaps.

Recently, we identified Rac protein, a small GTPase member of Rho family, as an important actor of asthma pathophysiology. Indeed, we demonstrated that Rac activation was mandatory in airway smooth cells (aSMC) contraction through the regulation of intracellular Ca<sup>2+</sup> (11). In parallel, Rac is also activated while human aSMC proliferate in response to EGF and VEGF (12). Inhaled Rac inhibitor significantly reduced AHR and bronchial remodeling in an allergic murine asthma model to house dust mite (HDM) confirming its role in such mechanisms (11, 12). Interestingly, nebulization of a Rac inhibitor also resulted in reduced bronchial inflammatory cells in

murine models. Nevertheless, Rac-dependent inflammatory mechanisms during asthma need to be identified and deciphered.

Here, we hypothesized that Rac is active within inflammatory cells and plays an important role in immune cell functions and in the constitution of the pro-inflammatory cytokine environment. We evaluated Rac activation in bronchial biopsies from severe asthmatic patients and in a murine model of severe allergic asthma. Next, we assessed the activation of Rac and its impact on the maturation and degranulation abilities of eosinophils.



## C. Methods

**Human bronchial biopsies.** Bronchial biopsies were obtained by bronchial endoscopy in severe asthmatics under NARACAS protocol (NCT03325088). All enrolled patients gave written approval. Clinical protocols were previously approved by relevant ethic committees.

**Histology and immunohistochemistry.** To assess bronchial remodeling and qualify immune infiltrates, paraffin-embedded bronchial biopsies were deparaffinized and stained by hematoxylin and eosin. To assess smooth muscle hypertrophy/hyperplasia, sections were stained by immunohistochemistry with SM22 $\alpha$  antibody (Abcam) (1/500 O/N at RT). Sections were acquired under white light microscopy and analyzed with ImageJ software (v1.53n). Histological grade (/12 points) was determined by blinded operator as previously described (13).

**Rac activity assessment by immune fluorescence.** Human bronchial biopsies paraffin-embedded sections were deparaffinized and permeabilized (phosphate buffered saline (PBS)+0.1% Triton- X100) before incubation with anti-Rac-GTP antibody (NewEast Biosciences, King of Prussia, Pennsylvania, ref 26903) (1/500) overnight at room temperature (RT). After three washes in PBS, sections were incubated for 1 hour at RT with the secondary Alexa647-labelled anti-mouse IgM antibody (1/500). Anti-SM22 $\alpha$  antibody (Abcam) (1/500 O/N at RT) with Alexa488-labelled anti-mouse antibody (1/1000 1 hour at RT) were used to localize smooth muscle. DAPI staining was performed and slides were mounted with Prolong®. Blind operator analyzed images using QuPath (v. 0.3.2). Briefly, smooth muscle, epithelial and immune infiltrates area were manually determined thanks to SM22 $\alpha$  signal and reticular basement membrane along with HE stained sections. Within each area, cells were detected thanks to DAPI staining (threshold 100, minimum area 10 $\mu$ m<sup>2</sup>, expansion 5 $\mu$ m). Sum Rac-GTP fluorescence was then determined at a cell level.

**Mice.** All experimental procedures and animal care were performed in accordance with the Regional Ethical Committee for Animal Experiments of the Pays de la Loire.

**Allergic asthma model.** To induce asthma model, eight weeks old male C57Bl/6J (JANVIER labs) were sensitized on days D0, D7, D14 and D21 by skin application of 500 $\mu$ g of house dust mite extracts (house dust mite extract *Dermatophagoides farinae*

[Der f], from Stallergenes Greer, Antony, France) in 20 $\mu$ L of DiMethyl SulfOxide (DMSO, SIGMA-ALDRICH) or DMSO alone (control mice) on ears (Supp Figure 1). Bronchial inflammation was induced by intranasal challenges on days 26 to 28 and 32 to 34 with 250 $\mu$ g of HDM extracts in 40 $\mu$ L of sterile Phosphate Buffered Saline (PBS) or PBS alone (control mice). When indicated, Rac inhibition was carried out with the Rac inhibitor A.41 (25mg/kg) diluted in 10% DMSO in PBS nebulized 4 hours before each challenge or with PBS (untreated control).

**Airways reactivity ex vivo.** Murine primary bronchi were cleaned, cut in rings, and mounted on a multichannel isometric myograph in Krebs-Henseleit physiological solution (118.4 mM NaCl, 4.7 mM KCl, 2 mM CaCl<sub>2</sub>, 1.2 mM MgSO<sub>4</sub>, 1.2 mM KH<sub>2</sub>PO<sub>4</sub>, 25 mM NaHCO<sub>3</sub>, and 11 mM glucose) at 37°C and gassed with a mixture of 95%O<sub>2</sub>/5%CO<sub>2</sub>. A pretension of 0.5 mN was applied. We constructed dose-response curves to methacholine (Sigma-Aldrich, Paris, France). The wire myograph was connected to a digital data recorder (MacLab/4e, AD Instruments, Paris, France) and recordings were analyzed using LabChart v7 software (AD Instruments).

**Isolation of lung cells for flow cytometry and culture.** Mice were exsanguinated and their right ventricle flushed with 5mL of PBS to ensure proper elimination of intravascular immune cells. Lungs were removed and dissociated using a mouse Lung Dissociation Kit (Miltenyi Biotec, ref 130-095-9247) with a gentleMACS $\square$  Octo Dissociator (Miltenyi Biotec, ref 130-095-937) for 45 minutes at 37°C. Lung cells were then passed through a 40 $\mu$ m cell strainer and red blood cell lysis was performed using Red Blood Cell Lysis Buffer (eBiosciences $\square$ , ref 00-4333-57). Viable lung cells were counted on a Cellometer (Nexcelom<sup>TM</sup>) with Trypan Blue.

**Flow Cytometry staining and data analysis.** Staining was performed on a 96-well conical bottom plate. Cells were stained with Fixable Viability Dye UV450 (BD Biosciences) for dead cells exclusion. After FcRs blocking by mouse CD16/32 mAbs (BD Biosciences), surface staining was performed with the following antibodies: CD4 BUV 395, CD45 BUV 805, CD11b BV 480, CD11c BUV 737, Ly6G PE, Siglec-F BV 750, CD8a APC-H7, IA-IE BUV 563, CD19 BV 711, CD3 PE-Cy7 and CD34 BV 786 from BD Biosciences. Cells were then fixed and permeabilized with Fix/Perm Buffer and intracellular staining was completed with CD206 BV 421 (BioLegend) and home-made Rac-GTP Alexa Fluor 647 (NewEast Biosciences, ref 26903, Alexa Fluor<sup>TM</sup> 647

Labeling kit, ThermoFisher ref A20186). Cells were acquired on a Symphony flow cytometer (BD Biosciences). Standardization of photo-multiplier voltage over time was carried out thanks to 8 peaks calibration beads (BD Biosciences).

Flow cytometry data were analyzed with FlowJo software (v10.8.1) using the following plugins: DownSample (v3.3.1), UMAP (v3.1) (14). Data were cleaned (signal stability over time) and doublets and dead cells excluded before further analysis. Compensations were calculated from single stained cells when available or compensation beads. Unmarked cells and Fluorescence Minus One (FMO) controls were used to determine the positivity threshold of markers. CountBright™ Plus Absolute Counting Beads (ThermoFisher Scientific, ref C36995) allowed absolute cells counting according to the manufacturer's instructions. Detailed gating strategy are detailed on Supp Figure 2 and Supp Figure 3. Median fluorescence intensity (MFI) of Rac-GTP was used to describe levels of Rac activity. Dimension reduction was performed using UMAP algorithm with the following parameters: (Nearest Neighbors = 15, Minimum Distance = 0.5, Number of Components =2).

#### **Murine cytokines quantification from bronchoalveolar lavage fluid and lungs.**

Bronchoalveolar lavage fluid was performed by instilling 1mL of cold PBS after tracheal canulation and supernatants were recovered by centrifugation. Right upper lobe was removed, weighed and snap-frozen on liquid nitrogen. Cytokines concentrations were determined by using the Bio-Plex Pro Mouse Cytokine 23-plex Assay (BioRad™, ref #M60009RDPD) according to the manufacturer's instruction. Concentrations obtained on the lung lobes were normalized to their respective weights.

**Bone-marrow derived eosinophil culture.** Culture protocol from Dyer et al. was adapted as follow (Supp Figure 4 A) (15). At day 1, bone-marrow from 8 to 12 weeks old male C57Bl/6J (JANVIER labs) was recovered by flushing the femur and tibia with bone marrow (BM) culture medium (RPMI, 20% FCS, 100UI/mL penicillin/streptomycin, 2mmol/L glutamin, HEPES buffer 25mmol/L, non-essential amino acids 1X, sodium pyruvate 1mM, beta-mercaptoethanol 50µmol/L). Cells were then cultured in suspension at 37°C 5% CO<sub>2</sub> in BM culture medium at a concentration of 1x10<sup>6</sup> live cells/mL and stimulated with mouse recombinant Stem-Cell Factor (SCF, 100ng/mL, StemCell™Technologies ref # 78064) and mouse recombinant Fms-like tyrosine kinase 3/fetal liver kinase-2 (Flt3/Flk-2 ligand, 100ng/mL

StemCell™Technologies ref # 78011). At day 4, SCF and Flt3/Flk-2 ligand were replaced by IL-5 (10ng/mL, StemCell™Technologies ref 78049). From the 8th day, half of the medium was daily replaced. Purity of eosinophil culture was assessed at the 14th day by Wright-Giemsa staining.

**Degranulation assay.** To induce degranulation, 100µL of cell suspension at 2.5x10<sup>6</sup> cells/mL were seeded on a 96-well flat-bottom plate with or without Rac inhibitor (A.41) diluted in phenol-red free RPMI for 30 minutes at 37°C and 5% CO<sub>2</sub>. Degranulation was then induced by adding Platelet Activating Factor (PAF) at a final concentration of 2.5µM for an incubation of 30 minutes. The degradation reaction of o-phenylenediamine in the presence of recovered supernatants was monitored by measuring the absorbance at 415nm every 45 seconds for 1 hour and a half on a Varioskan™ LUX (ThermoScientific™). Area under the curve (AUC) derived from unstimulated and PAF-stimulated cells were then calculated (Supp Figure 5).

**Rac activity assessment by immunoblotting.** Cultured cells were incubated on ice with lysis buffer supplemented with proteases and phosphatases inhibitor cocktails (Sigma Aldrich). A fraction of the lysate was set aside to determine the total amount of Rac (Total Cell Lysate). Activated Rac (Rac-GTP) was pulled-down by incubating protein suspensions for 1 hour with glutathion sepharose beads loaded with Glutathion-S-transferase (GST) - Pak-Binding-Domain constructs produced in E. coli (16). Lysates were boiled, subjected to SDS-PAGE and transferred to nitrocellulose membranes. Membranes were consecutively incubated with primary Rac1 antibody (BD Biosciences™, ref 610651) and secondary antibody HRP anti-mouse IgG (Cell Signaling™ ref 7076S). Chemiluminescence reaction (Clarity ECL BioRad) was measured and protein band intensities were quantified using ImageJ software (NIH software, Bethesda, Md).

**Statistics.** Mann-Whitney tests were performed for two-group comparisons of numerical data. Multiple-group comparison of numerical data was done with a Kruskal-Wallis test. Correction for multiple comparisons was made with a false discovery rate (FDR) approach (FDR = 0.05) based on two-stage step-up of Benjamini, Krieger and Yekutieli. To compare categorical variables distribution between groups, chi-square test was used. Data analyses were performed using the GraphPad Prism software (v9.4.1), Rstudio software (v 2022.02.2+485), R software (v 4.2.0) along with the

following plugins: arsenal, FactoMineR, questionr, readxl and tidyverse (17-21). The threshold for statistical significance of unique comparisons was set at  $p < 0.05$ .

## **D. Results**

- ***Rac is activated in epithelial cells, smooth muscle cells and infiltrating cells in severe asthma patients.***

To assess Rac activation during severe asthma, we stained bronchial biopsies from severe asthmatic patients obtained in the NARACAS protocol. Detailed characteristics of patients are described in Table 1. Patients were severe asthmatics confirmed by high intensity treatment according to ATS/ERS guidelines along with good adherence. They displayed remodeling lesions involving epithelium and bronchial smooth muscle associated with significant chorion inflammation mainly composed of lymphocytes and eosinophils (Figure 1A). Intense membrane Rac-GTP signal was observed in epithelial and infiltrating immune cells. Rac was also activated in bronchial smooth muscle cells as previously described (12). Distribution of Rac-GTP total cell fluorescence was heterogeneous within each cell population (Figure 1B). Few infiltrating cells displayed high Rac-GTP signal suggesting specific activation within limited cell populations. High variability of Rac-GTP positive cells frequency was observed between patients (Figure 1C).

- ***Rac is overactivated in macrophages and eosinophils in a murine model of severe asthma***

To identify the immune cells that overactivated Rac, we assessed its activity in inflammatory cells in our murine allergic asthma model to house dust mite by flow cytometry. Mixed eosinophilic and neutrophilic lung inflammation associated with CD4+ and CD8+ T cells infiltration were observed in HDM asthmatic mice compared with control (DMSO PBS) mice (corrected  $p < 0.01$ , Mann-Whitney tests) (Figure 2A). More than 90% of studied cell populations were Rac-GTP positive compared to their respective FMO controls, both in control and asthmatic mice (Figure 2B). High heterogeneity of Rac-GTP levels could be observed in the different cell populations with intensities varying from 1 to 10-fold. Asthmatic mice displayed increased Rac-GTP MFI in alveolar macrophages and eosinophils populations compared to control mice (corrected  $p = 0.03$ , Mann-Whitney tests) (Figure 2C). A trend toward decreased Rac-GTP levels could be observed in neutrophils from asthmatic mice compared with

control mice (corrected  $p = 0.21$ ). No difference was observed in neutrophils or in T and B cells. These results confirmed that Rac was indeed activated in inflammatory cells infiltrating lungs in asthma especially in eosinophils and macrophages.

- ***Nebulization of a Rac inhibitor did not significantly modify bronchial and lung cytokine environment.***

To decipher the role of Rac activation in bronchial inflammation during asthma, we assessed the effect of nebulization of a Rac inhibitor prior to each challenge. As previously described with the Rac inhibitor NSC23766, A.41 lowered aSMC contraction in response to methacholine, neutrophils and eosinophils in BALF and bronchial remodeling in our murine model of severe allergic asthma (Supp Figure 6) (11, 12). We hypothesized that such effect would be linked with decreased pro-inflammatory cytokine environment. We quantified type 1, type 2 and type 17 cytokines along with chemokines and growth factors in BALF and lungs in our murine allergic asthma model treated or not with A.41. In BALF, the 2 first dimensions explained 49.7 % and 19.7% of the total variance (Figure 3 A and B). Dimension 1 mainly correlated with type 1 cytokines IL12p70 and IFN $\gamma$ , and TNF $\alpha$  on the one hand and with chemokines MIP1a/MIP1b, RANTES, MCP-1 and type 17 cytokines (IL-17) on the other hand (Figure 3 A). Dimension 2 was associated with type 2 cytokines IL-4 and IL-5 and eosinophilic chemokines eotaxin on one side and growth factors GM-CSF on the other side (Figure 3 A). On the individuals graph, whereas control mice were clearly separated from asthmatic mice, treatment with a Rac inhibitor did not clearly modify the position of the groups (Figure 3 A). Regarding the principal component analysis of cytokines measured in lung extracts, first dimension (53.1% of explained variance) mainly represented type 1 and 2 cytokines along macrophage associated chemokines (MCP-1, MIP1a and MIP1b). The second dimension (13.5% of explained variance) opposed type 2 cytokines (IL-4, IL-5 and IL-13) on the one hand and type 1 and type 17 cytokines on the other hand (Figure 3 B). Asthmatic mice were clearly different from control mice considering the first-dimension axis (Figure 3 B). These findings were confirmed with no significant difference of cytokine concentrations between treated and non-treated mice in control and asthmatic mice (Supplemental Table 1 & Supplemental Table 2). Strikingly, these results demonstrated that inhaled Rac inhibitor did not significantly modify the production of pro-inflammatory cytokines in an asthma context.

- ***Rac is activated in eosinophils while differentiating***

To explain decreased number of immune cells in BALF, we next hypothesized that Rac activation could be essential to the production of immune cells. Given the major role played by eosinophils in type 2 severe asthma, we chose to focus on that population. We evaluated Rac activity by mean of RacGTP/total Rac ratio during the maturation of murine eosinophils in culture. Culture of bone marrow total cells subsequently stimulated with SCF/ Flt3/Flk-2 ligand and IL-5 gave rise to 95% pure murine eosinophils at the 14th day of culture (Supp Figure 4). Rac-GTP/total Rac ratio and total Rac levels significantly differed throughout the culture (Friedman test,  $p=0.001$  and  $p<0.001$  respectively) (Figure 4A). Rac-GTP/Total Rac ratio increased by 5-fold between the 4th and the 11th day of culture ( $p=0.009$ ). Conversely, a 1.9 fold decrease of total Rac occurred between the 4th and the 11th day ( $p=0.009$  respectively). In flow cytometry, we observed on UMAP maps a fall of the heterogeneity of the cells while differentiation occurred from the first to the 11th day of culture associated with a global increase of Rac-GTP fluorescence intensity (Figure 4B). Looking specifically within eosinophils, almost all cells were Rac-GTP positive. An overall increase in fluorescence intensities was noted during differentiation (Kruskal-Wallis test,  $p<0.001$ ) (Figure 4C). Noteworthy, a bi-modal distribution of Rac-GTP fluorescence intensities could be noted with a switch appearing between the 4th and the 8th day. Quartiles of distribution of Rac-GTP fluorescence intensity, determined with the whole eosinophils set, was significantly different between the different selected days of culture (chi-square test,  $p<0.0001$ ). The 3rd and 4th quartiles were predominantly observed on the 8th and 11th day of culture. These results revealed that a global Rac activation occurred in eosinophils while they differentiate in presence of SCF/ Flt3/Flk-2 ligand followed by IL-5.

- ***Rac is essential to the degranulation of eosinophils***

To explain the lack of global effect on the cytokine environment, we assumed that Rac activation could be linked with specific functions such as degranulation. To estimate its role in degranulation processes in bronchial infiltrating immune cells, we isolated total cells from lungs of mice subjected to our severe allergic asthma model. Total cells were subjected to a degranulation assay induced by Platelet Activating



Factor (PAF). Low degradation of o-phenylene-diamine was observed in total cell lysates from control mice compared to asthmatic mice (Figure 5). PAF did not induce any significant degranulation in cells from DMSO PBS. In cells from HDM-sensitized mice, PAF induced significant degranulation compared to unstimulated condition although representing a low fraction of the total peroxidase activity of total cell lysate. In presence of Rac inhibitor (A.41), we observed a slight reduction of degranulation abilities in cells from HDM-sensitized mice. Based on our observations of increased Rac activation in differentiating eosinophils and infiltrating lungs eosinophils, we postulated that Rac activation could be linked with eosinophils degranulation. To explore that hypothesis, we induced the degranulation of differentiated eosinophils at day 14th by PAF in presence or absence of a Rac inhibitor. We observed a decrease of degranulation efficiency evaluated by the difference of AUC between stimulated and unstimulated eosinophils with increasing concentrations of Rac inhibitor (Figure 6). At  $10^{-4}$  M of Rac inhibitor, induced degranulation by PAF was almost completely abolished. Those results are in favor of an important role of Rac in the degranulation of eosinophils produced in vitro but also from inflammatory lungs cells in an asthma context.

## ***E. Discussion***

This study demonstrated that Rac was activated in infiltrating cells in bronchial biopsies from asthmatic patients and in a murine severe allergic asthma model, especially in alveolar macrophages and eosinophils. Moreover, its activation was also observed in the maturation and degranulation abilities of eosinophils in vitro.

A paradoxical aspect of our observations is the absence of effect of a Rac inhibitor on the bronchopulmonary cytokine environment in our murine model of asthma while a reduction of inflammatory populations was observed (11, 12). One potential explanation would be that Rac inhibition may have different effects depending on cell populations. Indeed, whereas Rac was activated in almost all inflammatory cells infiltrating the lungs, both in control and asthmatic mice, slight variations between those groups were restricted to limited cell types. If the state of Rac activation depends on involved mechanisms, high variability of Rac-GTP signal in a population could be correlated with the wide variety of ongoing activities (migration, maturation, cytokine production, degranulation). It could also explain why only high Rac-GTP fluorescence values seem to differ in macrophages and eosinophils between control and asthmatic mice as only limited cellular activities specifically happen during asthma. High Rac-GTP signal observed in macrophages could be linked to their polarization state as Rac2 have already been linked to M2 development in other pathological context like pulmonary fibrosis and cancer (22, 23). Finally, aside inflammatory cells, it has been shown that the reduction of Rac expression in epithelial cells in a mouse model of asthma increased their production and release of IL-33, an alarmin stimulating type 2 inflammatory responses (24). Influence of Rac activation on inflammation to tolerance balance might be more complex than expected.

To our knowledge, there was no available study evaluating the activity of Rac during eosinophil maturation. Though, studies already demonstrated in vivo in healthy mice that Rac activation was essential for the maturation of some hematopoietic cells especially T and B cells (25-27). Mechanisms underlying eosinophilopoiesis are well described (28). CD34<sup>+</sup> granulocyte monocyte progenitor (GMP) first differentiates into CD34<sup>+</sup> IL-5R $\alpha$  Eosinophil Progenitor (EoP) under the activation of a complex transcription factors program implicating PU.1, C/EBP and GATA 1. Concomitant

stimulation of EoP by IL-5 along with GM-CSF and IL-33 induces the final differentiation in eosinophils (29, 30). Interestingly, Vav protein, a described activator of Rho/Rac GTPase has been described as associated with IL-5R and activated by phosphorylation after IL-5 stimulation (31). Moreover, IL-5 binding to its receptor activates a signaling cascade leading to eosinophils survival, migration, and recruitment involving JAK-STAT, MAPK, PI3K/Erk and NFκB which are described potential downstream pathways activated by Rac-GTPase (32, 33). Rac-GTPase could be one of the molecular switches activated in IL-5 pathways. Whether Rac is indeed essential to eosinophil differentiation and survival remains unanswered and need to be addressed.

Rac activation plays essential role in immune functions that can be observed in asthma such as dendritic cell mediated T cell priming, T helper cell polarization, mast cells chemotaxis and exocytosis and neutrophils recruitment in tissues (34-37). Nevertheless, few data concerning eosinophil's function in asthma are available. Here, we observed a significant reduction of the degranulation abilities of eosinophils in vitro which argues in favor of a major role played by Rac. The involvement of Rac in degranulation mechanisms is probably less dependent on the activation stimulus than on its impact on the regulation of the actin cytoskeleton. Indeed, pharmacological inhibition of Rac by EHT1864 also lead to a decrease in RNase secretion by murine eosinophils in response to another stimulation (eotaxin/CCR3) (38). Rac2<sup>-/-</sup> eosinophils from CD2-IL5 transgenic mice presented decreased induced degranulation by PAF associated with impaired actin polymerization (39). Interestingly, previous data showed that Rac was also involved in the oxidative capabilities of the eosinophil. Indeed, eosinophils present a higher expression of Rac within their NADPH oxidase complex than neutrophils (40). Moreover, Rac2 translocates to the membrane alongside other components of NADPH oxidase, notably p47phox and p67phox, in response to stimulation by PMA (41). These findings were confirmed in eosinophils from induced sputum of asthmatic patients with a reduction of their oxidative capacity in the presence of a non-specific inhibitor of Rho GTPases (toxin B) (42). Targeting Rac activation could be an attractive way to both reduce the oxidative power of the eosinophil while also limiting its ability to release pro-inflammatory compounds into the

tissues. In order to prove such concept, it appears interesting to directly target Rac expression in eosinophils in mouse models of asthma.

To conclude, we were able to show that Rac activation plays an important role in the inflammatory mechanisms present in asthma, particularly those dependent on eosinophils. The discovery of Rac activators involved in the degranulation abilities of eosinophils could lead to the development of new specific innovative treatments in eosinophilic severe asthma.

## **F. References**

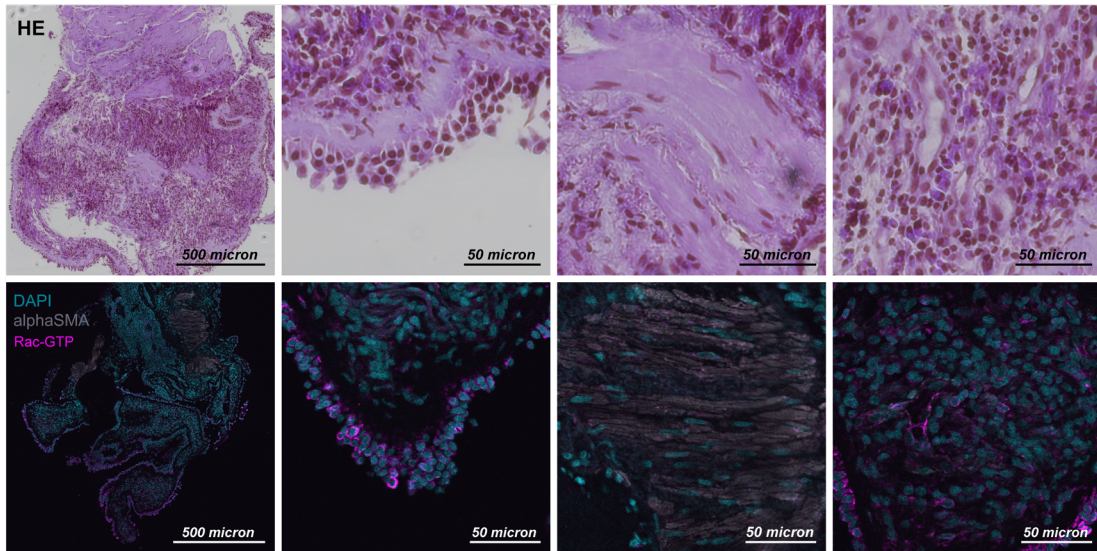
1. Safiri S, Carson-Chahhoud K, Karamzad N, Sullman MJM, Nejadghaderi SA, Taghizadieh A, Bell AW, Kolahi AA, Ansarin K, Mansournia MA, Collins GS, Kaufman JS. Prevalence, Deaths, and Disability-Adjusted Life-Years Due to Asthma and Its Attributable Risk Factors in 204 Countries and Territories, 1990-2019. *Chest* 2022; 161: 318-329.
2. Hekking PP, Wener RR, Amelink M, Zwinderman AH, Bouvy ML, Bel EH. The prevalence of severe refractory asthma. *J Allergy Clin Immunol* 2015; 135: 896-902.
3. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, Adcock IM, Bateman ED, Bel EH, Bleecker ER, Boulet LP, Brightling C, Chanez P, Dahlen SE, Djukanovic R, Frey U, Gaga M, Gibson P, Hamid Q, Jajour NN, Mauad T, Sorkness RL, Teague WG. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J* 2014; 43: 343-373.
4. Moore WC, Bleecker ER, Curran-Everett D, Erzurum SC, Ameredes BT, Bacharier L, Calhoun WJ, Castro M, Chung KF, Clark MP, Dweik RA, Fitzpatrick AM, Gaston B, Hew M, Hussain I, Jarjour NN, Israel E, Levy BD, Murphy JR, Peters SP, Teague WG, Meyers DA, Busse WW, Wenzel SE, National Heart Lungs and Blood Institute's Severe Asthma Research Program. Characterization of the severe asthma phenotype by the National Heart, Lung, and Blood Institute's Severe Asthma Research Program. *J Allergy Clin Immunol* 2007; 119: 405-413.
5. Lane S, Molina J, Plusa T. An international observational prospective study to determine the cost of asthma exacerbations (COAX). *Respir Med* 2006; 100: 434-450.
6. Lambrecht BN, Hammad H, Fahy JV. The Cytokines of Asthma. *Immunity* 2019; 50: 975-991.
7. Reddel HK, Bacharier LB, Bateman ED, Brightling CE, Brusselle GG, Buhl R, Cruz AA, Duijts L, Drazen JM, Fitzgerald JM, Fleming LJ, Inoue H, Ko FW, Krishnan JA, Levy ML, Lin J, Mortimer K, Pitrez PM, Sheikh A, Yorgancioglu AA, Boulet L-P. Global Initiative for Asthma Strategy 2021: executive summary and rationale for key changes. *European Respiratory Journal* 2022; 59: 2102730.
8. Holguin F, Cardet JC, Chung KF, Diver S, Ferreira DS, Fitzpatrick A, Gaga M, Kellermeyer L, Khurana S, Knight S, McDonald VM, Morgan RL, Ortega VE, Rigau D, Subbarao P, Tonia T, Adcock IM, Bleecker ER, Brightling C, Boulet LP, Cabana M, Castro M, Chanez P, Custovic A, Djukanovic R, Frey U, Frankemolle B, Gibson P, Hamerlijnck D, Jarjour N, Konno S, Shen H, Vitary C, Bush A. Management of severe asthma: a European Respiratory Society/American Thoracic Society guideline. *Eur Respir J* 2020; 55.
9. Busse WW. Biological treatments for severe asthma: A major advance in asthma care. *Allergol Int* 2019; 68: 158-166.
10. Haldar P, Brightling CE, Singapuri A, Hargadon B, Gupta S, Monteiro W, Bradding P, Green RH, Wardlaw AJ, Ortega H, Pavord ID. Outcomes after cessation of mepolizumab therapy in severe eosinophilic asthma: a 12-month follow-up analysis. *J Allergy Clin Immunol* 2014; 133: 921-923.
11. Andre-Gregoire G, Dilasser F, Chesne J, Braza F, Magnan A, Loirand G, Sauzeau V. Targeting of Rac1 prevents bronchoconstriction and airway hyperresponsiveness. *J Allergy Clin Immunol* 2018; 142: 824-833 e823.
12. Dilasser F, Rose L, Hassoun D, Klein M, Rousselle M, Brosseau C, Guignabert C, Taille C, Dombret MC, Di Candia L, Heddebaut N, Bouchaud G, Pretolani M, Magnan A, Loirand G, Sauzeau V. Essential role of smooth muscle Rac1 in severe asthma-associated airway remodelling. *Thorax* 2021.

13. Castan L, Cheminant MA, Colas L, Brouard S, Magnan A, Bouchaud G. Food allergen-sensitized CCR9(+) lymphocytes enhance airways allergic inflammation in mice. *Allergy* 2018; 73: 1505-1514.
14. McInnes L, Healy J, Melville J. UMAP: Uniform Manifold Approximation and Projection for Dimension Reduction. *arXiv:180203426* 2018.
15. Dyer KD, Moser JM, Czapiga M, Siegel SJ, Percopo CM, Rosenberg HF. Functionally competent eosinophils differentiated ex vivo in high purity from normal mouse bone marrow. *J Immunol* 2008; 181: 4004-4009.
16. Guilluy C, Dubash AD, Garcia-Mata R. Analysis of RhoA and Rho GEF activity in whole cells and the cell nucleus. *Nat Protoc* 2011; 6: 2050-2060.
17. Wickham H, Bryan J. `_readxl: Read Excel Files_`. R package version 1.4.0. 2022.
18. Heinzen E, Sinnwell J, Atkinson E, Gunderson T, Dougherty G. `_arsenal: An Arsenal of 'R' Functions for Large-Scale Statistical Summaries_`. R package version 3.6.3. 2021.
19. Le S, Josse J, Husson F. FactoMineR: An R package for multivariate analysis. *Journal of Statistical Software* 2008; 25: 1-18.
20. Barnier J, Briatte F, Larmarange J. `_questionr: Functions to Make Surveys Processing Easier_`. R package version 0.7.7. 2022.
21. Wickham H, Averick M, Bryan J, Chang W, McGowan L, François R, Golemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen T, Miller E, Bache S, Müller K, Ooms J, Robinson D, Seidel D, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, Yutani H. Welcome to the Tidyverse. *Journal of Open Source Software* 2019; 4.
22. Joshi S, Singh AR, Zulcic M, Bao L, Messer K, Ideker T, Dutkowski J, Durden DL. Rac2 controls tumor growth, metastasis and M1-M2 macrophage differentiation in vivo. *PLoS One* 2014; 9: e95893.
23. Joshi S, Singh AR, Wong SS, Zulcic M, Jiang M, Pardo A, Selman M, Hagood JS, Durden DL. Rac2 is required for alternative macrophage activation and bleomycin induced pulmonary fibrosis; a macrophage autonomous phenotype. *PLoS One* 2017; 12: e0182851.
24. Juncadella IJ, Kadl A, Sharma AK, Shim YM, Hochreiter-Hufford A, Borish L, Ravichandran KS. Apoptotic cell clearance by bronchial epithelial cells critically influences airway inflammation. *Nature* 2013; 493: 547-551.
25. Dumont C, Corsoni-Tadrzak A, Ruf S, de Boer J, Williams A, Turner M, Kioussis D, Tybulewicz VL. Rac GTPases play critical roles in early T-cell development. *Blood* 2009; 113: 3990-3998.
26. Guo F, Cancelas JA, Hildeman D, Williams DA, Zheng Y. Rac GTPase isoforms Rac1 and Rac2 play a redundant and crucial role in T-cell development. *Blood* 2008; 112: 1767-1775.
27. Walmsley MJ, Ooi SK, Reynolds LF, Smith SH, Ruf S, Mathiot A, Vanes L, Williams DA, Cancro MP, Tybulewicz VL. Critical roles for Rac1 and Rac2 GTPases in B cell development and signaling. *Science* 2003; 302: 459-462.
28. Iwasaki H, Mizuno S, Mayfield R, Shigematsu H, Arinobu Y, Seed B, Gurish MF, Takatsu K, Akashi K. Identification of eosinophil lineage-committed progenitors in the murine bone marrow. *J Exp Med* 2005; 201: 1891-1897.
29. Johnston LK, Hsu CL, Krier-Burris RA, Chhiba KD, Chien KB, McKenzie A, Berdnikovs S, Bryce PJ. IL-33 Precedes IL-5 in Regulating Eosinophil Commitment and Is Required for Eosinophil Homeostasis. *J Immunol* 2016; 197: 3445-3453.
30. Kopf M, Brombacher F, Hodgkin PD, Ramsay AJ, Milbourne EA, Dai WJ, Ovington KS, Behm CA, Kohler G, Young IG, Matthaei KI. IL-5-Deficient mice have a

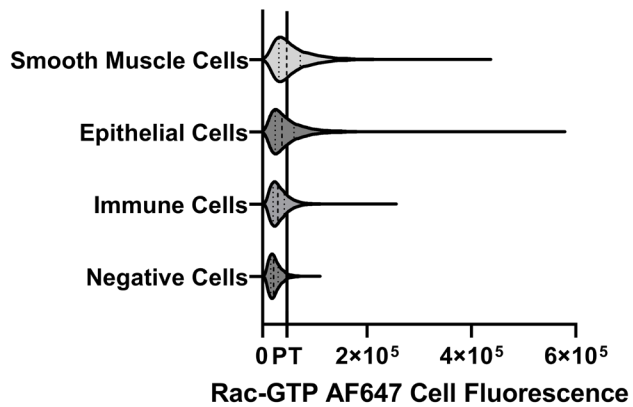
- developmental defect in CD5(+) B-1 cells and lack eosinophilia but have normal antibody and cytotoxic T cell responses. *Immunity* 1996; 4: 15-24.
31. Sato S, Katagiri T, Takaki S, Kikuchi Y, Hitoshi Y, Yonehara S, Tsukada S, Kitamura D, Watanabe T, Witte O, Takatsu K. IL-5 receptor-mediated tyrosine phosphorylation of SH2/SH3-containing proteins and activation of Bruton's tyrosine and Janus 2 kinases. *J Exp Med* 1994; 180: 2101-2111.
32. Kouro T, Kikuchi Y, Kanazawa H, Hirokawa K, Harada N, Shiiba M, Wakao H, Takaki S, Takatsu K. Critical proline residues of the cytoplasmic domain of the IL-5 receptor alpha chain and its function in IL-5-mediated activation of JAK kinase and STAT5. *Int Immunol* 1996; 8: 237-245.
33. Pazdrak K, Adachi T, Alam R. Src homology 2 protein tyrosine phosphatase (SHPTP2)/Src homology 2 phosphatase 2 (SHP2) tyrosine phosphatase is a positive regulator of the interleukin 5 receptor signal transduction pathways leading to the prolongation of eosinophil survival. *J Exp Med* 1997; 186: 561-568.
34. Pantarelli C, Welch HCE. Rac-GTPases and Rac-GEFs in neutrophil adhesion, migration and recruitment. *Eur J Clin Invest* 2018; 48 Suppl 2: e12939.
35. Kurdi AT, Bassil R, Olah M, Wu C, Xiao S, Taga M, Frangieh M, Buttrick T, Orent W, Bradshaw EM, Khoury SJ, Elyaman W. Tiam1/Rac1 complex controls IL17a transcription and autoimmunity. *Nat Commun* 2016; 7: 13048.
36. Chesne J, Braza F, Chadeuf G, Mahay G, Cheminant MA, Loy J, Brouard S, Sauzeau V, Loirand G, Magnan A. Prime role of IL-17A in neutrophilia and airway smooth muscle contraction in a house dust mite-induced allergic asthma model. *J Allergy Clin Immunol* 2015; 135: 1643-1643 e1643.
37. Benvenuti F, Hugues S, Walmsley M, Ruf S, Fetler L, Popoff M, Tybulewicz VL, Amigorena S. Requirement of Rac1 and Rac2 expression by mature dendritic cells for T cell priming. *Science* 2004; 305: 1150-1153.
38. Shamri R, Young KM, Weller PF. Rho and Rac, but not ROCK, are required for secretion of human and mouse eosinophil-associated RNases. *Clin Exp Allergy* 2019; 49: 190-198.
39. Lacy P, Willetts L, Kim JD, Lo AN, Lam B, Maclean EI, Moqbel R, Rothenberg ME, Zimmermann N. Agonist activation of f-actin-mediated eosinophil shape change and mediator release is dependent on Rac2. *Int Arch Allergy Immunol* 2011; 156: 137-147.
40. Someya A, Nishijima K, Nunoi H, Irie S, Nagaoka I. Study on the superoxide-producing enzyme of eosinophils and neutrophils--comparison of the NADPH oxidase components. *Arch Biochem Biophys* 1997; 345: 207-213.
41. Lacy P, Mahmudi-Azer S, Bablitz B, Gilchrist M, Fitzharris P, Cheng D, Man SF, Bokoch GM, Moqbel R. Expression and translocation of Rac2 in eosinophils during superoxide generation. *Immunology* 1999; 98: 244-252.
42. Lacy P, Abdel-Latif D, Steward M, Musat-Marcu S, Man SF, Moqbel R. Divergence of mechanisms regulating respiratory burst in blood and sputum eosinophils and neutrophils from atopic subjects. *J Immunol* 2003; 170: 2670-2679.

## G. List of figures

**A**



**B**



**C**

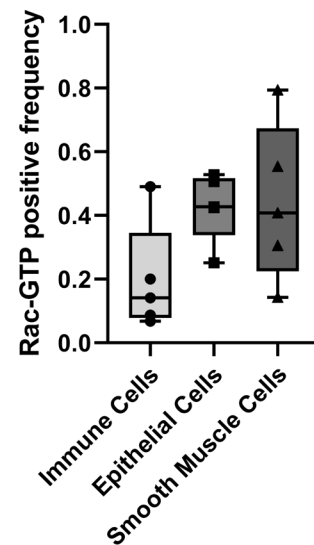


Figure 1 A) Representative HE staining and alphaSMA/Rac-GTP signal from bronchial biopsies of severe asthmatic patients (n=5). B) Quantification of Rac-GTP AF647 cell fluorescence in immune cells, epithelial cells and smooth muscle cells from bronchi of severe asthmatic patients (n=5). PT = Positive Threshold.



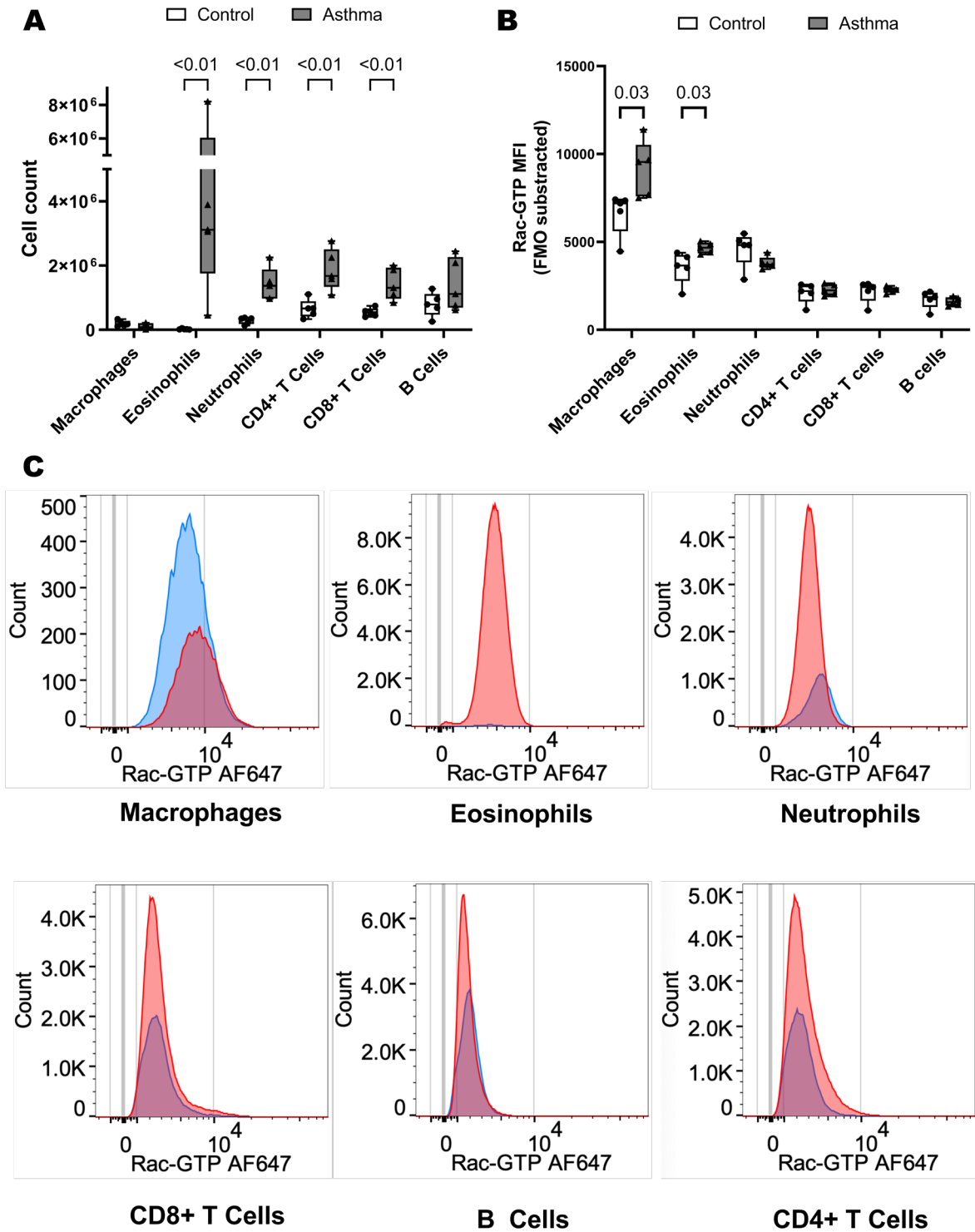
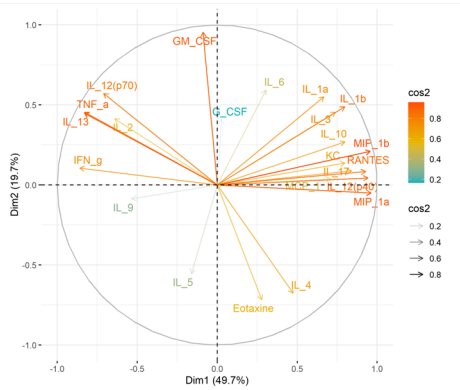
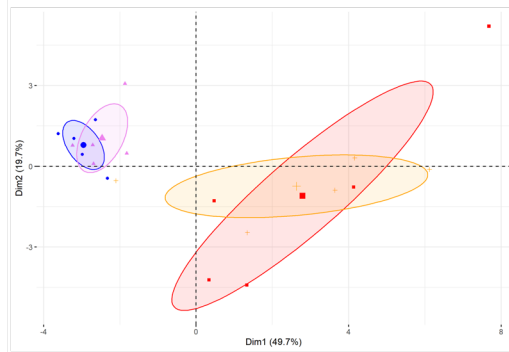


Figure 2 Rac is activated in alveolar macrophages and eosinophils in a murine asthma model. A) Infiltrating cells in lungs of mice subjected to a murine severe allergic asthma model to house dust mite (n=5 mice/group). B) Rac-GTP fluorescence distribution by cell population in concatenated data from control and asthmatic mice along with related cell counts. C) Rac-GTP median of fluorescence (MFI) by cell population in control and asthmatic mice (n=5 mice/group). Data are represented as mean $\pm$ SD. Mann-Whitney tests were used corrected with a False Discovery Rate approach (FDR=0.05) by Benjamini, Krieger and Yekutieli.

### A BALF - Variables

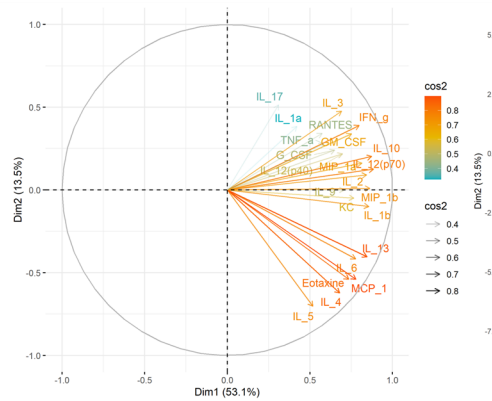


### BALF - Individuals

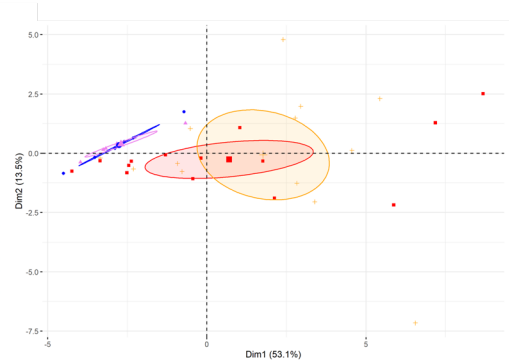


CTL Untreated  
 CTL + Rac Inh.  
 Asthma Untreated  
 Asthma + Rac Inh.

### B Lungs - Variables



### Lungs - Individuals



CTL Untreated  
 CTL + Rac Inh.  
 Asthma Untreated  
 Asthma + Rac Inh.

Figure 3 Principal component analyses of cytokines concentration from BALF (A) and lungs (B). Variables and individual graphs are represented side-by-side.

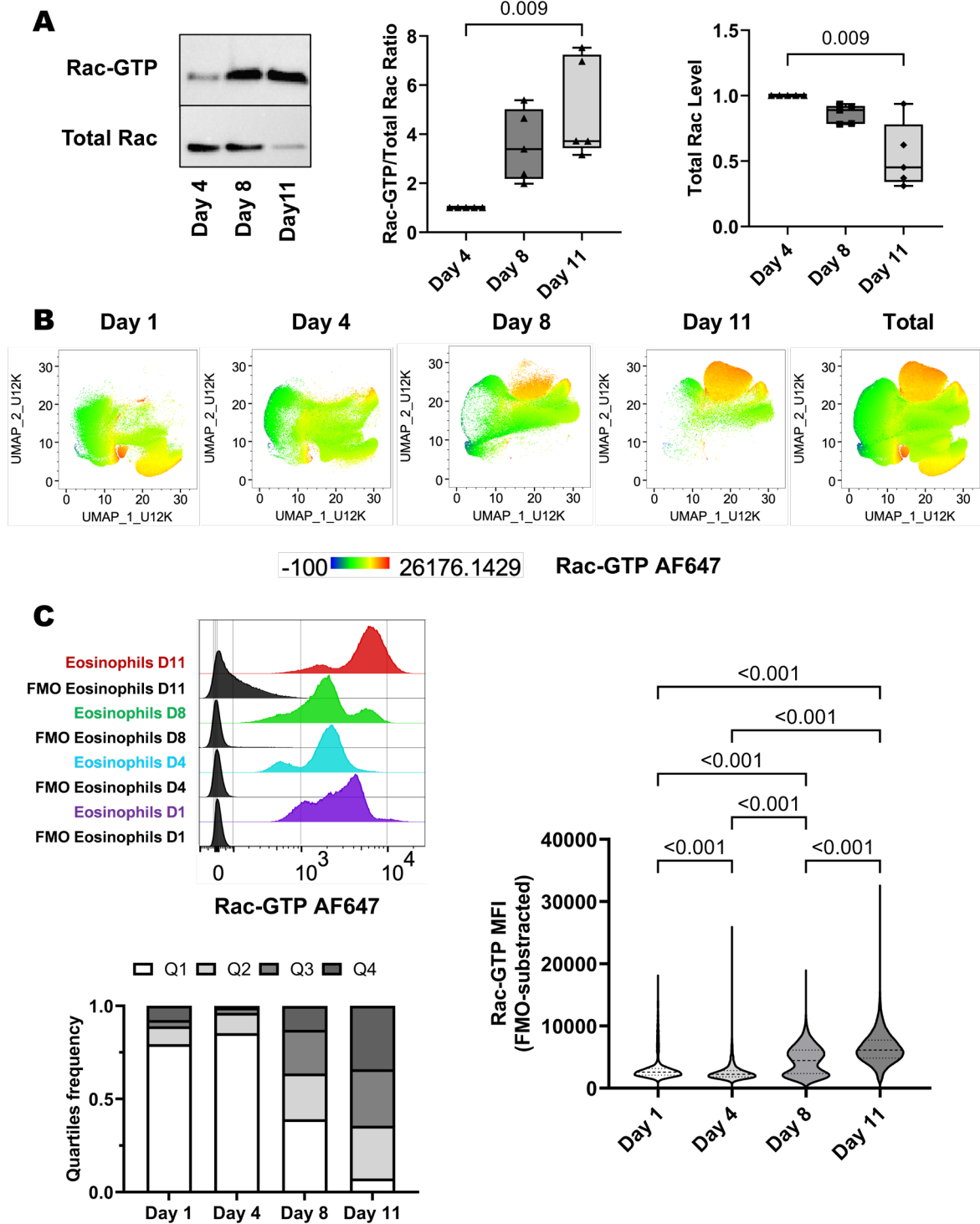


Figure 4 Rac is activated while eosinophils differentiate from bone marrow precursors. A) Immunoblot analysis and quantification of Rac-GTP and total Rac at different time of the eosinophil differentiation. B) UMAP maps of Rac-GTP fluorescence intensity of whole cells while eosinophils differentiate. C) Distribution and quantification of Rac-GTP fluorescence intensity in differentiating eosinophils with relative FMO controls. Kruskal-Wallis or Friedman test (paired data) followed by multiple comparisons controlled by a False Discovery Rate approach (FDR = 0.05).

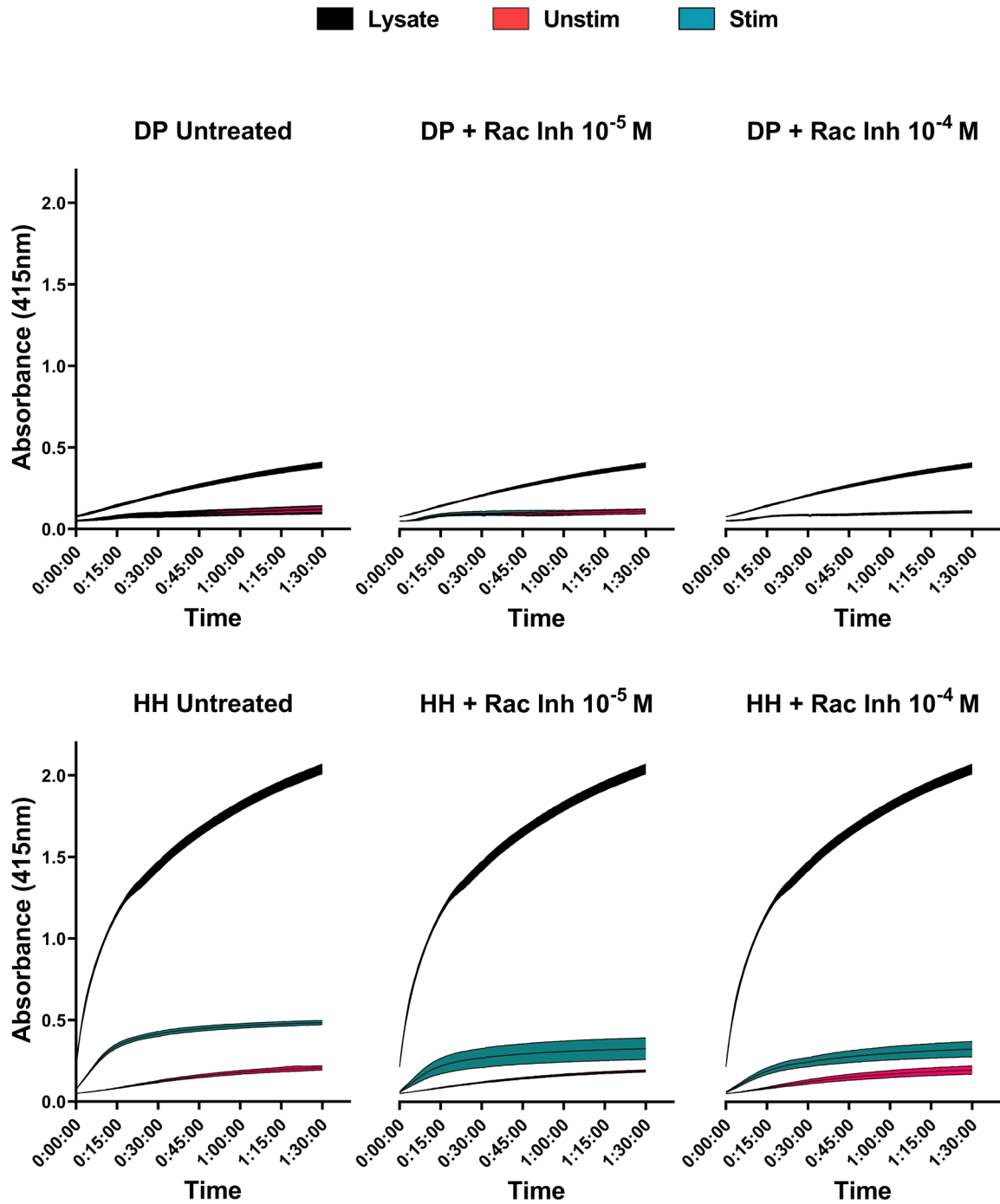


Figure 5 Rac inhibition led to decreased degranulation capacities of total lung cells from DMSO PBS (DP) and HDM HDM (HH) mice at different concentrations of Rac Inhibitor A.41 (n=5 pooled mice per group).

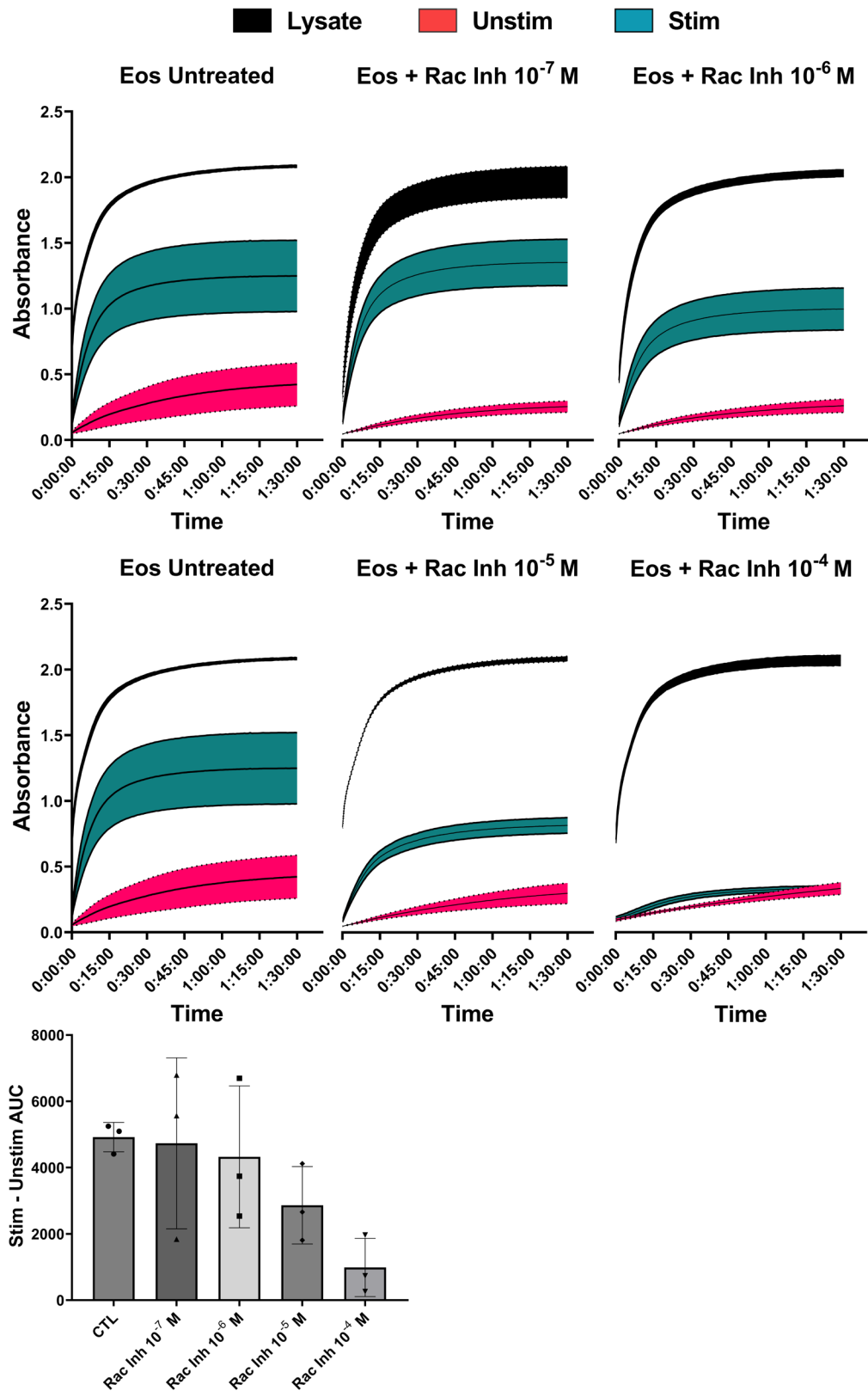


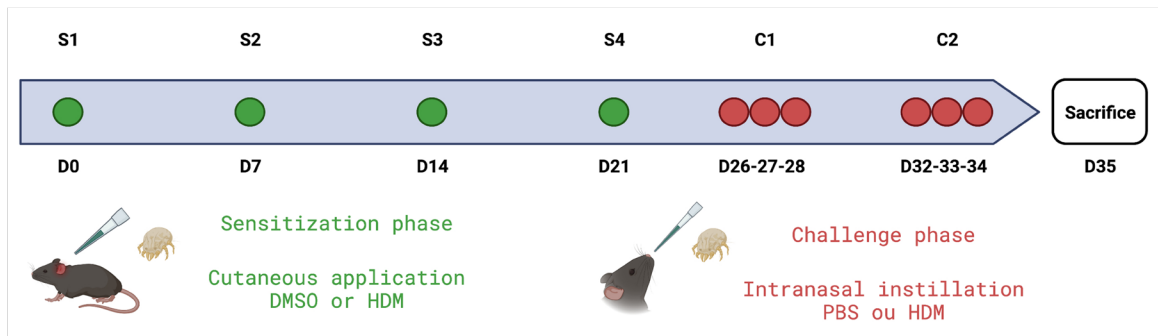
Figure 6 Decreased degranulation capacities of eosinophils (Eos) treated at different concentration of Rac inhibitor. Quantifications of stimulated minus unstimulated area under the curve (AUC) at different concentrations of Rac inhibitor.

## H. List of tables

	Total (n=5)
Sex (male)	3/5
Age (years)	63.0 (39.0, 64.0)
BMI (kg.m <sup>-2</sup> )	26.8 (25.3, 29.2)
Non smoker	5/5
Atopic	5/5
Morisky Medication Adherence Scale	8.0 (7.0, 8.0)
High Dose ICS	5/5
LABA	5/5
OCS	1/5
ACQ score	3.0 (2.2, 3.2)
ACT score	14.0 (12.0, 21.0)
AQLQ total score	4.1 (4.0, 6.2)
Annual exacerbation rate	1.0 (1.0, 2.0)
Eosinophils (G/L)	0.6 (0.3, 0.6)
FeNO (ppb)	72.5 (62.5, 100.0)
Total IgE (kUI/L)	109.0 (103.0, 224.0)
FEV1 (% theoretic)	83.0 (57.0, 87.0)
Max CV (% theoretic)	97.0 (80.0, 112.3)
FEV1/max CV	0.6 (0.6, 0.7)
TLC (% theoretic)	103.0 (100.0, 110.0)
RV/TLC (% theoretic)	96.0 (87.0, 105.0)
Goblet cell hyperplasia	2/5
RBM thickening	4/5
Chorion edema	2/5
Chorion inflammation	4/5
Lymphocytes	4/4
Neutrophils	1/4
Eosinophils	3/4

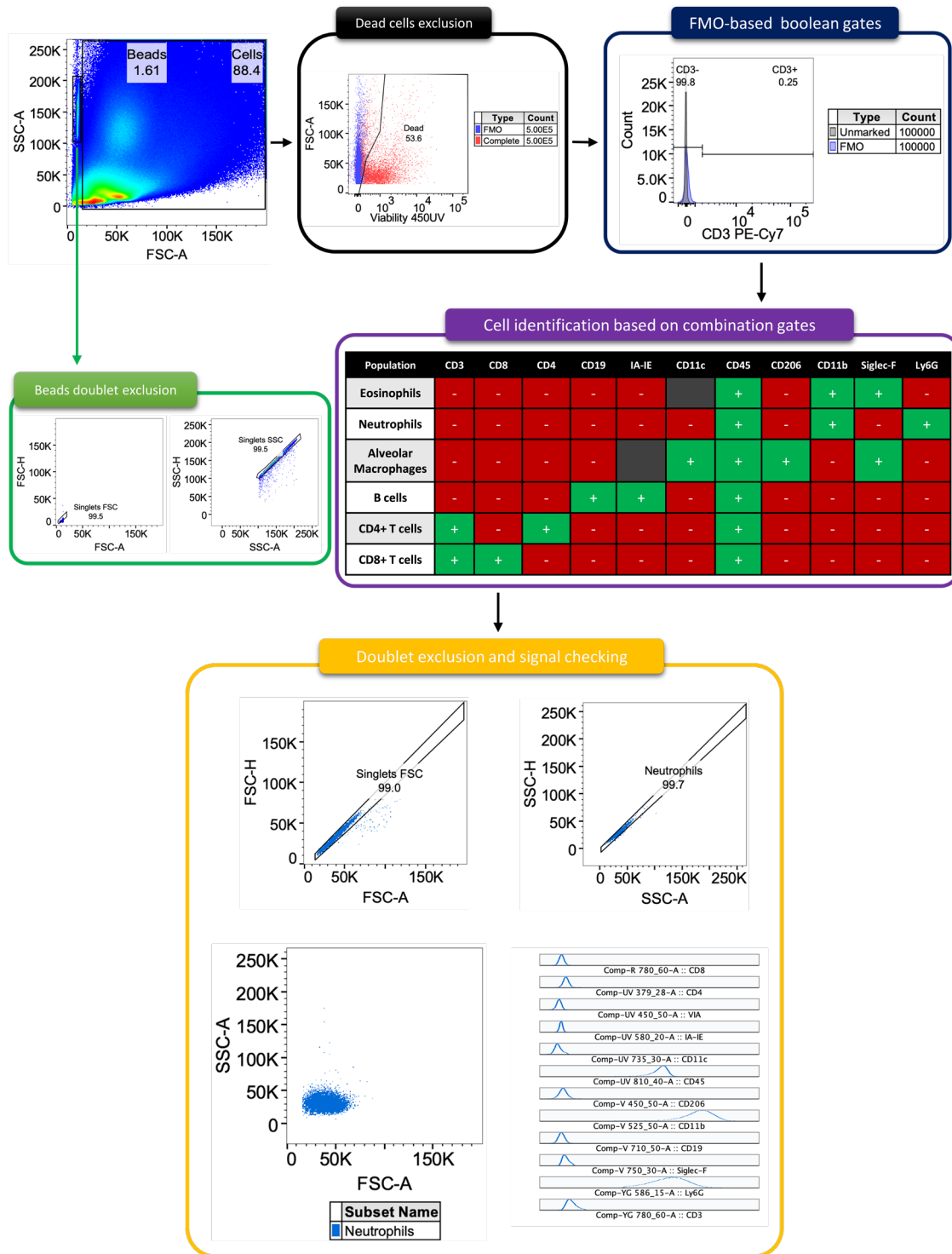
*Table 1 Clinical, functional and biological characteristics of severe asthmatic patients included.*

## I. Supplemental Figures



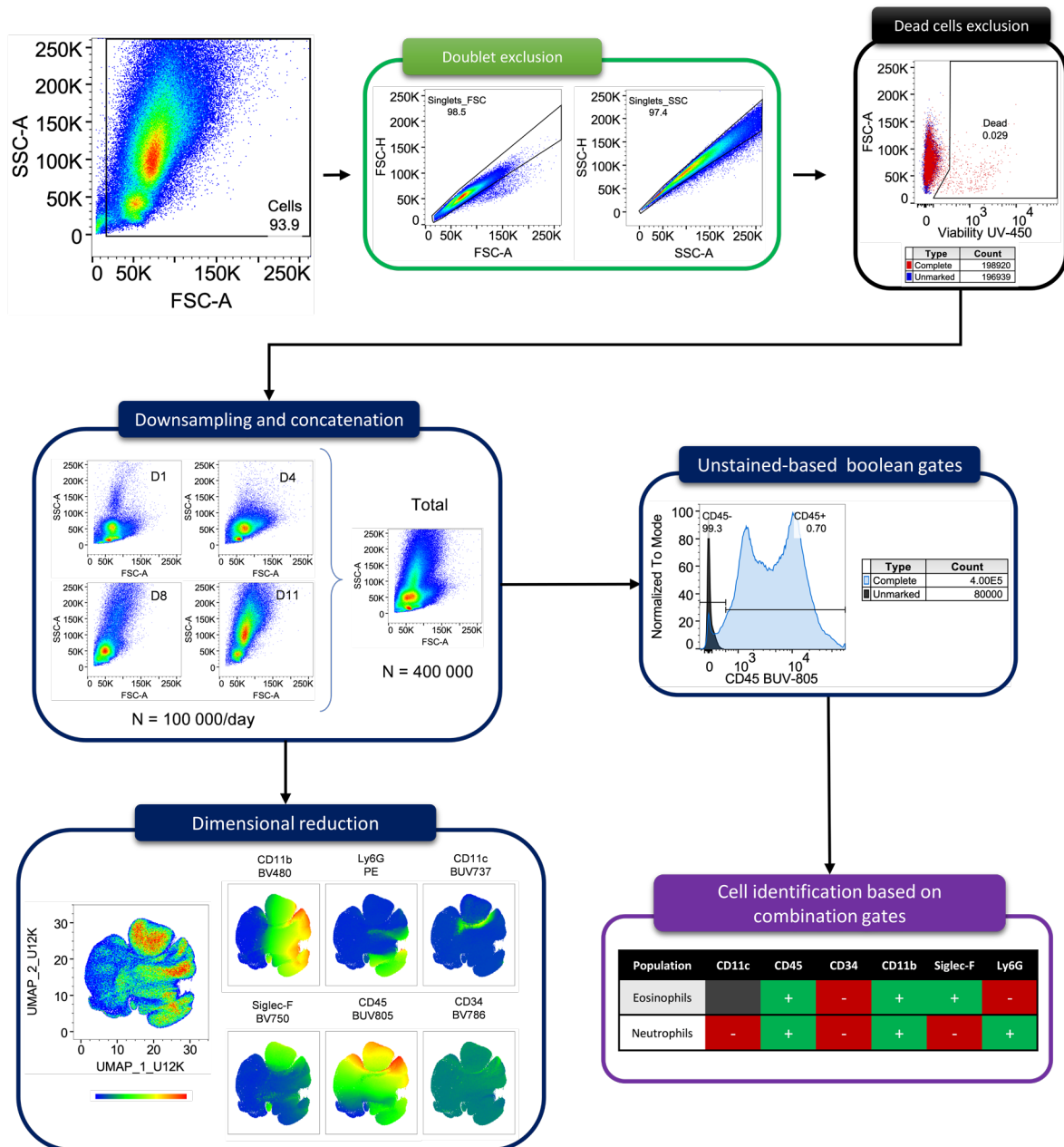
Supp Figure 1 Murine model of severe allergic asthma to house dust mite (HDM).

Eight weeks old male C57Bl/6J are sensitized per-cutaneously with 500 $\mu$ g of HDM or with DMSO 70% (control) on days 0, 7, 14 and 21. Asthma is then triggered by intranasal instillation of 250 $\mu$ g of HDM or with PBS (control) on days 26 to 28 and 32 to 34. Mice were sacrificed on day 35 to perform bronchoalveolar lavage fluid and to harvest lungs.



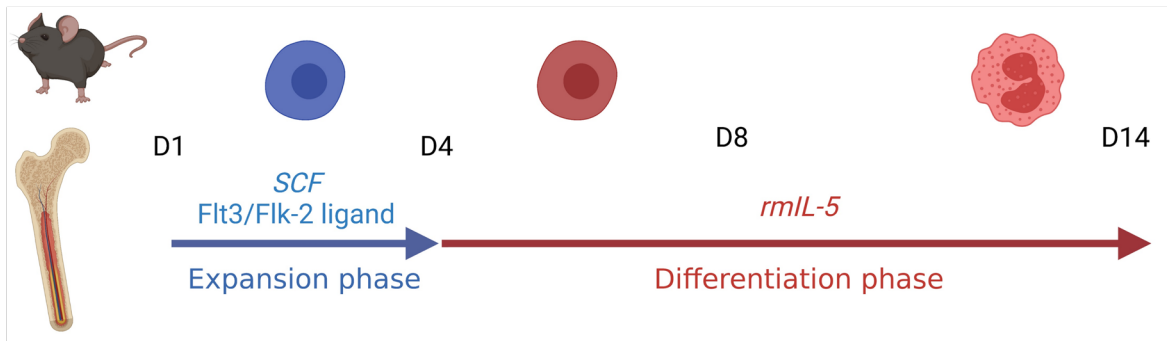
Supp Figure 2 Flow Cytometry gating strategy for inflammatory cells identification within total lung cells. First, cells and beads were identified in terms of FSC and SSC values. Dead cells were excluded and positive threshold were determined according to the relative Fluorescence Minus One controls. Using combination gates, the following populations were identified: eosinophils, neutrophils, alveolar macrophages, B cells, CD4+ and CD8+ T cells. Doublets were excluded and markers signature was checked for each population.



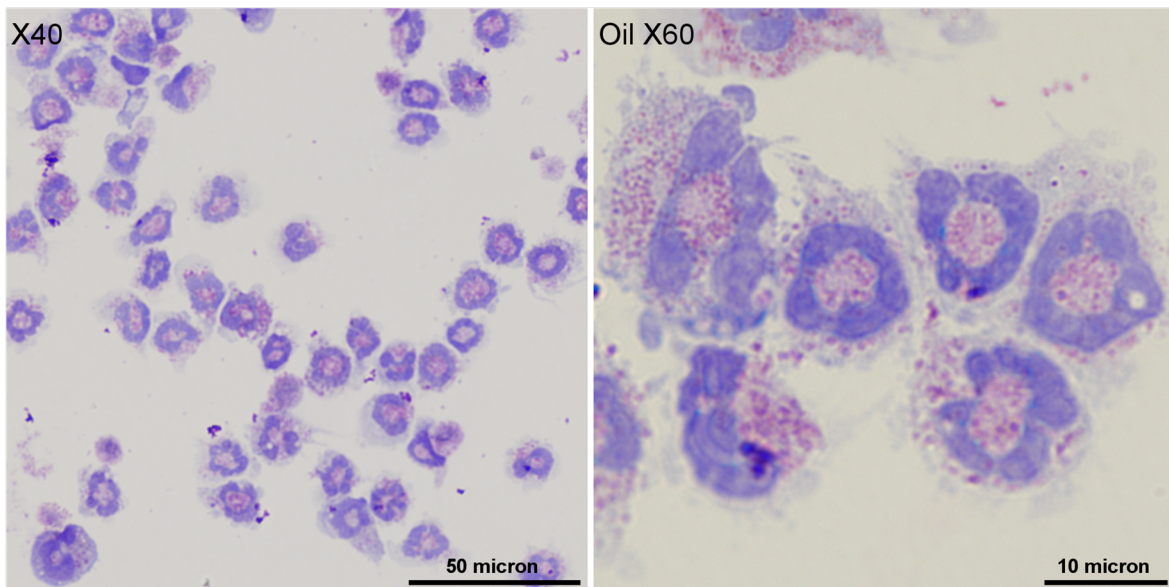


Supp Figure 3 Flow cytometry gating strategy for bone marrow derived eosinophils culture. Cells were selected thanks to their FSC/SSC structure. Doublets and dead cells were removed. Downsampled fractions ( $n = 105$  cells) of each day were concatenated. Based on positive threshold fixed by unmarked cells, eosinophils and neutrophils were identified. In parallel, dimensional reduction was performed using UMAP algorithm to get an overall look at cell populations throughout the culture.

**A**

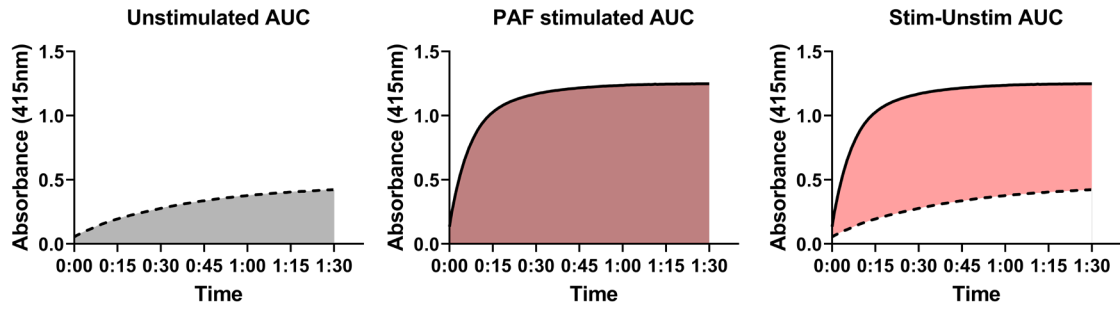


**B**

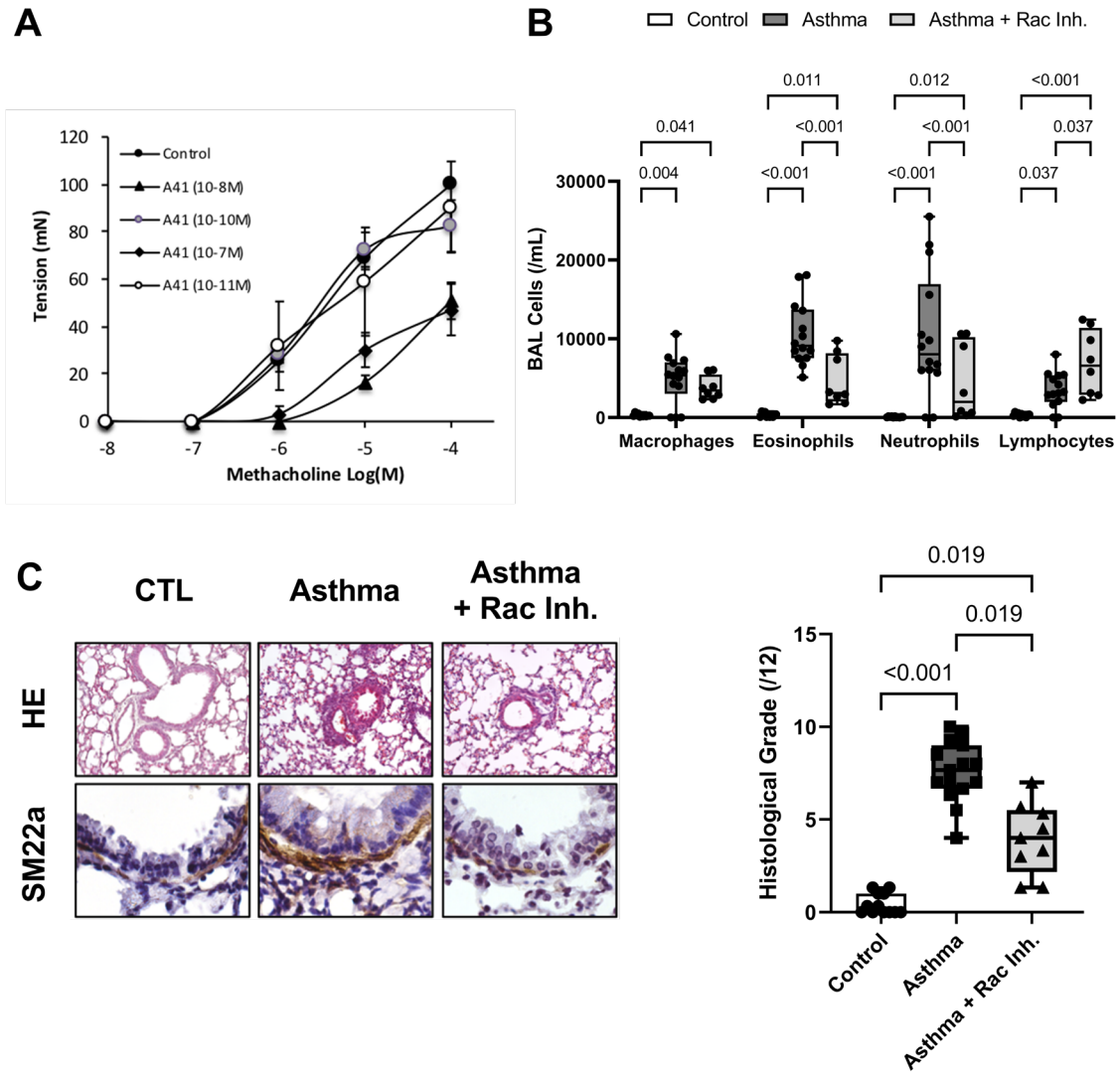


Supp Figure 4 A) Bone-marrow derived eosinophil culture model. B) Wright-Giemsa staining of eosinophils at the 14th day.

Abbreviations: FLT3-L = Fms-Like Tyrosine kinase 3 Ligand, rmIL-5 = recombinant mouse Interleukine 5, SCF = Stem Cell Factor.



*Supp Figure 5 Area Under the Curve (AUC) calculation method for degranulation assays*



Supp Figure 6 Rac inhibition by A.41 reduced aSMC contraction and bronchial inflammation.

A) Contractile responses to methacholine in bronchi from control mice treated or not with a Rac inhibitor (A.41) B) Bronchoalveolar lavage fluid inflammatory cells from control and asthmatic mice treated or not with a Rac inhibitor (A.41) ( $n = 8-14$  mice/group) C) Hematoxylin/Eosin (HE) staining and SM22 $\alpha$  immunohistochemistry of lung sections from control and asthmatic mice treated or not with a Rac inhibitor (A.41) Images are representative of 9-10 mice of each experimental condition. Histological grade quantification.

## J. Supplemental tables

	DMSO/PBS/PBS (DPP, n=6)	DMSO/PBS/A41 (DPA, n=6)	HDM/HDM/PBS (HHP, n=14)	HDM/HDM/A41 (HHA, n=13)	p value	Multiple comparisons
<b>IL-1a</b>					0.056	
Median	2.985	2.940	1.730	2.240		NA
Q1, Q3	2.130, 3.413	2.562, 3.265	1.435, 2.065	1.790, 2.660		
<b>IL-1b</b>					0.008	ns
Median	1.620	1.620	1.775	2.100		
Q1, Q3	1.620, 1.620	1.620, 1.620	1.620, 2.070	1.640, 2.960		
<b>IL-2</b>					0.665	
Median	1.800	1.825	2.110	2.160		NA
Q1, Q3	1.373, 2.228	1.690, 2.245	1.470, 2.692	1.850, 2.390		
<b>IL-3</b>					0.144	
Median	0.380	0.380	0.385	0.450		NA
Q1, Q3	0.380, 0.380	0.380, 0.380	0.380, 0.475	0.380, 0.550		
<b>IL-4</b>					< 0.001	DPP vs HPP ***
Median	0.370	0.370	5.930	4.040		
Q1, Q3	0.370, 0.370	0.370, 0.370	2.810, 12.745	1.470, 13.260		
<b>IL-5</b>					< 0.001	DPP vs HPP **
Median	0.985	1.060	4.190	4.180		
Q1, Q3	0.950, 1.163	0.950, 1.275	3.210, 7.125	3.300, 10.640		
<b>IL-6</b>					< 0.001	DPP vs HPP *
Median	1.675	1.225	26.830	40.220		
Q1, Q3	1.150, 2.440	1.185, 1.400	8.555, 88.342	19.790, 57.570		
<b>IL-9</b>					0.500	
Median	3.610	3.610	3.610	3.610		NA
Q1, Q3	3.610, 3.610	3.610, 3.610	3.610, 3.610	3.610, 4.310		
<b>IL-10</b>					< 0.001	DPP vs HPP *
Median	7.390	6.830	14.635	17.730		
Q1, Q3	6.905, 8.100	6.105, 8.605	12.090, 18.570	12.360, 19.790		
<b>IL-12(p40)</b>					< 0.001	DPP vs HPP *
Median	194.950	136.655	618.660	751.600		
Q1, Q3	127.338, 228.670	80.395, 232.785	468.948, 927.152	624.110, 1085.690		
<b>IL-12(p70)</b>					0.306	
Median	13.135	9.630	11.870	18.020		NA
Q1, Q3	10.488, 13.210	8.662, 10.822	8.065, 19.378	10.230, 21.650		
<b>IL-13</b>					0.003	DPP vs HPP *
Median	14.870	14.870	33.040	31.470		
Q1, Q3	14.870, 16.168	14.870, 14.870	16.867, 54.275	28.590, 63.490		
<b>IL-17</b>					< 0.001	DPP vs HPP **
Median	2.125	1.845	5.800	8.130		
Q1, Q3	1.643, 2.607	1.455, 2.610	5.250, 12.860	6.020, 17.560		
<b>Eotaxin</b>					< 0.001	DPP vs HPP *
Median	4.445	4.105	21.010	27.910		
Q1, Q3	3.010, 5.662	3.513, 5.245	15.645, 36.655	18.680, 50.650		
<b>G-CSF</b>					0.607	
Median	59.590	52.520	56.450	78.990		NA
Q1, Q3	30.230, 83.377	38.538, 77.078	38.085, 113.170	49.870, 103.090		
<b>GM-CSF</b>					0.805	
Median	6.025	6.450	5.925	7.100		NA
Q1, Q3	5.535, 6.852	5.933, 6.697	4.880, 7.935	5.760, 9.680		
<b>IFN<math>\gamma</math></b>					0.298	
Median	2.610	3.155	2.700	3.930		NA
Q1, Q3	2.060, 3.032	2.768, 3.340	1.722, 3.632	2.920, 4.600		
<b>KC</b>					< 0.001	DPP vs HPP *
Median	14.385	13.195	36.470	42.130		
Q1, Q3	11.732, 16.152	10.088, 16.078	24.503, 50.260	36.910, 68.520		

	DMSO/PBS/PBS (DPP, n=6)	DMSO/PBS/A41 (DPA, n=6)	HDM/HDM/PBS (HHP, n=14)	HDM/HDM/A41 (HHA, n=13)	p value	Multiple comparisons
<b>MCP-1</b>					< 0.001	DPP vs HPP *
Median	25.110	26.680	120.210	191.920		
Q1, Q3	22.922, 27.935	23.447, 29.642	79.752, 206.782	57.050, 355.260		
<b>MIP-1a</b>					< 0.001	DPP vs HPP *
Median	3.405	2.150	13.555	16.980		
Q1, Q3	1.615, 4.865	1.865, 3.545	7.660, 23.282	11.820, 26.040		
<b>MIP-1b</b>					< 0.001	DPP vs HPP *
Median	26.090	22.125	143.840	164.090		
Q1, Q3	15.500, 41.623	15.338, 32.670	94.925, 169.030	112.360, 223.370		
<b>RANTES</b>					< 0.001	DPP vs HPP **
Median	12.500	12.745	160.365	259.030		
Q1, Q3	10.378, 20.645	10.457, 18.100	72.267, 255.907	176.330, 308.310		
<b>TNF<math>\alpha</math></b>					0.067	
Median	6.715	7.080	13.710	12.470		NA
Q1, Q3	5.970, 8.120	6.268, 9.228	5.367, 18.852	7.750, 28.010		

*Supplemental Table 1 Cytokines concentration from bronchoalveolar fluid (murine severe asthma model). Kruskal Wallis test results and Dunn's test for multiple comparisons: \* =  $p < 0.05$ , \*\* =  $p < 0.01$ .*

*Abbreviations: DMSO = Dimethyl Sulfoxide, G-CSF = Granulocyte Colony- Stimulating Factor, GM-CSF = Granulocyte-Macrophage Colony Stimulating Factor, HDM = House Dust Mite, IFN = Interferon, IL = Interleukin, KC = Keratinocyte-derived Chemokine, MCP = Monocyte Chemoattractant Protein, MIP = Macrophage Inflammatory Protein, PBS = Phosphate Buffered Saline, RANTES = Regulated upon Activation, Normal T cell Expressed and Secreted.*

	DMSO/PBS/PBS (DPP, n=5)	DMSO/PBS/A41 (DPA, n=5)	HDM/HDM/PBS (HHP, n=5)	HDM/HDM/A41 (HHA, n=5)	pvalue	Multiple comparisons
<b>IL-1a</b>					0.927	
Median	509.756	494.683	492.801	467.822		NA
Q1, Q3	506.724, 526.008	492.007, 528.345	489.325, 613.790	456.614, 613.793		
<b>IL-1b</b>					0.032	ns
Median	84.566	90.398	200.168	296.044		
Q1, Q3	70.321, 92.089	62.892, 99.344	168.211, 355.397	188.879, 374.911		
<b>IL-2</b>					0.193	
Median	416.342	419.579	335.561	231.189		NA
Q1, Q3	412.050, 444.885	405.079, 426.930	333.348, 347.457	226.143, 333.393		
<b>IL-3</b>					0.125	
Median	15.069	15.126	19.086	19.972		NA
Q1, Q3	14.151, 16.450	14.540, 16.348	14.412, 20.603	18.888, 20.587		
<b>IL-4</b>					0.002	DPP vs HHP **
Median	28.975	31.735	527.731	222.312		
Q1, Q3	28.200, 30.855	28.065, 33.854	458.548, 655.747	218.937, 282.492		
<b>IL_5</b>					0.128	
Median	1979.023	1865.122	2894.211	1478.404		NA
Q1, Q3	1858.437, 2107.740	1781.801, 1986.605	2048.675, 3377.776	1000.973, 1640.835		
<b>IL-6</b>					0.187	
Median	3258.427	3345.560	2957.183	2250.949		NA
Q1, Q3	3088.683, 3389.728	3237.057, 3470.223	2031.217, 4863.320	2056.386, 2463.545		
<b>IL-9</b>					0.782	
Median	1132.380	1113.538	1120.274	717.884		NA
Q1, Q3	1058.675, 1265.053	1097.747, 1158.770	969.050, 1418.389	681.620, 1267.682		
<b>IL-10</b>					0.014	ns
Median	1120.588	1225.602	1481.448	1546.734		
Q1, Q3	849.133, 1304.519	1098.245, 1304.752	1370.396, 1773.553	1369.304, 1585.842		
<b>IL-12(p40)</b>					0.003	DPP vs HHP *
Median	7443.857	6915.622	19999.819	22254.221		
Q1, Q3	5812.839, 8751.171	6432.576, 7067.287	18349.086, 27973.155	21516.117, 26456.826		
<b>IL-12(p70)</b>					0.002	ns
Median	1768.767	2009.295	1278.872	996.799		
Q1, Q3	1755.341, 1846.634	1999.151, 2164.009	1108.641, 1387.335	696.781, 1342.355		
<b>IL-13</b>					0.001	DPP vs HHP *
Median	79572.076	64243.057	29113.705	19322.625		
Q1, Q3	64526.789, 84504.089	61425.285, 70625.784	28817.224, 30246.275	17557.732, 27707.159		
<b>IL-17</b>					0.001	DPP vs HHP **
Median	70.679	104.846	1100.563	5451.252		
Q1, Q3	60.708, 71.427	87.517, 122.426	528.508, 2851.548	1176.977, 5878.431		
<b>Eotaxin</b>					0.007	ns
Median	32708.412	23724.736	57610.679	34073.165		
Q1, Q3	28078.192, 34871.325	21955.622, 27240.050	57592.655, 61409.041	31014.193, 41007.500		
<b>G-CSF</b>					0.497	
Median	865.583	1584.314	1237.269	1046.158		NA
Q1, Q3	681.321, 1429.204	747.266, 2260.716	1167.602, 5818.557	862.692, 1054.747		
<b>GM-CSF</b>					0.069	
Median	802.588	756.396	613.386	655.421		NA
Q1, Q3	753.685, 854.789	722.605, 805.663	558.838, 686.329	653.691, 665.437		
<b>IFN<math>\gamma</math></b>					0.067	
Median	73399.862	68631.248	42554.744	22397.854		NA
Q1, Q3	73029.383, 74444.294	57500.020, 68996.360	37685.973, 60438.558	21948.637, 53662.140		

	DMSO/PBS/PBS (DPP, n=5)	DMSO/PBS/A41 (DPA, n=5)	HDM/HDM/PBS (HHP, n=5)	HDM/HDM/A41 (HHA, n=5)	pvalue	Multiple comparisons
<b>KC</b>					0.017	
Median	948.225	1030.398	4165.107	8115.142		NA
Q1, Q3	820.885, 1513.553	810.158, 1564.990	2457.557, 11060.529	3825.668, 8909.253		
<b>MCP-1</b>					0.034	
Median	23571.397	19194.728	67117.670	51387.033		NA
Q1, Q3	21193.799, 25487.814	18363.813, 29287.445	62928.869, 81355.344	41238.710, 54814.703		
<b>MIP_1a</b>					0.003	
Median	1825.082	2007.494	14006.878	11854.322		NA
Q1, Q3	1590.950, 1914.443	1514.983, 2314.161	11216.545, 17614.913	10283.564, 13429.711		
<b>MIP-1b</b>					0.006	
Median	8587.683	10507.471	30807.904	36791.019		NA
Q1, Q3	7050.674, 11602.183	9797.966, 11345.706	28875.113, 45457.799	23821.752, 38748.428		
<b>RANTES</b>					0.003	
Median	24346.392	33445.265	46008.706	71307.538		NA
Q1, Q3	22205.900, 33968.443	29562.324, 35178.950	45508.235, 60583.215	56929.519, 80667.425		
<b>TNF<math>\alpha</math></b>					0.002	
Median	20112.943	17366.331	7567.323	4874.753		NA
Q1, Q3	16870.810, 22386.883	14389.203, 17899.409	7427.073, 7636.982	4138.075, 6236.944		

*Supplemental Table 2 Cytokines concentration (pg/mL/g tissue) in right upper lobe (murine severe asthma model). Kruskal Wallis test results and Dunn's test for multiple comparisons: \* =  $p < 0.05$ , \*\* =  $p < 0.01$ .*

*Abbreviations: DMSO = Dimethyl Sulfoxide, G-CSF = Granulocyte Colony- Stimulating Factor, GM-CSF = Granulocyte-Macrophage Colony Stimulating Factor, HDM = House Dust Mite, IFN = Interferon, IL = Interleukin, KC = Keratinocyte-derived Chemokine, MCP = Monocyte Chemoattractant Protein, MIP = Macrophage Inflammatory Protein, PBS = Phosphate Buffered Saline, RANTES = Regulated upon Activation, Normal T cell Expressed and Secreted.*



### III. Discussion

La **GTPase Rac** régule le cytosquelette d'actine impliqué dans les mécanismes de migration cellulaire, de phagocytose et d'exocytose nécessaires au développement des réponses immunes (316, 394). Néanmoins, les données dans des contextes pathologiques inflammatoires manquent. Mes résultats apportent de nouvelles connaissances sur l'activation de Rac dans l'asthme sévère et ouvrent de nouvelles perspectives thérapeutiques.

Nous avons observé **une activation de Rac** dans les cellules infiltrantes bronchiques chez les patients asthmatiques sévères. La question se pose de savoir si cette activation serait liée à une intensification des réponses inflammatoires. En effet, celle-ci pourrait n'être qu'un marqueur de cellules immunitaires matures. Cette piste pourrait être explorée en comparant l'activation de Rac dans les cellules inflammatoires à l'état stable et en exacerbation. Enfin, il serait pertinent de confirmer que la suractivation de Rac au cours de l'asthme sévère concerne effectivement les polynucléaires éosinophiles ainsi que nous l'avons observé chez la souris.

Lors de cette étude, nos résultats *in vitro* montrent une activation de la GTPase Rac au cours de **la maturation des polynucléaires éosinophiles**. Il serait intéressant désormais de déterminer si cette activation de Rac est un acteur essentiel de la maturation éosinophilique ou bien seulement un marqueur. Il n'existe pas d'études l'ayant spécifiquement explorée. Toutefois, on note qu'une production d'éosinophiles est conservée lors de l'extinction complète de l'expression de Rac2 dans la souche de souris transgénique CD2-IL-5 (418). Dans cette étude, la conservation de l'expression de Rac1 ainsi que la surproduction d'IL-5 sont toutefois des biais importants. Par ailleurs, il n'a pas été décrit dans les modèles d'extinction combinée de Rac1 et de Rac2 de défaut de production des polynucléaires éosinophiles. Cependant, celle-ci n'a pas été évaluée dans des modèles inflammatoires favorisant la production d'éosinophiles. Mes expérimentations préliminaires montrent que l'inhibition de Rac (A.41) au cours de la différenciation éosinophilique entraînerait une apoptose des cellules. L'extinction sélective dans la lignée éosinophilique de Rac1 et Rac2 dans un modèle murin d'asthme permettrait d'explorer le rôle de ces 2 isoformes sur la maturation éosinophilique *in vivo* et en condition inflammatoire.

La production médullaire de polynucléaires éosinophiles dépend de l'**IL-5**, du **GM-CSF** et de l'**IL-33** (199, 423, 424). Bien qu'aucune de ces cytokines n'ait été décrite comme activant directement Rac, des études antérieures permettent de l'envisager. En effet, le récepteur de l'IL-5 phosphoryle la protéine Vav, GEF de Rac, qui active les voies d'aval JAK-STAT, MAPK, PI3K/Erk et NFκB (425, 426). Les données concernant le GM-CSF sont équivoques. En effet, le GM-CSF active Rac au sein de lignées cellulaires mastocytaires (409). Un dominant négatif de Rac réduit par ailleurs la prolifération de lignées cellulaires fibroblastiques en réponse au GM-CSF (427). En revanche, la stimulation de polynucléaires neutrophiles humains par le GM-CSF n'entraîne pas d'activation de Rac1 (428). Une analyse phospho-protéomique d'une lignée cellulaire macrophagique stimulée à l'IL-33 met en évidence la phosphorylation de TIAM1, GEF de Rac, et l'activation de la voie PAK1 (429). Toutefois, l'impact de cette voie dépendante de l'IL-33 sur les fonctions macrophagiques (phagocytose, migration, polarisation M1/M2) n'a pas été explorée. L'activation de Rac par ces différents récepteurs pouvant être spécifique du type cellulaire, il serait valable de les explorer dans les polynucléaires éosinophiles.

Nos données montrent que l'inhibition de Rac réduit significativement **les capacités de dégranulation** des polynucléaires éosinophiles *in vitro*. Une autre équipe a démontré que l'inhibition pharmacologique de Rac par l'EHT1864 réduit la sécrétion de RNase par les éosinophiles murins en réponse à une stimulation par l'éotaxine (430). En l'absence d'expression de Rac2, les éosinophiles issus d'une souche de souris transgéniques CD2-IL-5 présentent une réduction de la dégranulation induites par le PAF (418). Ces études démontrent que ces altérations sont secondaires à une dérégulation du cytosquelette d'actine. Les polynucléaires éosinophiles exprimant à la fois Rac1 et Rac2, la question du rôle spécifique de chacune de ces isoformes se pose. Pour le mastocyte, la dégranulation fait intervenir des formations membranaires (ruffles) dépendantes de l'activité de Rac1 ainsi que des influx de Ca<sup>2+</sup> régulés par Rac2 (421). Rac2 joue aussi un rôle dans le relargage des granules primaires des polynucléaires neutrophiles sans que l'implication de Rac1 ne soit connue (431). A l'heure actuelle, les inhibiteurs pharmacologiques de Rac disponibles ne sont pas spécifiques d'une isoforme particulière. Le recours à des extinctions sélectives de celles-ci constituerait une approche pertinente pour évaluer

leur rôle spécifique dans la dégranulation de l'éosinophile. On pourrait aussi explorer avec ces modèles d'autres formes de dégranulation telle que l'exocytose classique ou composée (compound exocytosis). En effet, le PAF et l'éotaxine sont décrits pour provoquer une dégranulation fragmentaire (432). Enfin, la découverte des activateurs de Rac impliqués dans la dégranulation éosinophilique pourrait apporter de nouvelles pistes physiopathologiques. Il n'existe pas de données concernant l'activation de GEF de Rac au décours d'une stimulation de cellules immunitaires par du PAF ou de l'éotaxine. Néanmoins, la stimulation de cellules endothéliales par le PAF est décrite pour activer la voie TIAM1-Rac1 qui module le cytosquelette d'actine (433). L'exploration de cette voie lors de la stimulation de la dégranulation de l'éosinophile pourrait être une piste intéressante.

Nous n'avons pas mis en évidence d'élévation de l'activation de Rac dans **les lymphocytes T et B** ni d'impact sur **les cytokines inflammatoires** d'un inhibiteur de Rac dans notre modèle murin d'asthme. Or, des études ont montré que Rac joue des rôles dans la réponse adaptative, de la migration cellulaire, à la présentation antigénique jusqu'à la prolifération et la polarisation lymphocytaire. En effet, la migration des lymphocytes T vers les ganglions lymphatiques dépend des Rac-GTPases (434, 435). Par ailleurs, l'extinction d'expression de Rac1 et de Rac2 diminue l'efficacité de présentation antigénique des cellules dendritiques aux lymphocytes T (399). Ensuite, la stimulation du TCR et l'induction de la prolifération lymphocytaire T par l'IL-2 font intervenir les voies DOCK2-Rac et Vav-Rac qui active les voies JAK/STAT, PI3K et MAPK aboutissant à l'exécution des programmes de transcription AP-1, NFAT et NFκB (344, 407, 408) (436-440). Sur le versant lymphocytaire B, la stimulation du BCR par un antigène entraîne l'activation de la voie Rac et de la PI3K (409). Au décours de l'activation des récepteurs spécifiques d'antigènes, la polarisation lymphocytaire T helper peut faire intervenir des voies Rac-dépendantes. Ainsi, l'activation de la voie TIAM1-Rac1 est décrite pour activer le facteur de transcription RORγT nécessaire au développement des lymphocytes Th17 (406). De plus, l'expression de TIAM1 par les lymphocytes T naïfs augmente en réponse à l'IL-6, cytokine impliquée dans la différenciation Th17 (441). Concernant la voie Th1, bien que la délétion de Rac2 réduise la production d'IFNγ *in vitro*, le développement de cette réponse *in vivo* n'est pas significativement altérée dans le cadre d'une infection

à *L. major* (442, 443). Enfin, la voie DOCK2-Rac régule la différenciation plasmocytaire et la production d'IgG *in vitro* et *in vivo* (411).

**L'utilisation de la voie inhalée** dans notre modèle a pu limiter les effets de l'inhibition de Rac à la sphère pulmonaire et donc l'impact sur la production médullaire des lymphocytes. De plus, on peut émettre l'hypothèse que la concentration d'inhibiteur de Rac au sein des ganglions lymphatiques ne soit pas suffisante pour limiter l'activation lymphocytaires. Nous pouvons aussi supposer que l'inhibition de Rac n'ait pas impacté les populations d'ILC tissulaires, sources potentielles d'IL-4, d'IL-5, d'IL-13 et d'IL-17. L'étude quantitative et qualitative (production de cytokines) des populations d'ILC et de lymphocytes T dans les tissus et les ganglions chez la souris pourrait fournir des pistes d'explication potentielles. Par ailleurs, si les lymphocytes B ne voit pas leur activité de Rac augmenter dans le cadre de notre modèle murin, il serait intéressant d'évaluer si l'inhibition de Rac réduit la différenciation plasmocytaire et a un impact sur la réponse humorale (production d'IgE spécifiques).

Mes résultats de thèse et les données de la littérature suggèrent que le ciblage des voies de signalisation dépendante de Rac constituerait **une nouvelle stratégie thérapeutique innovante** pour la prise en charge de l'asthme sévère.

Bien que les connaissances fondamentales se soient améliorées sur les 20 dernières années, peu de stratégies innovantes ciblant **le muscle lisse bronchique** sont actuellement disponibles (55). Seuls les bronchodilatateurs de courte ou de longue durée d'action sont communément accessibles. Le recours à la thermoplastie bronchique est réservé à une proportion limitée de patients et ne dispose pas de biomarqueur prédictif fiable (444). De plus, certains manques thérapeutiques persistent malgré l'arrivée des biothérapies. Tout d'abord, celles-ci ciblent principalement les asthmes T2 laissant **les formes non T2 orphelines** de stratégies innovantes. Si le tezepelumab a prouvé en phase III son efficacité chez des patients asthmatiques sévères avec des marqueurs T2 bas, il conviendra de confirmer ces observations dans le cadre d'essais pragmatiques. Par ailleurs, malgré l'utilisation de biomarqueurs, il persiste une faible proportion (15-25 %) de patients **ne répondant pas ou peu aux biothérapies** ciblant l'inflammation de type 2 (445-448). Les mécanismes de ces échecs de réponse restent méconnus. Une autre limite actuelle des biothérapies est liée **à leur caractère suspensif** posant la question de leur durée

d'utilisation (449, 450). Concernant l'arrêt des biothérapies après traitement prolongé, seul l'omalizumab dispose de données disponibles montrant un maintien du contrôle de la maladie au décours de l'arrêt (6 ans de traitement, n=16) (451). Ces résultats font actuellement l'objet d'une étude internationale de confirmation (SHORTEN, NCT NCT04763447).

Dans ce cadre, l'inhibition de Rac présenterait l'avantage de lutter **contre l'hyper-réactivité et le remodelage bronchique** mais aussi **contre l'inflammation éosinophilique et neutrophilique** (386, 389). Pour étayer la pertinence du développement d'inhibiteur des voies de signalisation dépendante de Rac, il serait intéressant d'évaluer l'activation de Rac chez des patients présentant des endotypes différents. Parallèlement, l'étude des interactions entre la corticothérapie et l'activation de Rac au sein des populations inflammatoires constituerait un axe de recherche important. En effet, nous pouvons supposer que l'activation de Rac au sein des cellules inflammatoires puisse diminuer leur sensibilité aux corticoïdes (promotion de la prolifération et de l'activation). Cette hypothèse pourrait être explorée par la comparaison des niveaux d'activation de Rac entre des patients asthmatiques modérés et sévères et tenant compte de leurs traitements corticoïdes. L'étude de l'impact *in vitro* des corticoïdes sur l'activation de Rac au sein des éosinophiles de sujet sain et de patients asthmatiques sévères compléterait cette approche. Enfin, au vu du caractère ubiquitaire de l'expression de Rac1 et hématopoïétique de Rac2, il serait essentiel d'évaluer leur activité dans d'autres pathologies bronchiques à valence inflammatoire telles que la BPCO, la mucoviscidose ou les dilatations des bronches.

Bien que cibler directement Rac puisse sembler prometteur, **certaines limites** doivent être envisagées. C'est le cas du risque infectieux notamment respiratoire. L'administration d'inhibiteur de Rac par voie systémique semblerait risquée du fait de **son rôle dans la production médullaire** de populations immunitaires. L'activation de Rac est en effet essentielle à la greffe médullaire, à la prolifération et à la survie des CSH (394, 452, 453). Son absence réduit la production des lymphocytes T et des lymphocytes B (blocage de maturation) (396, 405, 454). Néanmoins, aucun déficit de production des éosinophiles, des basophiles, des monocyte/macrophages et des mastocytes n'a été décrit chez la souris en l'absence de Rac. De plus, le recours à la voie inhalée pourrait constituer une stratégie pertinente pour limiter ces effets

secondaires. Nos résultats préliminaires n'ont pas retrouvé de réduction significative des leucocytes circulants. L'inhibition de Rac pourrait aussi réduire **l'efficacité anti-infectieuse** des cellules effectrices. Nous avons montré dans notre modèle murin que l'inhibition de Rac réduit l'infiltration bronchique neutrophilique. Or, la caractérisation des souris KO Rac2 retrouve une réduction significative des capacités chimiotactiques des polynucléaires neutrophiles avec susceptibilité aux infections fongiques invasives à *Aspergillus fumigatus* (336). Par ailleurs, l'extinction de l'expression de Rac2 au sein des polynucléaires neutrophiles réduit leurs capacités de production de radicaux libres (397). Chez l'Homme, une mutation constitutionnelle de Rac2 a été mise en évidence chez un patient souffrant d'infection récurrente liée à des déficits fonctionnels neutrophiliques (383). Toutefois, ces observations ont été réalisées dans des contextes d'extinction d'expression d'isoforme de Rac ou de mutations constitutionnelles. Une approche de réduction de l'activation de Rac par voie pharmacologique pourrait limiter l'importance de ces effets secondaires. Par ailleurs, elle offre l'avantage d'être réversible avec un arrêt potentiel en cas d'infection.

La recherche des **modulateurs de l'activation de Rac** (GEF, GAP et GDI) pourrait constituer une approche pertinente pour limiter les effets indésirables. Réduire l'activation des GEF serait une voie potentielle pour limiter l'activation des polynucléaires éosinophiles. Contrairement à l'expression ubiquitaire de Rac, les RacGEFs peuvent présenter une expression spécifique aux tissus et aux cellules. De plus, en fonction des signaux d'activation (facteurs solubles ou mécaniques), le ou les RacGEFs engagés pour activer Rac peuvent être spécifiques de fonction. La découverte des RacGEFs activés dans l'asthme pourrait fournir des cibles pharmacologiques supplémentaires permettant d'inhiber spécifiquement les voies de signalisation Rac-dépendantes au sein des cellules inflammatoires bronchiques.

De nombreux modulateurs de Rac peuvent être suspectés. **Les GEF de la famille Vav** ont été décrits comme activés dans plusieurs mécanismes immunitaires. Vav a été associée dans le cadre de modèle KO à la fois à la sélection négative mais aussi positive des thymocytes durant la maturation lymphocytaire T (455, 456). Son absence réduit aussi l'activation lymphocytaire T et B secondaire à la stimulation du TCR et du BCR respectivement (437, 457). Elle est impliquée dans la production d'IL-6 par les mastocytes et dans le chimiotactisme et la production de radicaux libres par les

neutrophiles (458-460). Elle est aussi décrite comme faisant partie de la voie de signalisation de l'IL-5 (425, 426). L'absence d'expression du GEF SWAP-70 réduit les capacités de migration des éosinophiles dans un modèle murin d'asthme par altération du cytosquelette d'actine (461). SWAP-70 est aussi nécessaire à la dégranulation mastocytaire médiée par IgE (462). Au vu de leurs implications dans les réponses lymphocytaires et neutrophiliques, les protéines TIAM et DOCK pourraient aussi constituer des candidats potentiels activant Rac au sein des éosinophiles (406, 459, 463). Enfin, on pourrait envisager des stratégies d'augmentation de l'activation des GAP comme Abr, dont la délétion est associée à une augmentation de la sévérité d'un modèle murin d'asthme (392).

**Au total**, l'exploration des voies de signalisation dépendante de Rac et impliquées dans l'inflammation éosinophilique et l'altération du phénotype des CML pourrait fournir de nouvelles pistes thérapeutiques pour la prise en charge de l'asthme sévère. L'identification des activateurs spécifiques de Rac et de ces fonctions constitue une piste de recherche à privilégier.

## **IV. Perspectives et retombées attendues**

Mes travaux de thèse seront poursuivis par l'exploration des voies de signalisation dépendantes de Rac impliquées spécifiquement **dans la maturation et la dégranulation éosinophilique**. Ce projet de recherche a pour objectifs principaux de confirmer le rôle de Rac dans la différenciation et l'activité de dégranulation du polynucléaire éosinophile, d'identifier les activateurs de Rac (GEF) impliqués dans la maturation et la fonction de dégranulation du polynucléaire éosinophile et de valider *in vivo* le rôle de Rac et de ses GEFs dans la maturation et les fonctions du polynucléaire éosinophile dans un contexte d'asthme sévère.

Nous émettons l'hypothèse que **les isoformes Rac1 et Rac2** pourraient avoir des rôles **différents mais complémentaires** durant *la différenciation et la dégranulation éosinophilique*. Pour explorer cette hypothèse, nous évaluerons en culture cellulaire les effets de l'extinction spécifique de ces isoformes par une approche de siRNA sur la proportion d'éosinophiles différenciés et matures (cytométrie en flux). Ces analyses seront complétées par l'étude de l'expression génique des enzymes et cytokines contenues dans les granules de l'éosinophile au cours de la culture. Par approche enzymatique, biochimique (ELISA) et d'immunofluorescence (marquage actine et CD63) nous estimerons les rôles spécifiques des isoformes Rac1 et Rac2 durant la dégranulation induite par le PAF et l'éotaxine. Pour évaluer si les résultats obtenus sont liés au niveau d'activité de Rac, ces protocoles de différenciation et de dégranulation des éosinophiles seront également réalisés en présence d'un inhibiteur pharmacologiques de Rac.

Au vu des connaissances actuelles, nous pouvons supposer que l'activation de Rac au sein des éosinophiles puisse être liée à l'activation de **GEF spécifiques**. Par des approches biochimiques et protéomiques (pull-down, nano LC-HRMS), nous identifierons *in vitro* les GEFs de Rac activés dans les polynucléaires éosinophiles durant leur maturation et la différenciation. Pour valider la pertinence de ces résultats, nous éteindrions ensuite, par siRNA, l'expression des RacGEFs identifiées pour confirmer le(s) rôle(s) de ces protéines dans les polynucléaires éosinophiles (maturation, polarisation, production d'interleukines et de cytokines) comme décrit précédemment.



Afin de confirmer la pertinence de nos résultats **dans le développement de l'asthme**, nous évaluerons l'activité de Rac et l'expression des GEF identifiés in vitro dans notre modèle murin d'asthme allergique sévère aux acariens. Nous quantifierons l'activité de Rac et de ses GEF au sein des progéniteurs myéloïdes médullaires, des polynucléaires éosinophiles circulants et tissulaires chez la souris. Afin d'évaluer la pertinence de Rac chez l'Homme dans le cadre de l'asthme sévère, nous utiliserons les biopsies bronchiques issues du protocole NARACAS (NCT03325088) mené actuellement au sein du service de pneumologie du CHU de Nantes. Par approche d'immunofluorescence multiplex (OPAL), nous évaluerons la proportion de polynucléaires éosinophiles infiltrants activant Rac ainsi que l'intensité de ce signal. Conjointement, nous effectuerons des calculs de corrélation des signaux Rac-GTP avec l'expression des différents contenus granulaires (EPX, EDN, ECP et cristaux de Charcot-Leyden).

**La réalisation de ce programme de recherche** permettra d'identifier le rôle de la protéine Rac dans la maturation et les fonctions de dégranulation du polynucléaire éosinophile dans le cadre de l'asthme sévère. Le décryptage des voies de signalisation impliquées dans ces fonctions permettra d'apporter de nouvelles connaissances sur les mécanismes moléculaires qui régissent la biologie de l'éosinophile. Dans le cas de figure où l'activation de Rac serait effectivement essentielle aux fonctions effectrices de l'éosinophile dans l'asthme, l'identification de ces activateurs (GEF) pourrait constituer des cibles thérapeutiques d'intérêt.

Au total, l'amélioration des connaissances concernant la signalisation intracellulaire sous-tendant les mécanismes inflammatoires de l'asthme éosinophilique pourrait aboutir au développement de **nouvelles classes thérapeutiques innovantes**.

## V. Bibliographie

1. Reddel HK, Bateman ED, Becker A, Boulet LP, Cruz AA, Drazen JM, Haahtela T, Hurd SS, Inoue H, de Jongste JC, Lemanske RF, Jr., Levy ML, O'Byrne PM, Paggiaro P, Pedersen SE, Pizzichini E, Soto-Quiroz M, Szeffler SJ, Wong GW, FitzGerald JM. A summary of the new GINA strategy: a roadmap to asthma control. *Eur Respir J* 2015; 46: 622-639.
2. Raheison-Semjen C, Guilleminault L, Billiard I, Chenivresse C, De Oliveira A, Izadifar A, Lorenzo A, Nocent C, Oster JP, Padovani M, Perez T, Russier M, Steinecker M, Didier A. [Update of the 2021 recommendations for the management and follow-up of adult asthmatic patients under the guidance of the French Society of Pulmonology and the Paediatric Society of Pulmonology and Allergology. Long version]. *Rev Mal Respir* 2021; 38: 1048-1083.
3. Gaillard EA, Kuehni CE, Turner S, Goutaki M, Holden KA, de Jong CCM, Lex C, Lo DKH, Lucas JS, Midulla F, Mozun R, Piacentini G, Rigau D, Rottier B, Thomas M, Tonia T, Usemann J, Yilmaz O, Zacharasiewicz A, Moeller A. European Respiratory Society clinical practice guidelines for the diagnosis of asthma in children aged 5-16 years. *Eur Respir J* 2021; 58.
4. Reddel HK, Bacharier LB, Bateman ED, Brightling CE, Brusselle GG, Buhl R, Cruz AA, Duijts L, Drazen JM, Fitzgerald JM, Fleming LJ, Inoue H, Ko FW, Krishnan JA, Levy ML, Lin J, Mortimer K, Pitrez PM, Sheikh A, Yorgancioglu AA, Boulet L-P. Global Initiative for Asthma Strategy 2021: executive summary and rationale for key changes. *European Respiratory Journal* 2022; 59: 2102730.
5. Deschildre A, Abou Taam R, Drummond D, Giovannini-Chami L, Labouret G, Lejeune S, Lezmi G, Lecam MT, Marguet C, Petat H, Taille C, Wanin S, Corvol H, Epaud R. [Update guidelines for management of asthmatic patients (from 12 years and older). Short version]. *Rev Mal Respir* 2022; 39: 179-187.
6. Société pédiatrique de pneumologie et d'allergologie (SP2A). Asthme de l'enfant de moins de 36 mois : diagnostic, prise en charge et traitement en dehors des épisodes aigus: Haute autorité de santé; 2009.
7. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention; 2022.
8. Pakkasela J, Ilmarinen P, Honkamaki J, Tuomisto LE, Andersen H, Piirila P, Hisinger-Molkanen H, Sovijarvi A, Backman H, Lundback B, Ronmark E, Kankaanranta H, Lehtimaki L. Age-specific incidence of allergic and non-allergic asthma. *BMC Pulm Med* 2020; 20: 9.
9. European Respiratory Society. Childhood Asthma. The European Lung - White Book. p. 126-137.
10. Parameswaran K, Belda J, Sears MR. Use of peak flow variability and methacholine responsiveness in predicting changes from pre-test diagnosis of asthma. *Eur Respir J* 1999; 14: 1358-1362.
11. Graham BL, Steenbruggen I, Miller MR, Barjaktarevic IZ, Cooper BG, Hall GL, Hallstrand TS, Kaminsky DA, McCarthy K, McCormack MC, Oropez CE, Rosenfeld M, Stanojevic S, Swanney MP, Thompson BR. Standardization of Spirometry 2019 Update. An Official American Thoracic Society and European Respiratory Society Technical Statement. *Am J Respir Crit Care Med* 2019; 200: e70-e88.

12. Société de Pneumologie de Langue Française. Recommandations pour la pratique clinique concernant les explorations fonctionnelles respiratoires 2008-2010; 2011.
13. Coates AL, Wanger J, Cockcroft DW, Culver BH, Bronchoprovocation Testing Task Force: Kai-Hakon C, Diamant Z, Gauvreau G, Hall GL, Hallstrand TS, Horvath I, de Jongh FHC, Joos G, Kaminsky DA, Laube BL, Leuppi JD, Sterk PJ. ERS technical standard on bronchial challenge testing: general considerations and performance of methacholine challenge tests. *Eur Respir J* 2017; 49.
14. Hallstrand TS, Leuppi JD, Joos G, Hall GL, Carlsen KH, Kaminsky DA, Coates AL, Cockcroft DW, Culver BH, Diamant Z, Gauvreau GM, Horvath I, de Jongh FHC, Laube BL, Sterk PJ, Wanger J, American Thoracic Society /European Respiratory Society Bronchoprovocation Testing Task F. ERS technical standard on bronchial challenge testing: pathophysiology and methodology of indirect airway challenge testing. *Eur Respir J* 2018; 52.
15. Sumino K, Sugar EA, Irvin CG, Kaminsky DA, Shade D, Wei CY, Holbrook JT, Wise RA, Castro M, American Lung Association Asthma Clinical Research C. Methacholine challenge test: diagnostic characteristics in asthmatic patients receiving controller medications. *J Allergy Clin Immunol* 2012; 130: 69-75 e66.
16. Anderson SD, Charlton B, Weiler JM, Nichols S, Spector SL, Pearlman DS, Group AS. Comparison of mannitol and methacholine to predict exercise-induced bronchoconstriction and a clinical diagnosis of asthma. *Respir Res* 2009; 10: 4.
17. Aaron SD, Vandemheen KL, FitzGerald JM, Ainslie M, Gupta S, Lemiere C, Field SK, McIvor RA, Hernandez P, Mayers I, Mulpuru S, Alvarez GG, Pakhale S, Mallick R, Boulet LP, Canadian Respiratory Research N. Reevaluation of Diagnosis in Adults With Physician-Diagnosed Asthma. *JAMA* 2017; 317: 269-279.
18. Delmas M-C, Bénézet L, Ribet C, Iwatsubo Y, Zins M, Nadif R, Roche N, Leynaert B. Underdiagnosis of obstructive lung disease: findings from the French CONSTANCES cohort. *BMC Pulmonary Medicine* 2021; 21.
19. Annesi-Maesano I, Sterlin C, Caillaud D, De Blay F, Lavaud F, Charpin D, Raheerisson C. Factors related to under-diagnosis and under-treatment of childhood asthma in metropolitan France. *Multidisciplinary Respiratory Medicine* 2012; 7: 24.
20. Shaw D, Green R, Berry M, Mellor S, Hargadon B, Shelley M, Mckenna S, Thomas M, Pavord I. A cross-sectional study of patterns of airway dysfunction, symptoms and morbidity in primary care asthma. *Primary Care Respiratory Journal* 2012; 21: 283-287.
21. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, Adcock IM, Bateman ED, Bel EH, Bleecker ER, Boulet LP, Brightling C, Chanez P, Dahlen SE, Djukanovic R, Frey U, Gaga M, Gibson P, Hamid Q, Jajour NN, Mauad T, Sorkness RL, Teague WG. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J* 2014; 43: 343-373.
22. Safiri S, Carson-Chahhoud K, Karamzad N, Sullman MJM, Nejadghaderi SA, Taghizadieh A, Bell AW, Kolahi AA, Ansarin K, Mansournia MA, Collins GS, Kaufman JS. Prevalence, Deaths, and Disability-Adjusted Life-Years Due to Asthma and Its Attributable Risk Factors in 204 Countries and Territories, 1990-2019. *Chest* 2022; 161: 318-329.
23. Vos T, Lim SS, Abbafati C, Abbas KM, Abbasi M, Abbasifard M, Abbasi-Kangevari M, Abbastabar H, Abd-Allah F, Abdelalim A, Abdollahi M, Abdollahpour I, Abolhassani H, Aboyans V, Abrams EM, Abreu LG, Abrigo MRM, Abu-Raddad LJ, Abushouk AI, Acebedo A, Ackerman IN, Adabi M, Adamu AA, Adebayo OM, Adekanmbi V, Adelson JD, Adetokunboh OO, Adham D, Afshari M, Afshin A, Agardh EE, Agarwal G, Agesa

KM, Aghaali M, Aghamir SMK, Agrawal A, Ahmad T, Ahmadi A, Ahmadi M, Ahmadi H, Ahmadpour E, Akalu TY, Akinyemi RO, Akinyemiju T, Akombi B, Al-Aly Z, Alam K, Alam N, Alam S, Alam T, Alanzi TM, Albertson SB, Alcalde-Rabanal JE, Alema NM, Ali M, Ali S, Alicandro G, Alijanzadeh M, Alinia C, Alipour V, Aljunid SM, Alla F, Allebeck P, Almasi-Hashiani A, Alonso J, Al-Raddadi RM, Altirkawi KA, Alvis-Guzman N, Alvis-Zakzuk NJ, Amini S, Amini-Rarani M, Aminorroaya A, Amiri F, Amit AML, Amugsi DA, Amul GGH, Anderlini D, Andrei CL, Andrei T, Anjomshoa M, Ansari F, Ansari I, Ansari-Moghaddam A, Antonio CAT, Antony CM, Antriyandarti E, Anvari D, Anwer R, Arabloo J, Arab-Zozani M, Aravkin AY, Ariani F, Ärnlov J, Aryal KK, Arzani A, Asadi-Aliabadi M, Asadi-Pooya AA, Asghari B, Ashbaugh C, Atnafu DD, Atre SR, Ausloos F, Ausloos M, Ayala Quintanilla BP, Ayano G, Ayanore MA, Aynalem YA, Azari S, Azarian G, Azene ZN, Babae E, Badawi A, Bagherzadeh M, Bakhshaei MH, Bakhtiari A, Balakrishnan S, Balalla S, Balassyano S, Banach M, Banik PC, Bannick MS, Bante AB, Baraki AG, Barboza MA, Barker-Collo SL, Barthelemy CM, Barua L, Barzegar A, Basu S, Baune BT, Bayati M, Bazmandegan G, Bedi N, Beghi E, Béjot Y, Bello AK, Bender RG, Bennett DA, Bennitt FB, Bensenor IM, Benziger CP, Berhe K, Bernabe E, Bertolacci GJ, Bhageerathy R, Bhala N, Bhandari D, Bhardwaj P, Bhattacharyya K, Bhutta ZA, Bibi S, Biehl MH, Bikbov B, Bin Sayeed MS, Biondi A, Biriha BM, Bisanzio D, Bisignano C, Biswas RK, Bohlouli S, Bohluli M, Bolla SRR, Bolor A, Boon-Dooley AS, Borges G, Borzi AM, Bourne R, Brady OJ, Brauer M, Brayne C, Breitborde NJK, Brenner H, Briant PS, Briggs AM, Briko NI, Britton GB, Bryazka D, Buchbinder R, Bumgarner BR, Busse R, Butt ZA, Caetano Dos Santos FL, Cámara LLA, Campos-Nonato IR, Car J, Cárdenas R, Carreras G, Carrero JJ, Carvalho F, Castaldelli-Maia JM, Castañeda-Orjuela CA, Castelpietra G, Castle CD, Castro F, Catalá-López F, Causey K, Cederroth CR, Cercy KM, Cerin E, Chandan JS, Chang AR, Charlson FJ, Chattu VK, Chaturvedi S, Chimed-Ochir O, Chin KL, Cho DY, Christensen H, Chu D-T, Chung MT, Cicuttini FM, Ciobanu LG, Cirillo M, Collins EL, Compton K, Conti S, Cortesi PA, Costa VM, Cousin E, Cowden RG, Cowie BC, Cromwell EA, Cross DH, Crowe CS, Cruz JA, Cunningham M, Dahlawi SMA, Damiani G, Dandona L, Dandona R, Darwesh AM, Daryani A, Das JK, Das Gupta R, Das Neves J, Dávila-Cervantes CA, Davletov K, De Leo D, Dean FE, Decléene NK, Deen A, Degenhardt L, Dellavalle RP, Demeke FM, Demsie DG, Denova-Gutiérrez E, Dereje ND, Derveniz N, Desai R, Desalew A, Dessie GA, Dharmaratne SD, Dhungana GP, Dianatinasab M, Diaz D, Dibaji Forooshani ZS, Dingels ZV, Dirac MA, Djalalinia S, Do HT, Dokova K, Dorostkar F, Doshi CP, Doshmangir L, Douiri A, Doxey MC, Driscoll TR, Dunachie SJ, Duncan BB, Duraes AR, Eagan AW, Ebrahimi Kalan M, Edvardsson D, Ehrlich JR, El Nahas N, El Sayed I, El Tantawi M, Elbarazi I, Elgendy IY, Elhabashy HR, El-Jaafary SI, Elyazar IR, Emamian MH, Emmons-Bell S, Erskine HE, Eshrati B, Eskandarieh S, Esmaeilnejad S, Esmaeilzadeh F, Esteghamati A, Estep K, Etemadi A, Etillo AE, Farahmand M, Faraj A, Fareed M, Faridnia R, Farinha CSES, Farioli A, Faro A, Faruque M, Farzadfar F, Fattahi N, Fazlzadeh M, Feigin VL, Feldman R, Fereshtehnejad S-M, Fernandes E, Ferrari AJ, Ferreira ML, Filip I, Fischer F, Fisher JL, Fitzgerald R, Flohr C, Flor LS, Foigt NA, Folayan MO, Force LM, Fornari C, Foroutan M, Fox JT, Freitas M, Fu W, Fukumoto T, Furtado JM, Gad MM, Gakidou E, Galles NC, Gallus S, Gamkrelidze A, Garcia-Basteiro AL, Gardner WM, Geberemariam BS, Gebrehiwot AM, Gebremedhin KB, Gebreslassie AAAA, Gershberg Hayoon A, Gething PW, Ghadimi M, Ghadiri K, Ghafourifard M, Ghajar A, Ghamari F, Ghashghaee A, Ghasvand H, Ghith N, Gholamian A, Gilani SA, Gill PS, Gitimoghaddam M, Giussani G, Goli S, Gomez RS, Gopalani SV, Gorini G, Gorman

TM, Gottlich HC, Goudarzi H, Goulart AC, Goulart BNG, Grada A, Grivna M, Grosso G, Gubari MIM, Gugnani HC, Guimaraes ALS, Guimarães RA, Guled RA, Guo G, Guo Y, Gupta R, Haagsma JA, Haddock B, Hafezi-Nejad N, Hafiz A, Hagins H, Haile LM, Hall BJ, Halvaei I, Hamadeh RR, Hamagharib Abdullah K, Hamilton EB, Han C, Han H, Hankey GJ, Haro JM, Harvey JD, Hasaballah AI, Hasanzadeh A, Hashemian M, Hassanipour S, Hassankhani H, Havmoeller RJ, Hay RJ, Hay SI, Hayat K, Heidari B, Heidari G, Heidari-Soureshjani R, Hendrie D, Henrikson HJ, Henry NJ, Herteliu C, Heydarpour F, Hird TR, Hoek HW, Hole MK, Holla R, Hoogar P, Hosgood HD, Hosseinzadeh M, Hostiuc M, Hostiuc S, Househ M, Hoy DG, Hsairi M, Hsieh VC-R, Hu G, Huda TM, Hugo FN, Huynh CK, Hwang B-F, Iannucci VC, Ibitoye SE, Ikuta KS, Ilesanmi OS, Ilic IM, Ilic MD, Inbaraj LR, Ippolito H, Irvani SSN, Islam MM, Islam M, Islam SMS, Islami F, Iso H, Ivers RQ, Iwu CCD, Iyamu IO, Jaafari J, Jacobsen KH, Jadidi-Niaragh F, Jafari H, Jafarinia M, Jahagirdar D, Jahani MA, Jahanmehr N, Jakovljevic M, Jalali A, Jalilian F, James SL, Janjani H, Janodia MD, Jayatilleke AU, Jeemon P, Jenabi E, Jha RP, Jha V, Ji JS, Jia P, John O, John-Akinola YO, Johnson CO, Johnson SC, Jonas JB, Joo T, Joshi A, Jozwiak JJ, Jürisson M, Kabir A, Kabir Z, Kalani H, Kalani R, Kalankesh LR, Kalhor R, Kamiab Z, Kanchan T, Karami Matin B, Karch A, Karim MA, Karimi SE, Kassa GM, Kassebaum NJ, Katikireddi SV, Kawakami N, Kayode GA, Keddie SH, Keller C, Kereselidze M, Khafaie MA, Khalid N, Khan M, Khatab K, Khater MM, Khatib MN, Khayamzadeh M, Khodayari MT, Khundkar R, Kianipour N, Kieling C, Kim D, Kim Y-E, Kim YJ, Kimokoti RW, Kisa A, Kisa S, Kissimova-Skarbek K, Kivimäki M, Kneib CJ, Knudsen AKS, Kocarnik JM, Kolola T, Kopec JA, Kosen S, Koul PA, Koyanagi A, Kravchenko MA, Krishan K, Krohn KJ, Kuate Defo B, Kucuk Bicer B, Kumar GA, Kumar M, Kumar P, Kumar V, Kumares G, Kurmi OP, Kusuma D, Kyu HH, La Vecchia C, Lacey B, Lal DK, Laloo R, Lam JO, Lami FH, Landires I, Lang JJ, Lansingh VC, Larson SL, Larsson AO, Lasrado S, Lassi ZS, Lau KM-M, Lavados PM, Lazarus JV, Ledesma JR, Lee PH, Lee SWH, Legrand KE, Leigh J, Leonardi M, Lescinsky H, Leung J, Levi M, Lewington S, Li S, Lim L-L, Lin C, Lin R-T, Linehan C, Linn S, Liu H-C, Liu S, Liu Z, Looker KJ, Lopez AD, Lopukhov PD, Lorkowski S, Lotufo PA, Lucas TCD, Lugo A, Lunevicius R, Lyons RA, Ma J, Maclachlan JH, Maddison ER, Maddison R, Madotto F, Mahasha PW, Mai HT, Majeed A, Maled V, Maleki S, Malekzadeh R, Malta DC, Mamun AA, Manafi A, Manafi N, Manguerra H, Mansouri B, Mansournia MA, Mantilla Herrera AM, Maravilla JC, Marks A, Martins-Melo FR, Martopullo I, Masoumi SZ, Massano J, Massenburg BB, Mathur MR, Maulik PK, Mcalinden C, Mcgrath JJ, Mckee M, Mehndiratta MM, Mehri F, Mehta KM, Meitei WB, Memiah PTN, Mendoza W, Menezes RG, Mengesha EW, Mengesha MB, Mereke A, Meretoja A, Meretoja TJ, Mestrovic T, Miazgowski B, Miazgowski T, Michalek IM, Mihretie KM, Miller TR, Mills EJ, Mirica A, Mirrakhimov EM, Mirzaei H, Mirzaei M, Mirzaei-Alavijeh M, Misganaw AT, Mithra P, Moazen B, Moghadaszadeh M, Mohamadi E, Mohammad DK, Mohammad Y, Mohammad Gholi Mezerji N, Mohammadian-Hafshejani A, Mohammadifard N, Mohammadpourhodki R, Mohammed S, Mokdad AH, Molokhia M, Momen NC, Monasta L, Mondello S, Mooney MD, Moosazadeh M, Moradi G, Moradi M, Moradi-Lakeh M, Moradzadeh R, Moraga P, Morales L, Morawska L, Moreno Velásquez I, Morgado-Da-Costa J, Morrison SD, Mosser JF, Mouodi S, Mousavi SM, Mousavi Khaneghah A, Mueller UO, Munro SB, Muriithi MK, Musa KI, Muthupandian S, Naderi M, Nagarajan AJ, Nagel G, Naghshtabrizi B, Nair S, Nandi AK, Nangia V, Nansseu JR, Nayak VC, Nazari J, Negoï I, Negoï RI, Netsere HBN, Ngunjiri JW, Nguyen CT, Nguyen J, Nguyen M, Nguyen M, Nichols E, Nigatu D, Nigatu YT, Nikbakhsh R, Nixon MR, Nnaji CA, Nomura S, Norrving

B, Noubiap JJ, Nowak C, Nunez-Samudio V, Oțoiu A, Oancea B, Odell CM, Ogbo FA, Oh I-H, Okunga EW, Oladnabi M, Olagunju AT, Olusanya BO, Olusanya JO, Oluwasanu MM, Omar Bali A, Omer MO, Ong KL, Onwujekwe OE, Orji AU, Orpana HM, Ortiz A, Ostroff SM, Otstavnov N, Otstavnov SS, Øverland S, Owolabi MO, P A M, Padubidri JR, Pakhare AP, Palladino R, Pana A, Panda-Jonas S, Pandey A, Park E-K, Parmar PGK, Pasupula DK, Patel SK, Paternina-Caicedo AJ, Pathak A, Pathak M, Patten SB, Patton GC, Paudel D, Pazoki Toroudi H, Peden AE, Pennini A, Pepito VCF, Peprah EK, Pereira A, Pereira DM, Perico N, Pham HQ, Phillips MR, Pigott DM, Pilgrim T, Pilz TM, Pirsaeheb M, Plana-Ripoll O, Plass D, Pokhrel KN, Polibin RV, Polinder S, Polkinghorne KR, Postma MJ, Pourjafar H, Pourmalek F, Pourmirza Kalhori R, Pourshams A, Poznańska A, Prada SI, Prakash V, Pribadi DRA, Pupillo E, Quazi Syed Z, Rabiee M, Rabiee N, Radfar A, Rafiee A, Rafiei A, Raggi A, Rahimi-Movaghar A, Rahman MA, Rajabpour-Sanati A, Rajati F, Ramezanzadeh K, Ranabhat CL, Rao PC, Rao SJ, Rasella D, Rastogi P, Rathi P, Rawaf DL, Rawaf S, Rawal L, Razo C, Redford SB, Reiner RC, Reinig N, Reitsma MB, Remuzzi G, Renjith V, Renzaho AMN, Resnikoff S, Rezaei N, Rezai MS, Rezapour A, Rhinehart P-A, Riahi SM, Ribeiro ALP, Ribeiro DC, Ribeiro D, Rickard J, Roberts NLS, Roberts S, Robinson SR, Roeber L, Rolfe S, Ronfani L, Roshandel G, Roth GA, Rubagotti E, Rumisha SF, Sabour S, Sachdev PS, Saddik B, Sadeghi E, Sadeghi M, Saeidi S, Safi S, Safiri S, Sagar R, Sahebkar A, Sahraian MA, Sajadi SM, Salahshoor MR, Salamati P, Salehi Zahabi S, Salem H, Salem MRR, Salimzadeh H, Salomon JA, Salz I, Samad Z, Samy AM, Sanabria J, Santomauro DF, Santos IS, Santos JV, Santric-Milicevic MM, Saraswathy SYI, Sarmiento-Suárez R, Sarrafzadegan N, Sartorius B, Sarveazad A, Sathian B, Sathish T, Sattin D, Sbarra AN, Schaeffer LE, Schiavolin S, Schmidt MI, Schutte AE, Schwebel DC, Schwendicke F, Senbeta AM, Senthilkumaran S, Sepanlou SG, Shackelford KA, Shadid J, Shahabi S, Shaheen AA, Shaikh MA, Shalash AS, Shams-Beyranvand M, Shamsizadeh M, Shannawaz M, Sharafi K, Sharara F, Sheena BS, Sheikhtaheri A, Shetty RS, Shibuya K, Shiferaw WS, Shigematsu M, Shin JI, Shiri R, Shirkoohi R, Shrimme MG, Shuval K, Siabani S, Sigfusdottir ID, Sigurvinsdottir R, Silva JP, Simpson KE, Singh A, Singh JA, Skiadaresi E, Skou STS, Skryabin VY, Sobngwi E, Sokhan A, Soltani S, Sorensen RJD, Soriano JB, Sorrie MB, Soyiri IN, Sreeramareddy CT, Stanaway JD, Stark BA, Ștefan SC, Stein C, Steiner C, Steiner TJ, Stokes MA, Stovner LJ, Stubbs JL, Sudaryanto A, Sufiyan MAB, Sulo G, Sultan I, Sykes BL, Sylte DO, Szócska M, Tabarés-Seisdedos R, Tabb KM, Tadakamadla SK, Taherkhani A, Tajdini M, Takahashi K, Taveira N, Teagle WL, Teame H, Tehrani-Banihashemi A, Teklehaimanot BF, Terrason S, Tessema ZT, Thankappan KR, Thomson AM, Tohidinik HR, Tonelli M, Topor-Madry R, Torre AE, Touvier M, Tovani-Palone MRR, Tran BX, Travillian R, Troeger CE, Truelsen TC, Tsai AC, Tsatsakis A, Tudor Car L, Tyrovolas S, Uddin R, Ullah S, Undurraga EA, Unnikrishnan B, Vacante M, Vakilian A, Valdez PR, Varughese S, Vasankari TJ, Vasseghian Y, Venketasubramanian N, Violante FS, Vlassov V, Vollset SE, Vongpradith A, Vukovic A, Vukovic R, Waheed Y, Walters MK, Wang J, Wang Y, Wang Y-P, Ward JL, Watson A, Wei J, Weintraub RG, Weiss DJ, Weiss J, Westerman R, Whisnant JL, Whiteford HA, Wiangkham T, Wiens KE, Wijeratne T, Wilner LB, Wilson S, Wojtyniak B, Wolfe CDA, Wool EE, Wu A-M, Wulf Hanson S, Wunrow HY, Xu G, Xu R, Yadgir S, Yahyazadeh Jabbari SH, Yamagishi K, Yaminfirooz M, Yano Y, Yaya S, Yazdi-Feyzabadi V, Yearwood JA, Yeheyis TY, Yeshitila YG, Yip P, Yonemoto N, Yoon S-J, Yoosefi Lebni J, Younis MZ, Younker TP, Yousefi Z, Yousefifard M, Yousefinezhadi T, Yousuf AY, Yu C, Yusefzadeh H, Zahirian Moghadam T, Zaki L, Zaman SB, Zamani

- M, Zamanian M, Zandian H, Zangeneh A, Zastrozhin MS, Zewdie KA, Zhang Y, Zhang Z-J, Zhao JT, Zhao Y, Zheng P, Zhou M, Ziapour A, Zimsen SRM, Naghavi M, Murray CJL. Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *The Lancet* 2020; 396: 1204-1222.
24. Beran D, Zar HJ, Perrin C, Menezes AM, Burney P. Burden of asthma and chronic obstructive pulmonary disease and access to essential medicines in low-income and middle-income countries. *The Lancet Respiratory Medicine* 2015; 3: 159-170.
25. Asher MI, Rutter CE, Bissell K, Chiang C-Y, El Sony A, Ellwood E, Ellwood P, García-Marcos L, Marks GB, Morales E, Mortimer K, Pérez-Fernández V, Robertson S, Silverwood RJ, Strachan DP, Pearce N, Bissell K, Chiang C-Y, Ellwood E, Ellwood P, Marks GB, Masekela R, Morales E, Mortimer K, Pearce N, Strachan D, Ellwood P, Ellwood E, Martínez-Torres A, Morales E, Pérez-Fernández V, Pearce N, Robertson S, Rutter C, Silverwood R, Strachan D, Mallol J, Soto-Martínez M, Cabrera Aguilar A, Douros K, Sabir M, Singh M, Singh V, Sukumaran TU, Awasthi S, Kabra SK, Salvi S, García-Almaráz R, Mérida-Palacio JV, Del Río Navarro BE, González-Díaz SN, Navarrete-Rodríguez EM, Sánchez JF, Falade AG, Zar HJ, López-Silvarrey Varela A, González Díaz C, Nour M, Dib G, Mohammad Y, Huang J-L, Chinratanapisit S, Soto-Quirós ME, Vichyanond P, Aguilar P, Barba S, Kumar L, Sharma SK, Linares-Zapién FJ, Onadeko BO, Musa OAA, Aguirre V, Baeza-Bacab M, Mohammad S, Cortez E, Gratiou CH, Chopra K, Hanumante NM, Nelson H, Rubio AD, Hsieh K-H, Shah J. Worldwide trends in the burden of asthma symptoms in school-aged children: Global Asthma Network Phase I cross-sectional study. *The Lancet* 2021; 398: 1569-1580.
26. Hekking PP, Wener RR, Amelink M, Zwinderman AH, Bouvy ML, Bel EH. The prevalence of severe refractory asthma. *J Allergy Clin Immunol* 2015; 135: 896-902.
27. Moore WC, Bleecker ER, Curran-Everett D, Erzurum SC, Ameredes BT, Bacharier L, Calhoun WJ, Castro M, Chung KF, Clark MP, Dweik RA, Fitzpatrick AM, Gaston B, Hew M, Hussain I, Jarjour NN, Israel E, Levy BD, Murphy JR, Peters SP, Teague WG, Meyers DA, Busse WW, Wenzel SE. Characterization of the severe asthma phenotype by the National Heart, Lung, and Blood Institute's Severe Asthma Research Program. *J Allergy Clin Immunol* 2007; 119: 405-413.
28. Lane S, Molina J, Plusa T. An international observational prospective study to determine the cost of asthma exacerbations (COAX). *Respir Med* 2006; 100: 434-450.
29. Israel E, Reddel HK. Severe and Difficult-to-Treat Asthma in Adults. *N Engl J Med* 2017; 377: 965-976.
30. Delmas MCaCF. Asthma in France: a review of descriptive epidemiological data. *Rev Mal Respir* 2009; 27: 151-159.
31. Riviere S, Delmas MC, Iwatsubo Y. [Asthma and socioeconomic characteristics in France in 2012]. *Rev Mal Respir* 2018; 35: 287-294.
32. Delmas MC, Guignon N, Leynaert B, Moisy M, Marguet C, Fuhrman C. [Increase in asthma prevalence among young children in France]. *Rev Mal Respir* 2017; 34: 525-534.
33. Bourdin A, Fabry-Vendrand C, Ostinelli J, Ait-Yahia M, Darnal E, Bouee S, Laurendeau C, Bureau I, Gourmelen J, Chouaid C. The Burden of Severe Asthma in France: A Case-Control Study Using a Medical Claims Database. *J Allergy Clin Immunol Pract* 2019; 7: 1477-1487.
34. Raherison-Semjen C, Izadifar A, Russier M, Rolland C, Aubert JP, Touboul C, Leynaud D, Fabry-Vendrand C, Didier A. Self-reported asthma prevalence and management in adults in France in 2018: ASTHMAPOP survey. *Respir Med Res* 2021;

80: 100864.

35. Nordon C, Grimaldi-Bensouda L, Pribil C, Nachbaur G, Amzal B, Thabut G, Marthan R, Aubier M, Aubier M, Crestani B, Taillé C, Dombret MC, Pretolani M, Berger P, Bourdin A, Vachier I, Molinari N, Chanez P, Similowski T, Didier A, De Blay F, Humbert M, Garcia G, Magnan A, Maitre B, Roche N, Tsicopoulos A, Chenivesse C, Deslée G, Marquette CH, Devouassoux G. Clinical and economic burden of severe asthma: A French cohort study. *Respiratory Medicine* 2018; 144: 42-49.
36. Moore WC, Meyers DA, Wenzel SE, Teague WG, Li H, Li X, D'Agostino R, Jr., Castro M, Curran-Everett D, Fitzpatrick AM, Gaston B, Jarjour NN, Sorkness R, Calhoun WJ, Chung KF, Comhair SA, Dweik RA, Israel E, Peters SP, Busse WW, Erzurum SC, Bleecker ER, National Heart L, Blood Institute's Severe Asthma Research P. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. *Am J Respir Crit Care Med* 2010; 181: 315-323.
37. Haldar P, Pavord ID, Shaw DE, Berry MA, Thomas M, Brightling CE, Wardlaw AJ, Green RH. Cluster analysis and clinical asthma phenotypes. *Am J Respir Crit Care Med* 2008; 178: 218-224.
38. Raheison-Semjen C, Parrat E, Nocent-Eijnani C, Mangiapan G, Prudhomme A, Oster JP, Aperre de Vecchi C, Maurer C, Debieuvre D, Portel L, Cphg. FASE-CPHG Study: identification of asthma phenotypes in the French Severe Asthma Study using cluster analysis. *Respir Res* 2021; 22: 136.
39. Spycher BD, Silverman M, Pescatore AM, Beardsmore CS, Kuehni CE. Comparison of phenotypes of childhood wheeze and cough in 2 independent cohorts. *J Allergy Clin Immunol* 2013; 132: 1058-1067.
40. Just J, Saint-Pierre P, Gouvis-Echraghi R, Laoudi Y, Roufai L, Momas I, Annesi Maesano I. Childhood allergic asthma is not a single phenotype. *J Pediatr* 2014; 164: 815-820.
41. Loza MJ, Djukanovic R, Chung KF, Horowitz D, Ma K, Branigan P, Barnathan ES, Susulic VS, Silkoff PE, Sterk PJ, Baribaud F, Adept, U. Biopred investigators. Validated and longitudinally stable asthma phenotypes based on cluster analysis of the ADEPT study. *Respir Res* 2016; 17: 165.
42. Lefaudeux D, De Meulder B, Loza MJ, Peffer N, Rowe A, Baribaud F, Bansal AT, Lutter R, Sousa AR, Corfield J, Pandis I, Bakke PS, Caruso M, Chanez P, Dahlen SE, Fleming LJ, Fowler SJ, Horvath I, Krug N, Montuschi P, Sanak M, Sandstrom T, Shaw DE, Singer F, Sterk PJ, Roberts G, Adcock IM, Djukanovic R, Auffray C, Chung KF, Group UBS. U-BIOPRED clinical adult asthma clusters linked to a subset of sputum omics. *J Allergy Clin Immunol* 2017; 139: 1797-1807.
43. Cunha F, Amaral R, Jacinto T, Sousa-Pinto B, Fonseca JA. A Systematic Review of Asthma Phenotypes Derived by Data-Driven Methods. *Diagnostics (Basel)* 2021; 11.
44. Kaur R, Chupp G. Phenotypes and endotypes of adult asthma: Moving toward precision medicine. *Journal of Allergy and Clinical Immunology* 2019; 144: 1-12.
45. Wenzel SE. Asthma: defining of the persistent adult phenotypes. *Lancet* 2006; 368: 804-813.
46. Kupczyk M, Dahlén B, Sterk PJ, Nizankowska-Mogilnicka E, Papi A, Bel EH, Chanez P, Howarth PH, Holgate ST, Brusselle G, Siafakas NM, Gjomarkaj M, Dahlén S-E. Stability of phenotypes defined by physiological variables and biomarkers in adults with asthma. *Allergy* 2014; 69: 1198-1204.
47. Newby C, Heaney LG, Menzies-Gow A, Niven RM, Mansur A, Bucknall C, Chaudhuri R, Thompson J, Burton P, Brightling C, British Thoracic Society Severe



Refractory Asthma N. Statistical cluster analysis of the British Thoracic Society Severe refractory Asthma Registry: clinical outcomes and phenotype stability. *PLoS One* 2014; 9: e102987.

48. Fleming L, Tsartsali L, Wilson N, Regamey N, Bush A. Sputum inflammatory phenotypes are not stable in children with asthma. *Thorax* 2012; 67: 675-681.

49. Tsang YP, Marchant JM, Li AM, Chang AB. Stability of sputum inflammatory phenotypes in childhood asthma during stable and exacerbation phases. *Pediatric Pulmonology* 2021; 56: 1484-1489.

50. Bourdin A, Molinari N, Vachier I, Varrin M, Marin G, Gamez AS, Paganin F, Chanez P. Prognostic value of cluster analysis of severe asthma phenotypes. *J Allergy Clin Immunol* 2014; 134: 1043-1050.

51. Boudier A, Chanoine S, Accordini S, Anto JM, Basagana X, Bousquet J, Demoly P, Garcia-Aymerich J, Gormand F, Heinrich J, Janson C, Kunzli N, Matran R, Pison C, Raheison C, Sunyer J, Varraso R, Jarvis D, Leynaert B, Pin I, Siroux V. Data-driven adult asthma phenotypes based on clinical characteristics are associated with asthma outcomes twenty years later. *Allergy* 2018.

52. Badri KR, Zhou Y, Schuger L. Embryological Origin of Airway Smooth Muscle. *Proceedings of the American Thoracic Society* 2008; 5: 4-10.

53. Cieri RL. Pulmonary Smooth Muscle in Vertebrates: A Comparative Review of Structure and Function. *Integr Comp Biol* 2019; 59: 10-28.

54. Mitzner W. Airway smooth muscle: the appendix of the lung. *Am J Respir Crit Care Med* 2004; 169: 787-790.

55. Hassoun D, Rose L, Blanc FX, Magnan A, Loirand G, Sauzeau V. Bronchial smooth muscle cell in asthma: where does it fit? *BMJ Open Respir Res* 2022; 9.

56. Mazzone SB, Udem BJ. Vagal Afferent Innervation of the Airways in Health and Disease. *Physiol Rev* 2016; 96: 975-1024.

57. Berger P, Marthan R, Tunon de Lara JM. [The pathophysiological role of smooth muscle cells in bronchial inflammation]. *Rev Mal Respir* 2002; 19: 778-794.

58. Haga T. Molecular properties of muscarinic acetylcholine receptors. *Proc Jpn Acad Ser B Phys Biol Sci* 2013; 89: 226-256.

59. Ouedraogo N, Roux E. Physiology of Airway Smooth Muscle Contraction: An Overview. *J Pulm Respir Med* 2014; 4.

60. Bergner A, Sanderson MJ. Acetylcholine-induced calcium signaling and contraction of airway smooth muscle cells in lung slices. *J Gen Physiol* 2002; 119: 187-198.

61. Barnes PJ. Airway receptors. *Postgrad Med J* 1989; 65: 532-542.

62. Tanaka DT, Grunstein MM. Mechanisms of substance P-induced contraction of rabbit airway smooth muscle. *J Appl Physiol Respir Environ Exerc Physiol* 1984; 57: 1551-1557.

63. Nakajima T, Hazama H, Hamada E, Omata M, Kurachi Y. Ionic basis of neurokinin-A-induced depolarization in single smooth muscle cells isolated from guinea-pig trachea. *Pflugers Arch* 1995; 430: 552-562.

64. Grandordy BM, Frossard N, Rhoden KJ, Barnes PJ. Tachykinin-induced phosphoinositide breakdown in airway smooth muscle and epithelium: relationship to contraction. *Mol Pharmacol* 1988; 33: 515-519.

65. Stretton D. Non-adrenergic, non-cholinergic neural control of the airways. *Clin Exp Pharmacol Physiol* 1991; 18: 675-684.

66. Said SI. Vasoactive intestinal polypeptide (VIP): current status. *Peptides* 1984; 5: 143-150.

67. Ellis JL, Farmer SG. Modulation of cholinergic neurotransmission by vasoactive intestinal peptide and peptide histidine isoleucine in guinea-pig tracheal smooth muscle. *Pulm Pharmacol* 1989; 2: 107-112.
68. Kistemaker LEM, Prakash YS. Airway Innervation and Plasticity in Asthma. *Physiology (Bethesda)* 2019; 34: 283-298.
69. Udem BJ, Taylor-Clark T. Mechanisms underlying the neuronal-based symptoms of allergy. *J Allergy Clin Immunol* 2014; 133: 1521-1534.
70. Sanico AM, Atsuta S, Proud D, Togias A. Dose-dependent effects of capsaicin nasal challenge: in vivo evidence of human airway neurogenic inflammation. *J Allergy Clin Immunol* 1997; 100: 632-641.
71. Chen C-Y, Bonham AC, Schelegle ES, Gershwin LJ, Plopper CG, Joad JP. Extended allergen exposure in asthmatic monkeys induces neuroplasticity in nucleus tractus solitarius. *Journal of Allergy and Clinical Immunology* 2001; 108: 557-562.
72. Udem BJ, Kajekar R, Hunter DD, Myers AC. Neural integration and allergic disease. *J Allergy Clin Immunol* 2000; 106: S213-220.
73. Santing RE, Pasman Y, Olymulder CG, Roffel AF, Meurs H, Zaagsma J. Contribution of a cholinergic reflex mechanism to allergen-induced bronchial hyperreactivity in permanently instrumented, unrestrained guinea-pigs. *Br J Pharmacol* 1995; 114: 414-418.
74. Ma X, Cheng Z, Kong H, Wang Y, Unruh H, Stephens NL, Laviolette M. Changes in biophysical and biochemical properties of single bronchial smooth muscle cells from asthmatic subjects. *Am J Physiol Lung Cell Mol Physiol* 2002; 283: L1181-1189.
75. Matsumoto H, Moir LM, Oliver BG, Burgess JK, Roth M, Black JL, McParland BE. Comparison of gel contraction mediated by airway smooth muscle cells from patients with and without asthma. *Thorax* 2007; 62: 848-854.
76. Sanderson MJ, Delmotte P, Bai Y, Perez-Zogbi JF. Regulation of airway smooth muscle cell contractility by Ca<sup>2+</sup> signaling and sensitivity. *Proc Am Thorac Soc* 2008; 5: 23-31.
77. Deshpande DA, Walseth TF, Panettieri RA, Kannan MS. CD38/cyclic ADP-ribose-mediated Ca<sup>2+</sup> signaling contributes to airway smooth muscle hyper-responsiveness. *FASEB J* 2003; 17: 452-454.
78. Deshpande DA, White TA, Guedes AG, Milla C, Walseth TF, Lund FE, Kannan MS. Altered airway responsiveness in CD38-deficient mice. *Am J Respir Cell Mol Biol* 2005; 32: 149-156.
79. Benayoun L, Druilhe A, Dombret MC, Aubier M, Pretolani M. Airway structural alterations selectively associated with severe asthma. *Am J Respir Crit Care Med* 2003; 167: 1360-1368.
80. Deshpande DA, Dogan S, Walseth TF, Miller SM, Amrani Y, Panettieri RA, Kannan MS. Modulation of calcium signaling by interleukin-13 in human airway smooth muscle: role of CD38/cyclic adenosine diphosphate ribose pathway. *Am J Respir Cell Mol Biol* 2004; 31: 36-42.
81. Feng J, Ito M, Ichikawa K, Isaka N, Nishikawa M, Hartshorne DJ, Nakano T. Inhibitory phosphorylation site for Rho-associated kinase on smooth muscle myosin phosphatase. *J Biol Chem* 1999; 274: 37385-37390.
82. Sakai H, Chiba Y, Hirano T, Misawa M. Possible involvement of CPI-17 in augmented bronchial smooth muscle contraction in antigen-induced airway hyper-responsive rats. *Mol Pharmacol* 2005; 68: 145-151.
83. Chiba Y, Ueno A, Shinozaki K, Takeyama H, Nakazawa S, Sakai H, Misawa M. Involvement of RhoA-mediated Ca<sup>2+</sup> sensitization in antigen-induced bronchial

- smooth muscle hyperresponsiveness in mice. *Respir Res* 2005; 6: 4.
84. Chiba Y, Tanoue G, Suto R, Suto W, Hanazaki M, Katayama H, Sakai H. Interleukin-17A directly acts on bronchial smooth muscle cells and augments the contractility. *Pharmacol Rep* 2017; 69: 377-385.
85. Chiba Y, Nakazawa S, Todoroki M, Shinozaki K, Sakai H, Misawa M. Interleukin-13 augments bronchial smooth muscle contractility with an up-regulation of RhoA protein. *Am J Respir Cell Mol Biol* 2009; 40: 159-167.
86. Schaafsma D, Bos IS, Zuidhof AB, Zaagsma J, Meurs H. The inhaled Rho kinase inhibitor Y-27632 protects against allergen-induced acute bronchoconstriction, airway hyperresponsiveness, and inflammation. *Am J Physiol Lung Cell Mol Physiol* 2008; 295: L214-219.
87. Pascual RM, Peters SP. Airway remodeling contributes to the progressive loss of lung function in asthma: an overview. *J Allergy Clin Immunol* 2005; 116: 477-486; quiz 487.
88. Lambrecht BN, Hammad H. The airway epithelium in asthma. *Nat Med* 2012; 18: 684-692.
89. Saglani S, Payne DN, Zhu J, Wang Z, Nicholson AG, Bush A, Jeffery PK. Early detection of airway wall remodeling and eosinophilic inflammation in preschool wheezers. *Am J Respir Crit Care Med* 2007; 176: 858-864.
90. Regamey N, Ochs M, Hilliard TN, Muhlfeld C, Cornish N, Fleming L, Saglani S, Alton EW, Bush A, Jeffery PK, Davies JC. Increased airway smooth muscle mass in children with asthma, cystic fibrosis, and non-cystic fibrosis bronchiectasis. *Am J Respir Crit Care Med* 2008; 177: 837-843.
91. Bossley CJ, Fleming L, Gupta A, Regamey N, Frith J, Oates T, Tsartsali L, Lloyd CM, Bush A, Saglani S. Pediatric severe asthma is characterized by eosinophilia and remodeling without T(H)2 cytokines. *J Allergy Clin Immunol* 2012; 129: 974-982 e913.
92. Bumbacea D, Campbell D, Nguyen L, Carr D, Barnes PJ, Robinson D, Chung KF. Parameters associated with persistent airflow obstruction in chronic severe asthma. *Eur Respir J* 2004; 24: 122-128.
93. Tillie-Leblond I, de Blic J, Jaubert F, Wallaert B, Scheinmann P, Gosset P. Airway remodeling is correlated with obstruction in children with severe asthma. *Allergy* 2008; 63: 533-541.
94. Siddiqui S, Shikotra A, Richardson M, Doran E, Choy D, Bell A, Austin CD, Eastham-Anderson J, Hargadon B, Arron JR, Wardlaw A, Brightling CE, Heaney LG, Bradding P. Airway pathological heterogeneity in asthma: Visualization of disease microclusters using topological data analysis. *J Allergy Clin Immunol* 2018; 142: 1457-1468.
95. Gupta S, Hartley R, Khan UT, Singapuri A, Hargadon B, Monteiro W, Pavord ID, Sousa AR, Marshall RP, Subramanian D, Parr D, Entwisle JJ, Siddiqui S, Raj V, Brightling CE. Quantitative computed tomography-derived clusters: redefining airway remodeling in asthmatic patients. *J Allergy Clin Immunol* 2014; 133: 729-738 e718.
96. Wadsworth S, Sin D, Dorscheid D. Clinical update on the use of biomarkers of airway inflammation in the management of asthma. *J Asthma Allergy* 2011; 4: 77-86.
97. Loxham M, Davies DE. Phenotypic and genetic aspects of epithelial barrier function in asthmatic patients. *Journal of Allergy and Clinical Immunology* 2017; 139: 1736-1751.
98. Thomas B, Rutman A, Hirst RA, Haldar P, Wardlaw AJ, Bankart J, Brightling CE, O'Callaghan C. Ciliary dysfunction and ultrastructural abnormalities are features of severe asthma. *J Allergy Clin Immunol* 2010; 126: 722-729 e722.

99. Lachowicz-Scroggins ME, Yuan S, Kerr SC, Dunican EM, Yu M, Carrington SD, Fahy JV. Abnormalities in MUC5AC and MUC5B Protein in Airway Mucus in Asthma. *Am J Respir Crit Care Med* 2016; 194: 1296-1299.
100. de Boer WI, Sharma HS, Baelemans SM, Hoogsteden HC, Lambrecht BN, Braunstahl GJ. Altered expression of epithelial junctional proteins in atopic asthma: possible role in inflammation. *Can J Physiol Pharmacol* 2008; 86: 105-112.
101. Adam E, Hansen KK, Astudillo Fernandez O, Coulon L, Bex F, Duhant X, Jaumotte E, Hollenberg MD, Jacquet A. The house dust mite allergen Der p 1, unlike Der p 3, stimulates the expression of interleukin-8 in human airway epithelial cells via a proteinase-activated receptor-2-independent mechanism. *J Biol Chem* 2006; 281: 6910-6923.
102. Uehara A, Fujimoto Y, Fukase K, Takada H. Various human epithelial cells express functional Toll-like receptors, NOD1 and NOD2 to produce anti-microbial peptides, but not proinflammatory cytokines. *Mol Immunol* 2007; 44: 3100-3111.
103. Sha Q, Truong-Tran AQ, Plitt JR, Beck LA, Schleimer RP. Activation of airway epithelial cells by toll-like receptor agonists. *Am J Respir Cell Mol Biol* 2004; 31: 358-364.
104. Whetstone CE, Ranjbar M, Omer H, Cusack RP, Gauvreau GM. The Role of Airway Epithelial Cell Alarmins in Asthma. *Cells* 2022; 11.
105. Soumelis V, Reche PA, Kanzler H, Yuan W, Edward G, Homey B, Gilliet M, Ho S, Antonenko S, Lauerma A, Smith K, Gorman D, Zurawski S, Abrams J, Menon S, McClanahan T, de Waal-Malefyt Rd R, Bazan F, Kastelein RA, Liu YJ. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol* 2002; 3: 673-680.
106. Shikotra A, Choy DF, Ohri CM, Doran E, Butler C, Hargadon B, Shelley M, Abbas AR, Austin CD, Jackman J, Wu LC, Heaney LG, Arron JR, Bradding P. Increased expression of immunoreactive thymic stromal lymphopoietin in patients with severe asthma. *J Allergy Clin Immunol* 2012; 129: 104-111 e101-109.
107. Kato A, Favoreto S, Jr., Avila PC, Schleimer RP. TLR3- and Th2 cytokine-dependent production of thymic stromal lymphopoietin in human airway epithelial cells. *J Immunol* 2007; 179: 1080-1087.
108. Corrigan CJ, Wang W, Meng Q, Fang C, Eid G, Caballero MR, Lv Z, An Y, Wang YH, Liu YJ, Kay AB, Lee TH, Ying S. Allergen-induced expression of IL-25 and IL-25 receptor in atopic asthmatic airways and late-phase cutaneous responses. *J Allergy Clin Immunol* 2011; 128: 116-124.
109. Cheng D, Xue Z, Yi L, Shi H, Zhang K, Huo X, Bonser LR, Zhao J, Xu Y, Erle DJ, Zhen G. Epithelial interleukin-25 is a key mediator in Th2-high, corticosteroid-responsive asthma. *Am J Respir Crit Care Med* 2014; 190: 639-648.
110. Prefontaine D, Lajoie-Kadoch S, Foley S, Audusseau S, Olivenstein R, Halayko AJ, Lemiere C, Martin JG, Hamid Q. Increased expression of IL-33 in severe asthma: evidence of expression by airway smooth muscle cells. *J Immunol* 2009; 183: 5094-5103.
111. Chustz RT, Nagarkar DR, Poposki JA, Favoreto S, Jr., Avila PC, Schleimer RP, Kato A. Regulation and function of the IL-1 family cytokine IL-1F9 in human bronchial epithelial cells. *Am J Respir Cell Mol Biol* 2011; 45: 145-153.
112. Prefontaine D, Nadigel J, Chouiali F, Audusseau S, Semlali A, Chakir J, Martin JG, Hamid Q. Increased IL-33 expression by epithelial cells in bronchial asthma. *J Allergy Clin Immunol* 2010; 125: 752-754.
113. Bartemes KR, Iijima K, Kobayashi T, Kephart GM, McKenzie AN, Kita H. IL-33-

- responsive lineage- CD25+ CD44(hi) lymphoid cells mediate innate type 2 immunity and allergic inflammation in the lungs. *J Immunol* 2012; 188: 1503-1513.
114. Barlow JL, Peel S, Fox J, Panova V, Hardman CS, Camelo A, Bucks C, Wu X, Kane CM, Neill DR, Flynn RJ, Sayers I, Hall IP, McKenzie AN. IL-33 is more potent than IL-25 in provoking IL-13-producing nuocytes (type 2 innate lymphoid cells) and airway contraction. *J Allergy Clin Immunol* 2013; 132: 933-941.
115. Grainge CL, Davies DE. Epithelial injury and repair in airways diseases. *Chest* 2013; 144: 1906-1912.
116. Erjefalt JS, Erjefalt I, Sundler F, Persson CG. In vivo restitution of airway epithelium. *Cell Tissue Res* 1995; 281: 305-316.
117. Puddicombe SM, Polosa R, Richter A, Krishna MT, Howarth PH, Holgate ST, Davies DE. Involvement of the epidermal growth factor receptor in epithelial repair in asthma. *FASEB J* 2000; 14: 1362-1374.
118. Amishima M, Munakata M, Nasuhara Y, Sato A, Takahashi T, Homma Y, Kawakami Y. Expression of epidermal growth factor and epidermal growth factor receptor immunoreactivity in the asthmatic human airway. *Am J Respir Crit Care Med* 1998; 157: 1907-1912.
119. Holgate ST, Davies DE, Lackie PM, Wilson SJ, Puddicombe SM, Lordan JL. Epithelial-mesenchymal interactions in the pathogenesis of asthma. *Journal of Allergy and Clinical Immunology* 2000; 105: 193-204.
120. Hackett TL, Warner SM, Stefanowicz D, Shaheen F, Pechkovsky DV, Murray LA, Argentieri R, Kicic A, Stick SM, Bai TR, Knight DA. Induction of epithelial-mesenchymal transition in primary airway epithelial cells from patients with asthma by transforming growth factor-beta1. *Am J Respir Crit Care Med* 2009; 180: 122-133.
121. Knight DA, Grainge CL, Stick SM, Kicic A, Schuliga M. Epithelial Mesenchymal Transition in Respiratory Disease: Fact or Fiction. *Chest* 2020; 157: 1591-1596.
122. Halayko AJ, Salari H, Ma X, Stephens NL. Markers of airway smooth muscle cell phenotype. *Am J Physiol* 1996; 270: L1040-1051.
123. Berair R, Saunders R, Brightling CE. Origins of increased airway smooth muscle mass in asthma. *BMC Med* 2013; 11: 145.
124. Zou H, Fang QH, Ma YM, Wang XY. Analysis of growth factors in serum and induced sputum from patients with asthma. *Exp Ther Med* 2014; 8: 573-578.
125. Shan L, Redhu NS, Saleh A, Halayko AJ, Chakir J, Gounni AS. Thymic stromal lymphopoietin receptor-mediated IL-6 and CC/CXC chemokines expression in human airway smooth muscle cells: role of MAPKs (ERK1/2, p38, and JNK) and STAT3 pathways. *J Immunol* 2010; 184: 7134-7143.
126. Lajoie-Kadoch S, Joubert P, Letuve S, Halayko AJ, Martin JG, Soussi-Gounni A, Hamid Q. TNF-alpha and IFN-gamma inversely modulate expression of the IL-17E receptor in airway smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 2006; 290: L1238-1246.
127. Malavia NK, Raub CB, Mahon SB, Brenner M, Panettieri RA, Jr., George SC. Airway epithelium stimulates smooth muscle proliferation. *Am J Respir Cell Mol Biol* 2009; 41: 297-304.
128. Lan B, Mitchel JA, O'Sullivan MJ, Park CY, Kim JH, Cole WC, Butler JP, Park JA. Airway epithelial compression promotes airway smooth muscle proliferation and contraction. *Am J Physiol Lung Cell Mol Physiol* 2018; 315: L645-L652.
129. Risse PA, Jo T, Suarez F, Hirota N, Tolloczko B, Ferraro P, Grutter P, Martin JG. Interleukin-13 inhibits proliferation and enhances contractility of human airway smooth muscle cells without change in contractile phenotype. *Am J Physiol Lung Cell Mol*

*Physiol* 2011; 300: L958-966.

130. Espinosa K, Bosse Y, Stankova J, Rola-Pleszczynski M. CysLT1 receptor upregulation by TGF-beta and IL-13 is associated with bronchial smooth muscle cell proliferation in response to LTD4. *J Allergy Clin Immunol* 2003; 111: 1032-1040.

131. Hirst SJ, Barnes PJ, Twort CH. Quantifying proliferation of cultured human and rabbit airway smooth muscle cells in response to serum and platelet-derived growth factor. *Am J Respir Cell Mol Biol* 1992; 7: 574-581.

132. Hirst SJ, Barnes PJ, Twort CH. PDGF isoform-induced proliferation and receptor expression in human cultured airway smooth muscle cells. *Am J Physiol* 1996; 270: L415-428.

133. Simeone-Penney MC, Severgnini M, Roza L, Takahashi S, Cochran BH, Simon AR. PDGF-induced human airway smooth muscle cell proliferation requires STAT3 and the small GTPase Rac1. *Am J Physiol Lung Cell Mol Physiol* 2008; 294: L698-704.

134. Stamatiou R, Paraskeva E, Gourgoulisanis K, Molyvdas PA, Hatziefthimiou A. Cytokines and growth factors promote airway smooth muscle cell proliferation. *ISRN Inflamm* 2012; 2012: 731472.

135. Ediger TL, Toews ML. Synergistic stimulation of airway smooth muscle cell mitogenesis. *J Pharmacol Exp Ther* 2000; 294: 1076-1082.

136. Bosse Y, Thompson C, Stankova J, Rola-Pleszczynski M. Fibroblast growth factor 2 and transforming growth factor beta1 synergism in human bronchial smooth muscle cell proliferation. *Am J Respir Cell Mol Biol* 2006; 34: 746-753.

137. Zou H, Nie XH, Zhang Y, Hu M, Zhang YA. Effect of basic fibroblast growth factor on the proliferation, migration and phenotypic modulation of airway smooth muscle cells. *Chin Med J (Engl)* 2008; 121: 424-429.

138. Krymskaya VP, Hoffman R, Eszterhas A, Kane S, Ciocca V, Panettieri RA, Jr. EGF activates ErbB-2 and stimulates phosphatidylinositol 3-kinase in human airway smooth muscle cells. *Am J Physiol* 1999; 276: L246-255.

139. Chen G, Khalil N. TGF-beta1 increases proliferation of airway smooth muscle cells by phosphorylation of map kinases. *Respir Res* 2006; 7: 2.

140. Cohen MD, Ciocca V, Panettieri RA, Jr. TGF-beta 1 modulates human airway smooth-muscle cell proliferation induced by mitogens. *Am J Respir Cell Mol Biol* 1997; 16: 85-90.

141. Xie S, Sukkar MB, Issa R, Khorasani NM, Chung KF. Mechanisms of induction of airway smooth muscle hyperplasia by transforming growth factor-beta. *Am J Physiol Lung Cell Mol Physiol* 2007; 293: L245-253.

142. Stewart AG, Tomlinson PR, Fernandes DJ, Wilson JW, Harris T. Tumor necrosis factor alpha modulates mitogenic responses of human cultured airway smooth muscle. *Am J Respir Cell Mol Biol* 1995; 12: 110-119.

143. Knobloch J, Yanik SD, Korber S, Stoelben E, Jungck D, Koch A. TNFalpha-induced airway smooth muscle cell proliferation depends on endothelin receptor signaling, GM-CSF and IL-6. *Biochem Pharmacol* 2016; 116: 188-199.

144. Li X, Zou F, Lu Y, Fan X, Wu Y, Feng X, Sun X, Liu Y. Notch1 contributes to TNF-alpha-induced proliferation and migration of airway smooth muscle cells through regulation of the Hes1/PTEN axis. *Int Immunopharmacol* 2020; 88: 106911.

145. De S, Zelazny ET, Souhrada JF, Souhrada M. Interleukin-1 beta stimulates the proliferation of cultured airway smooth muscle cells via platelet-derived growth factor. *Am J Respir Cell Mol Biol* 1993; 9: 645-651.

146. De S, Zelazny ET, Souhrada JF, Souhrada M. IL-1 beta and IL-6 induce

hyperplasia and hypertrophy of cultured guinea pig airway smooth muscle cells. *J Appl Physiol (1985)* 1995; 78: 1555-1563.

147. Amrani Y, Tliba O, Choubey D, Huang CD, Krymskaya VP, Eszterhas A, Lazaar AL, Panettieri RA, Jr. IFN-gamma inhibits human airway smooth muscle cell proliferation by modulating the E2F-1/Rb pathway. *Am J Physiol Lung Cell Mol Physiol* 2003; 284: L1063-1071.

148. Hawker KM, Johnson PR, Hughes JM, Black JL. Interleukin-4 inhibits mitogen-induced proliferation of human airway smooth muscle cells in culture. *Am J Physiol* 1998; 275: L469-477.

149. Shim JY, Park SW, Kim DS, Shim JW, Jung HL, Park MS. The effect of interleukin-4 and amphiregulin on the proliferation of human airway smooth muscle cells and cytokine release. *J Korean Med Sci* 2008; 23: 857-863.

150. Panettieri RA, Yadavish PA, Kelly AM, Rubinstein NA, Kotlikoff MI. Histamine stimulates proliferation of airway smooth muscle and induces c-fos expression. *Am J Physiol* 1990; 259: L365-371.

151. Chhabra J, Li YZ, Alkhouri H, Blake AE, Ge Q, Armour CL, Hughes JM. Histamine and tryptase modulate asthmatic airway smooth muscle GM-CSF and RANTES release. *Eur Respir J* 2007; 29: 861-870.

152. Noveral JP, Grunstein MM. Role and mechanism of thromboxane-induced proliferation of cultured airway smooth muscle cells. *Am J Physiol* 1992; 263: L555-561.

153. Capra V, Habib A, Accomazzo MR, Ravasi S, Citro S, Levy-Toledano S, Nicosia S, Rovati GE. Thromboxane prostanoid receptor in human airway smooth muscle cells: a relevant role in proliferation. *Eur J Pharmacol* 2003; 474: 149-159.

154. Ammit AJ, Hastie AT, Edsall LC, Hoffman RK, Amrani Y, Krymskaya VP, Kane SA, Peters SP, Penn RB, Spiegel S, Panettieri RA, Jr. Sphingosine 1-phosphate modulates human airway smooth muscle cell functions that promote inflammation and airway remodeling in asthma. *FASEB J* 2001; 15: 1212-1214.

155. Berger P, Perng DW, Thabrew H, Compton SJ, Cairns JA, McEuen AR, Marthan R, Tunon De Lara JM, Walls AF. Tryptase and agonists of PAR-2 induce the proliferation of human airway smooth muscle cells. *J Appl Physiol (1985)* 2001; 91: 1372-1379.

156. Panettieri RA, Jr., Hall IP, Maki CS, Murray RK. alpha-Thrombin increases cytosolic calcium and induces human airway smooth muscle cell proliferation. *Am J Respir Cell Mol Biol* 1995; 13: 205-216.

157. Huang CD, Chen HH, Wang CH, Chou CL, Lin SM, Lin HC, Kuo HP. Human neutrophil-derived elastase induces airway smooth muscle cell proliferation. *Life Sci* 2004; 74: 2479-2492.

158. Brar SS, Kennedy TP, Sturrock AB, Huecksteadt TP, Quinn MT, Murphy TM, Chitano P, Hoidal JR. NADPH oxidase promotes NF-kappaB activation and proliferation in human airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 2002; 282: L782-795.

159. Hedges JC, Dechert MA, Yamboliev IA, Martin JL, Hickey E, Weber LA, Gerthoffer WT. A role for p38(MAPK)/HSP27 pathway in smooth muscle cell migration. *J Biol Chem* 1999; 274: 24211-24219.

160. Carlin SM, Roth M, Black JL. Urokinase potentiates PDGF-induced chemotaxis of human airway smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 2003; 284: L1020-1026.

161. Parameswaran K, Cox G, Radford K, Janssen LJ, Sehmi R, O'Byrne PM.

- Cysteinyl leukotrienes promote human airway smooth muscle migration. *Am J Respir Crit Care Med* 2002; 166: 738-742.
162. Keglowich LF, Borger P. The Three A's in Asthma - Airway Smooth Muscle, Airway Remodeling & Angiogenesis. *Open Respir Med J* 2015; 9: 70-80.
163. Hirst SJ, Twort CH, Lee TH. Differential effects of extracellular matrix proteins on human airway smooth muscle cell proliferation and phenotype. *Am J Respir Cell Mol Biol* 2000; 23: 335-344.
164. Araujo BB, Dolhnikoff M, Silva LF, Elliot J, Lindeman JH, Ferreira DS, Mulder A, Gomes HA, Fernezlian SM, James A, Mauad T. Extracellular matrix components and regulators in the airway smooth muscle in asthma. *Eur Respir J* 2008; 32: 61-69.
165. Tran T, McNeill KD, Gerthoffer WT, Unruh H, Halayko AJ. Endogenous laminin is required for human airway smooth muscle cell maturation. *Respir Res* 2006; 7: 117.
166. Tliba O, Panettieri RA, Jr. Noncontractile functions of airway smooth muscle cells in asthma. *Annu Rev Physiol* 2009; 71: 509-535.
167. Yang W, Kaur D, Okayama Y, Ito A, Wardlaw AJ, Brightling CE, Bradding P. Human lung mast cells adhere to human airway smooth muscle, in part, via tumor suppressor in lung cancer-1. *J Immunol* 2006; 176: 1238-1243.
168. Girodet PO, Ozier A, Trian T, Begueret H, Ousova O, Vernejoux JM, Chanez P, Marthan R, Berger P, Tunon de Lara JM. Mast cell adhesion to bronchial smooth muscle in asthma specifically depends on CD51 and CD44 variant 6. *Allergy* 2010; 65: 1004-1012.
169. Hamawy MM, Mergenhagen SE, Siraganian RP. Adhesion molecules as regulators of mast-cell and basophil function. *Immunol Today* 1994; 15: 62-66.
170. Lazaar AL, Albelda SM, Pilewski JM, Brennan B, Pure E, Panettieri RA, Jr. T lymphocytes adhere to airway smooth muscle cells via integrins and CD44 and induce smooth muscle cell DNA synthesis. *J Exp Med* 1994; 180: 807-816.
171. Carr TF, Zeki AA, Kraft M. Eosinophilic and Noneosinophilic Asthma. *Am J Respir Crit Care Med* 2018; 197: 22-37.
172. Hammad H, Lambrecht BN. The basic immunology of asthma. *Cell* 2021; 184: 1469-1485.
173. Pulendran B, Artis D. New paradigms in type 2 immunity. *Science* 2012; 337: 431-435.
174. Gieseck RL, 3rd, Wilson MS, Wynn TA. Type 2 immunity in tissue repair and fibrosis. *Nat Rev Immunol* 2018; 18: 62-76.
175. Akdis CA, Arkwright PD, Bruggen MC, Busse W, Gadina M, Guttman-Yassky E, Kabashima K, Mitamura Y, Vian L, Wu J, Palomares O. Type 2 immunity in the skin and lungs. *Allergy* 2020; 75: 1582-1605.
176. Hassoun D, Malard O, Barbarot S, Magnan A, Colas L. Type 2 immunity-driven diseases: Towards a multidisciplinary approach. *Clin Exp Allergy* 2021; 51: 1538-1552.
177. Hammad H, Plantinga M, Deswarte K, Pouliot P, Willart MA, Kool M, Muskens F, Lambrecht BN. Inflammatory dendritic cells--not basophils--are necessary and sufficient for induction of Th2 immunity to inhaled house dust mite allergen. *J Exp Med* 2010; 207: 2097-2111.
178. Plantinga M, Guilliams M, Vanheerswynghe M, Deswarte K, Branco-Madeira F, Toussaint W, Vanhoutte L, Neyt K, Killeen N, Malissen B, Hammad H, Lambrecht BN. Conventional and monocyte-derived CD11b(+) dendritic cells initiate and maintain T helper 2 cell-mediated immunity to house dust mite allergen. *Immunity* 2013; 38: 322-335.
179. Dullaers M, Schuijjs MJ, Willart M, Fierens K, Van Moorlegheem J, Hammad H,



- Lambrecht BN. House dust mite-driven asthma and allergen-specific T cells depend on B cells when the amount of inhaled allergen is limiting. *J Allergy Clin Immunol* 2017; 140: 76-88 e77.
180. Girodet PO, Nguyen D, Mancini JD, Hundal M, Zhou X, Israel E, Cernadas M. Alternative Macrophage Activation Is Increased in Asthma. *Am J Respir Cell Mol Biol* 2016; 55: 467-475.
181. Zhu X, Cui J, Yi, Qin J, Tulake W, Teng F, Tang W, Wei Y, Dong J. The Role of T Cells and Macrophages in Asthma Pathogenesis: A New Perspective on Mutual Crosstalk. *Mediators Inflamm* 2020; 2020: 7835284.
182. Saradna A, Do DC, Kumar S, Fu QL, Gao P. Macrophage polarization and allergic asthma. *Transl Res* 2018; 191: 1-14.
183. Spits H, Artis D, Colonna M, Diefenbach A, Di Santo JP, Eberl G, Koyasu S, Locksley RM, McKenzie AN, Mebius RE, Powrie F, Vivier E. Innate lymphoid cells--a proposal for uniform nomenclature. *Nat Rev Immunol* 2013; 13: 145-149.
184. Neill DR, Wong SH, Bellosi A, Flynn RJ, Daly M, Langford TK, Bucks C, Kane CM, Fallon PG, Pannell R, Jolin HE, McKenzie AN. Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. *Nature* 2010; 464: 1367-1370.
185. Hoyler T, Klose CS, Souabni A, Turqueti-Neves A, Pfeifer D, Rawlins EL, Voehringer D, Busslinger M, Diefenbach A. The transcription factor GATA-3 controls cell fate and maintenance of type 2 innate lymphoid cells. *Immunity* 2012; 37: 634-648.
186. Wong SH, Walker JA, Jolin HE, Drynan LF, Hams E, Camelo A, Barlow JL, Neill DR, Panova V, Koch U, Radtke F, Hardman CS, Hwang YY, Fallon PG, McKenzie AN. Transcription factor RORalpha is critical for nuocyte development. *Nat Immunol* 2012; 13: 229-236.
187. Smith SG, Chen R, Kjarsgaard M, Huang C, Oliveria JP, O'Byrne PM, Gauvreau GM, Boulet LP, Lemiere C, Martin J, Nair P, Sehmi R. Increased numbers of activated group 2 innate lymphoid cells in the airways of patients with severe asthma and persistent airway eosinophilia. *J Allergy Clin Immunol* 2016; 137: 75-86 e78.
188. Chen R, Smith SG, Salter B, El-Gammal A, Oliveria JP, Obminski C, Watson R, O'Byrne PM, Gauvreau GM, Sehmi R. Allergen-induced Increases in Sputum Levels of Group 2 Innate Lymphoid Cells in Subjects with Asthma. *Am J Respir Crit Care Med* 2017; 196: 700-712.
189. Winkler C, Hochdorfer T, Israelsson E, Hasselberg A, Cavallin A, Thorn K, Muthas D, Shojaee S, Luer K, Muller M, Mjosberg J, Vaarala O, Hohlfeld J, Pardali K. Activation of group 2 innate lymphoid cells after allergen challenge in asthmatic patients. *J Allergy Clin Immunol* 2019; 144: 61-69 e67.
190. Liu S, Verma M, Michalec L, Liu W, Sripada A, Rollins D, Good J, Ito Y, Chu H, Gorska MM, Martin RJ, Alam R. Steroid resistance of airway type 2 innate lymphoid cells from patients with severe asthma: The role of thymic stromal lymphopoietin. *J Allergy Clin Immunol* 2018; 141: 257-268 e256.
191. Gour N, Wills-Karp M. IL-4 and IL-13 signaling in allergic airway disease. *Cytokine* 2015; 75: 68-78.
192. Grunewald SM, Werthmann A, Schnarr B, Klein CE, Brouckebach EB, Mohrs M, Brombacher F, Sebald W, Duschl A. An antagonistic IL-4 mutant prevents type I allergy in the mouse: inhibition of the IL-4/IL-13 receptor system completely abrogates humoral immune response to allergen and development of allergic symptoms in vivo. *J Immunol* 1998; 160: 4004-4009.
193. Bradding P, Walls AF, Holgate ST. The role of the mast cell in the pathophysiology of asthma. *J Allergy Clin Immunol* 2006; 117: 1277-1284.

194. Nakajima H, Sano H, Nishimura T, Yoshida S, Iwamoto I. Role of vascular cell adhesion molecule 1/very late activation antigen 4 and intercellular adhesion molecule 1/lymphocyte function-associated antigen 1 interactions in antigen-induced eosinophil and T cell recruitment into the tissue. *J Exp Med* 1994; 179: 1145-1154.
195. Kuperman DA, Huang X, Koth LL, Chang GH, Dolganov GM, Zhu Z, Elias JA, Sheppard D, Erle DJ. Direct effects of interleukin-13 on epithelial cells cause airway hyperreactivity and mucus overproduction in asthma. *Nat Med* 2002; 8: 885-889.
196. Sugita K, Steer CA, Martinez-Gonzalez I, Altunbulakli C, Morita H, Castro-Giner F, Kubo T, Wawrzyniak P, Ruckert B, Sudo K, Nakae S, Matsumoto K, O'Mahony L, Akdis M, Takei F, Akdis CA. Type 2 innate lymphoid cells disrupt bronchial epithelial barrier integrity by targeting tight junctions through IL-13 in asthmatic patients. *J Allergy Clin Immunol* 2018; 141: 300-310 e311.
197. Chibana K, Trudeau JB, Mustovich AT, Hu H, Zhao J, Balzar S, Chu HW, Wenzel SE. IL-13 induced increases in nitrite levels are primarily driven by increases in inducible nitric oxide synthase as compared with effects on arginases in human primary bronchial epithelial cells. *Clin Exp Allergy* 2008; 38: 936-946.
198. Walter DM, McIntire JJ, Berry G, McKenzie AN, Donaldson DD, DeKruyff RH, Umetsu DT. Critical role for IL-13 in the development of allergen-induced airway hyperreactivity. *J Immunol* 2001; 167: 4668-4675.
199. Kopf M, Brombacher F, Hodgkin PD, Ramsay AJ, Milbourne EA, Dai WJ, Ovington KS, Behm CA, Kohler G, Young IG, Matthaei KI. IL-5-Deficient mice have a developmental defect in CD5(+) B-1 cells and lack eosinophilia but have normal antibody and cytotoxic T cell responses. *Immunity* 1996; 4: 15-24.
200. Stirling RG, van Rensen EL, Barnes PJ, Chung KF. Interleukin-5 induces CD34(+) eosinophil progenitor mobilization and eosinophil CCR3 expression in asthma. *Am J Respir Crit Care Med* 2001; 164: 1403-1409.
201. Mould AW, Ramsay AJ, Matthaei KI, Young IG, Rothenberg ME, Foster PS. The effect of IL-5 and eotaxin expression in the lung on eosinophil trafficking and degranulation and the induction of bronchial hyperreactivity. *J Immunol* 2000; 164: 2142-2150.
202. Yang M, Hogan SP, Mahalingam S, Pope SM, Zimmermann N, Fulkerson P, Dent LA, Young IG, Matthaei KI, Rothenberg ME, Foster PS. Eotaxin-2 and IL-5 cooperate in the lung to regulate IL-13 production and airway eosinophilia and hyperreactivity. *J Allergy Clin Immunol* 2003; 112: 935-943.
203. Xu J, Jiang F, Nayeri F, Zetterstrom O. Apoptotic eosinophils in sputum from asthmatic patients correlate negatively with levels of IL-5 and eotaxin. *Respir Med* 2007; 101: 1447-1454.
204. Pazdrak K, Moon Y, Straub C, Stafford S, Kurosky A. Eosinophil resistance to glucocorticoid-induced apoptosis is mediated by the transcription factor NFIL3. *Apoptosis* 2016; 21: 421-431.
205. Persson EK, Verstraete K, Heyndrickx I, Gevaert E, Aegerter H, Percier JM, Deswarte K, Verschueren KHG, Dansercoer A, Gras D, Chanez P, Bachert C, Goncalves A, Van Gorp H, De Haard H, Blanchetot C, Saunders M, Hammad H, Savvides SN, Lambrecht BN. Protein crystallization promotes type 2 immunity and is reversible by antibody treatment. *Science* 2019; 364.
206. Canas JA, Sastre B, Rodrigo-Munoz JM, Fernandez-Nieto M, Barranco P, Quirce S, Sastre J, Del Pozo V. Eosinophil-derived exosomes contribute to asthma remodelling by activating structural lung cells. *Clin Exp Allergy* 2018; 48: 1173-1185.
207. Humbles AA, Lloyd CM, McMillan SJ, Friend DS, Xanthou G, McKenna EE,

- Ghiran S, Gerard NP, Yu C, Orkin SH, Gerard C. A critical role for eosinophils in allergic airways remodeling. *Science* 2004; 305: 1776-1779.
208. Lee JJ, Dimina D, Macias MP, Ochkur SI, McGarry MP, O'Neill KR, Protheroe C, Pero R, Nguyen T, Cormier SA, Lenkiewicz E, Colbert D, Rinaldi L, Ackerman SJ, Irvin CG, Lee NA. Defining a link with asthma in mice congenitally deficient in eosinophils. *Science* 2004; 305: 1773-1776.
209. Mamessier E, Nieves A, Lorec AM, Dupuy P, Pinot D, Pinet C, Vervloet D, Magnan A. T-cell activation during exacerbations: a longitudinal study in refractory asthma. *Allergy* 2008; 63: 1202-1210.
210. Josefowicz SZ, Niec RE, Kim HY, Treuting P, Chinen T, Zheng Y, Umetsu DT, Rudensky AY. Extrathymically generated regulatory T cells control mucosal TH2 inflammation. *Nature* 2012; 482: 395-399.
211. Wiest M, Upchurch K, Hasan MM, Cardenas J, Lanier B, Millard M, Turner J, Oh S, Joo H. Phenotypic and functional alterations of regulatory B cell subsets in adult allergic asthma patients. *Clin Exp Allergy* 2019; 49: 1214-1224.
212. Gao X, Ren X, Wang Q, Yang Z, Li Y, Su Z, Li J. Critical roles of regulatory B and T cells in helminth parasite-induced protection against allergic airway inflammation. *Clin Exp Immunol* 2019; 198: 390-402.
213. Braza F, Chesne J, Durand M, Dirou S, Brosseau C, Mahay G, Cheminant MA, Magnan A, Brouard S. A regulatory CD9(+) B-cell subset inhibits HDM-induced allergic airway inflammation. *Allergy* 2015; 70: 1421-1431.
214. Buhl R, Humbert M, Bjermer L, Chanez P, Heaney LG, Pavord I, Quirce S, Virchow JC, Holgate S. Severe eosinophilic asthma: a roadmap to consensus. *Eur Respir J* 2017; 49.
215. Holguin F, Cardet JC, Chung KF, Diver S, Ferreira DS, Fitzpatrick A, Gaga M, Kellermeyer L, Khurana S, Knight S, McDonald VM, Morgan RL, Ortega VE, Rigau D, Subbarao P, Tonia T, Adcock IM, Bleecker ER, Brightling C, Boulet LP, Cabana M, Castro M, Chanez P, Custovic A, Djukanovic R, Frey U, Frankemolle B, Gibson P, Hamerlijnck D, Jarjour N, Konno S, Shen H, Vitary C, Bush A. Management of severe asthma: a European Respiratory Society/American Thoracic Society guideline. *Eur Respir J* 2020; 55.
216. Schleich F, Brusselle G, Louis R, Vandenplas O, Michils A, Pilette C, Peche R, Manise M, Joos G. Heterogeneity of phenotypes in severe asthmatics. The Belgian Severe Asthma Registry (BSAR). *Respir Med* 2014; 108: 1723-1732.
217. Price DB, Rigazio A, Campbell JD, Bleecker ER, Corrigan CJ, Thomas M, Wenzel SE, Wilson AM, Small MB, Gopalan G, Ashton VL, Burden A, Hillyer EV, Kerkhof M, Pavord ID. Blood eosinophil count and prospective annual asthma disease burden: a UK cohort study. *Lancet Respir Med* 2015; 3: 849-858.
218. Lim S, Jatakanon A, Meah S, Oates T, Chung KF, Barnes PJ. Relationship between exhaled nitric oxide and mucosal eosinophilic inflammation in mild to moderately severe asthma. *Thorax* 2000; 55: 184-188.
219. Jatakanon A, Lim S, Kharitonov SA, Chung KF, Barnes PJ. Correlation between exhaled nitric oxide, sputum eosinophils, and methacholine responsiveness in patients with mild asthma. *Thorax* 1998; 53: 91-95.
220. Rajan JP, Wineinger NE, Stevenson DD, White AA. Prevalence of aspirin-exacerbated respiratory disease among asthmatic patients: A meta-analysis of the literature. *J Allergy Clin Immunol* 2015; 135: 676-681 e671.
221. Mascia K, Haselkorn T, Deniz YM, Miller DP, Bleecker ER, Borish L. Aspirin sensitivity and severity of asthma: evidence for irreversible airway obstruction in

- patients with severe or difficult-to-treat asthma. *J Allergy Clin Immunol* 2005; 116: 970-975.
222. McGrath KW, Icitovic N, Boushey HA, Lazarus SC, Sutherland ER, Chinchilli VM, Fahy JV, Asthma Clinical Research Network of the National Heart L, Blood I. A large subgroup of mild-to-moderate asthma is persistently noneosinophilic. *Am J Respir Crit Care Med* 2012; 185: 612-619.
223. Woodruff PG, Modrek B, Choy DF, Jia G, Abbas AR, Ellwanger A, Koth LL, Arron JR, Fahy JV. T-helper type 2-driven inflammation defines major subphenotypes of asthma. *Am J Respir Crit Care Med* 2009; 180: 388-395.
224. Ntontsi P, Loukides S, Bakakos P, Kostikas K, Papatheodorou G, Papathanassiou E, Hillas G, Koulouris N, Papis S, Papaioannou AI. Clinical, functional and inflammatory characteristics in patients with paucigranulocytic stable asthma: Comparison with different sputum phenotypes. *Allergy* 2017; 72: 1761-1767.
225. Demarche S, Schleich F, Henket M, Paulus V, Van Hees T, Louis R. Detailed analysis of sputum and systemic inflammation in asthma phenotypes: are paucigranulocytic asthmatics really non-inflammatory? *BMC Pulm Med* 2016; 16: 46.
226. Tliba O, Panettieri RA, Jr. Paucigranulocytic asthma: Uncoupling of airway obstruction from inflammation. *J Allergy Clin Immunol* 2019; 143: 1287-1294.
227. Moore WC, Hastie AT, Li X, Li H, Busse WW, Jarjour NN, Wenzel SE, Peters SP, Meyers DA, Bleecker ER. Sputum neutrophil counts are associated with more severe asthma phenotypes using cluster analysis. *J Allergy Clin Immunol* 2014; 133: 1557-1563 e1555.
228. Hastie AT, Moore WC, Meyers DA, Vestal PL, Li H, Peters SP, Bleecker ER, National Heart L, Blood Institute Severe Asthma Research P. Analyses of asthma severity phenotypes and inflammatory proteins in subjects stratified by sputum granulocytes. *J Allergy Clin Immunol* 2010; 125: 1028-1036 e1013.
229. Ogawa H, Azuma M, Tsunematsu T, Morimoto Y, Kondo M, Tezuka T, Nishioka Y, Tsuneyama K. Neutrophils induce smooth muscle hyperplasia via neutrophil elastase-induced FGF-2 in a mouse model of asthma with mixed inflammation. *Clin Exp Allergy* 2018; 48: 1715-1725.
230. Koga H, Miyahara N, Fuchimoto Y, Ikeda G, Waseda K, Ono K, Tanimoto Y, Kataoka M, Gelfand EW, Tanimoto M, Kanehiro A. Inhibition of neutrophil elastase attenuates airway hyperresponsiveness and inflammation in a mouse model of secondary allergen challenge: neutrophil elastase inhibition attenuates allergic airway responses. *Respir Res* 2013; 14: 8.
231. Vargas A, Roux-Dalvai F, Droit A, Lavoie JP. Neutrophil-Derived Exosomes: A New Mechanism Contributing to Airway Smooth Muscle Remodeling. *Am J Respir Cell Mol Biol* 2016; 55: 450-461.
232. Anticevich SZ, Hughes JM, Black JL, Armour CL. Induction of hyperresponsiveness in human airway tissue by neutrophils - mechanism of action. *Clinical & Experimental Allergy* 1996; 26: 549-556.
233. Green RH, Brightling CE, Woltmann G, Parker D, Wardlaw AJ, Pavord ID. Analysis of induced sputum in adults with asthma: identification of subgroup with isolated sputum neutrophilia and poor response to inhaled corticosteroids. *Thorax* 2002; 57: 875-879.
234. Annunziato F, Cosmi L, Liotta F, Maggi E, Romagnani S. Defining the human T helper 17 cell phenotype. *Trends Immunol* 2012; 33: 505-512.
235. Cosmi L, Maggi L, Santarlasci V, Capone M, Cardilicchia E, Frosali F, Querci V, Angeli R, Matucci A, Fambriini M, Liotta F, Parronchi P, Maggi E, Romagnani S,

- Annunziato F. Identification of a novel subset of human circulating memory CD4(+) T cells that produce both IL-17A and IL-4. *J Allergy Clin Immunol* 2010; 125: 222-230 e221-224.
236. Morita H, Moro K, Koyasu S. Innate lymphoid cells in allergic and nonallergic inflammation. *J Allergy Clin Immunol* 2016; 138: 1253-1264.
237. Kim HY, Lee HJ, Chang Y-J, Pichavant M, Shore SA, Fitzgerald KA, Iwakura Y, Israel E, Bolger K, Faul J, Dekruyff RH, Umetsu DT. Interleukin-17-producing innate lymphoid cells and the NLRP3 inflammasome facilitate obesity-associated airway hyperreactivity. *Nature Medicine* 2014; 20: 54-61.
238. Fricker M, Qin L, Sánchez-Ovando S, Simpson JL, Baines KJ, Riveros C, Scott HA, Wood LG, Wark PA, Kermani NZ, Chung KF, Gibson PG. An altered sputum macrophage transcriptome contributes to the neutrophilic asthma endotype. *Allergy* 2022; 77: 1204-1215.
239. Ouyang W, Kolls JK, Zheng Y. The biological functions of T helper 17 cell effector cytokines in inflammation. *Immunity* 2008; 28: 454-467.
240. Chesne J, Braza F, Mahay G, Brouard S, Aronica M, Magnan A. IL-17 in severe asthma. Where do we stand? *Am J Respir Crit Care Med* 2014; 190: 1094-1101.
241. Kudo M, Melton AC, Chen C, Engler MB, Huang KE, Ren X, Wang Y, Bernstein X, Li JT, Atabai K, Huang X, Sheppard D. IL-17A produced by alpha-beta T cells drives airway hyper-responsiveness in mice and enhances mouse and human airway smooth muscle contraction. *Nat Med* 2012; 18: 547-554.
242. Dos Santos TM, Righetti RF, Camargo LDN, Saraiva-Romanholo BM, Aristoteles L, de Souza FCR, Fukuzaki S, Alonso-Vale MIC, Cruz MM, Prado CM, Leick EA, Martins MA, Tiberio I. Effect of Anti-IL17 Antibody Treatment Alone and in Combination With Rho-Kinase Inhibitor in a Murine Model of Asthma. *Front Physiol* 2018; 9: 1183.
243. Camargo LDN, Righetti RF, Aristoteles L, Dos Santos TM, de Souza FCR, Fukuzaki S, Cruz MM, Alonso-Vale MIC, Saraiva-Romanholo BM, Prado CM, Martins MA, Leick EA, Tiberio I. Effects of Anti-IL-17 on Inflammation, Remodeling, and Oxidative Stress in an Experimental Model of Asthma Exacerbated by LPS. *Front Immunol* 2017; 8: 1835.
244. Chesne J, Braza F, Chadeuf G, Mahay G, Cheminant MA, Loy J, Brouard S, Sauzeau V, Loirand G, Magnan A. Prime role of IL-17A in neutrophilia and airway smooth muscle contraction in a house dust mite-induced allergic asthma model. *J Allergy Clin Immunol* 2015; 135: 1643-1643 e1643.
245. Xie Y, Abel PW, Casale TB, Tu Y. TH17 cells and corticosteroid insensitivity in severe asthma. *J Allergy Clin Immunol* 2022; 149: 467-479.
246. Schleich FN, Manise M, Sele J, Henket M, Seidel L, Louis R. Distribution of sputum cellular phenotype in a large asthma cohort: predicting factors for eosinophilic vs neutrophilic inflammation. *BMC Pulm Med* 2013; 13: 11.
247. Cowan DC, Cowan JO, Palmay R, Williamson A, Taylor DR. Effects of steroid therapy on inflammatory cell subtypes in asthma. *Thorax* 2010; 65: 384-390.
248. Nair P, Surette MG, Virchow JC. Neutrophilic asthma: misconception or misnomer? *The Lancet Respiratory Medicine* 2021.
249. Kermani NZ, Pavlidis S, Xie J, Sun K, Loza M, Baribaud F, Fowler SJ, Shaw DE, Fleming LJ, Howarth PH, Sousa AR, Corfield J, Auffray C, De Meulder B, Sterk PJ, Guo Y, Uddin M, Djukanovic R, Adcock IM, Chung KF, group UBs. Instability of sputum molecular phenotypes in U-BIOPRED severe asthma. *Eur Respir J* 2021; 57.
250. Nwaru BI, Ekstrom M, Hasvold P, Wiklund F, Telg G, Janson C. Overuse of short-acting beta2-agonists in asthma is associated with increased risk of exacerbation and

mortality: a nationwide cohort study of the global SABINA programme. *Eur Respir J* 2020; 55.

251. Kwong KY, Morpew T, Scott L, Guterman J, Jones CA. Asthma control and future asthma-related morbidity in inner-city asthmatic children. *Ann Allergy Asthma Immunol* 2008; 101: 144-152.

252. Raheison C, Bourdin A, Bonniaud P, Deslee G, Garcia G, Leroyer C, Taille C, De Blic J, Dubus JC, Tillie-Leblond I, Chanez P. Updated guidelines (2015) for management and monitoring of adult and adolescent asthmatic patients (from 12 years and older) of the Societe de pneumologie de langue francaise (SPLF) (summary). *Rev Mal Respir* 2016; 33: 271-278.

253. Juniper EF, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control. *Eur Respir J* 1999; 14: 902-907.

254. Nathan RA, Sorkness CA, Kosinski M, Schatz M, Li JT, Marcus P, Murray JJ, Pendergraft TB. Development of the asthma control test: a survey for assessing asthma control. *J Allergy Clin Immunol* 2004; 113: 59-65.

255. Reddel HK, Taylor DR, Bateman ED, Boulet LP, Boushey HA, Busse WW, Casale TB, Chanez P, Enright PL, Gibson PG, de Jongste JC, Kerstjens HA, Lazarus SC, Levy ML, O'Byrne PM, Partridge MR, Pavord ID, Sears MR, Sterk PJ, Stoloff SW, Sullivan SD, Szeffler SJ, Thomas MD, Wenzel SE, American Thoracic Society/European Respiratory Society Task Force on Asthma C, Exacerbations. An official American Thoracic Society/European Respiratory Society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. *Am J Respir Crit Care Med* 2009; 180: 59-99.

256. Schatz M, Sorkness CA, Li JT, Marcus P, Murray JJ, Nathan RA, Kosinski M, Pendergraft TB, Jhingran P. Asthma Control Test: reliability, validity, and responsiveness in patients not previously followed by asthma specialists. *J Allergy Clin Immunol* 2006; 117: 549-556.

257. Suissa S, Ernst P, Benayoun S, Baltzan M, Cai B. Low-dose inhaled corticosteroids and the prevention of death from asthma. *N Engl J Med* 2000; 343: 332-336.

258. Umland SP, Schleimer RP, Johnston SL. Review of the molecular and cellular mechanisms of action of glucocorticoids for use in asthma. *Pulm Pharmacol Ther* 2002; 15: 35-50.

259. Wallen N, Kita H, Weiler D, Gleich GJ. Glucocorticoids inhibit cytokine-mediated eosinophil survival. *J Immunol* 1991; 147: 3490-3495.

260. Meagher LC, Cousin JM, Seckl JR, Haslett C. Opposing effects of glucocorticoids on the rate of apoptosis in neutrophilic and eosinophilic granulocytes. *J Immunol* 1996; 156: 4422-4428.

261. Goldsmith AM, Hershenson MB, Wolbert MP, Bentley JK. Regulation of airway smooth muscle alpha-actin expression by glucocorticoids. *Am J Physiol Lung Cell Mol Physiol* 2007; 292: L99-L106.

262. Goto K, Chiba Y, Sakai H, Misawa M. Glucocorticoids inhibited airway hyperresponsiveness through downregulation of CPI-17 in bronchial smooth muscle. *Eur J Pharmacol* 2008; 591: 231-236.

263. Leung SY, Eynott P, Nath P, Chung KF. Effects of ciclesonide and fluticasone propionate on allergen-induced airway inflammation and remodeling features. *J Allergy Clin Immunol* 2005; 115: 989-996.

264. Chetta A, Zanini A, Foresi A, Del Donno M, Castagnaro A, D'Ippolito R, Baraldo

S, Testi R, Saetta M, Olivieri D. Vascular component of airway remodeling in asthma is reduced by high dose of fluticasone. *Am J Respir Crit Care Med* 2003; 167: 751-757.

265. Ward C, Pais M, Bish R, Reid D, Feltis B, Johns D, Walters EH. Airway inflammation, basement membrane thickening and bronchial hyperresponsiveness in asthma. *Thorax* 2002; 57: 309-316.

266. Humbert M, Beasley R, Ayres J, Slavin R, Hebert J, Bousquet J, Beeh KM, Ramos S, Canonica GW, Hedgcock S, Fox H, Blogg M, Surrey K. Benefits of omalizumab as add-on therapy in patients with severe persistent asthma who are inadequately controlled despite best available therapy (GINA 2002 step 4 treatment): INNOVATE. *Allergy* 2005; 60: 309-316.

267. Holgate ST, Chuchalin AG, Hebert J, Lotvall J, Persson GB, Chung KF, Bousquet J, Kerstjens HA, Fox H, Thirlwell J, Cioppa GD, Omalizumab 011 International Study G. Efficacy and safety of a recombinant anti-immunoglobulin E antibody (omalizumab) in severe allergic asthma. *Clin Exp Allergy* 2004; 34: 632-638.

268. Bel EH, Wenzel SE, Thompson PJ, Prazma CM, Keene ON, Yancey SW, Ortega HG, Pavord ID. Oral glucocorticoid-sparing effect of mepolizumab in eosinophilic asthma. *N Engl J Med* 2014; 371: 1189-1197.

269. Ortega HG, Liu MC, Pavord ID, Brusselle GG, FitzGerald JM, Chetta A, Humbert M, Katz LE, Keene ON, Yancey SW, Chanez P. Mepolizumab treatment in patients with severe eosinophilic asthma. *N Engl J Med* 2014; 371: 1198-1207.

270. Castro M, Zangrilli J, Wechsler ME, Bateman ED, Brusselle GG, Bardin P, Murphy K, Maspero JF, O'Brien C, Korn S. Reslizumab for inadequately controlled asthma with elevated blood eosinophil counts: results from two multicentre, parallel, double-blind, randomised, placebo-controlled, phase 3 trials. *Lancet Respir Med* 2015; 3: 355-366.

271. Bleecker ER, FitzGerald JM, Chanez P, Papi A, Weinstein SF, Barker P, Sproule S, Gilmartin G, Aurivillius M, Werkstrom V, Goldman M. Efficacy and safety of benralizumab for patients with severe asthma uncontrolled with high-dosage inhaled corticosteroids and long-acting beta2-agonists (SIROCCO): a randomised, multicentre, placebo-controlled phase 3 trial. *Lancet* 2016; 388: 2115-2127.

272. FitzGerald JM, Bleecker ER, Nair P, Korn S, Ohta K, Lommatzsch M, Ferguson GT, Busse WW, Barker P, Sproule S, Gilmartin G, Werkstrom V, Aurivillius M, Goldman M. Benralizumab, an anti-interleukin-5 receptor alpha monoclonal antibody, as add-on treatment for patients with severe, uncontrolled, eosinophilic asthma (CALIMA): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet* 2016; 388: 2128-2141.

273. Nair P, Wenzel S, Rabe KF, Bourdin A, Lugogo NL, Kuna P, Barker P, Sproule S, Ponnarambil S, Goldman M. Oral Glucocorticoid-Sparing Effect of Benralizumab in Severe Asthma. *N Engl J Med* 2017; 376: 2448-2458.

274. Castro M, Corren J, Pavord ID, Maspero J, Wenzel S, Rabe KF, Busse WW, Ford L, Sher L, FitzGerald JM, Katelaris C, Tohda Y, Zhang B, Staudinger H, Pirozzi G, Amin N, Ruddy M, Akinlade B, Khan A, Chao J, Martincova R, Graham NMH, Hamilton JD, Swanson BN, Stahl N, Yancopoulos GD, Teper A. Dupilumab Efficacy and Safety in Moderate-to-Severe Uncontrolled Asthma. *N Engl J Med* 2018; 378: 2486-2496.

275. Rabe KF, Nair P, Brusselle G, Maspero JF, Castro M, Sher L, Zhu H, Hamilton JD, Swanson BN, Khan A, Chao J, Staudinger H, Pirozzi G, Antoni C, Amin N, Ruddy M, Akinlade B, Graham NMH, Stahl N, Yancopoulos GD, Teper A. Efficacy and Safety of Dupilumab in Glucocorticoid-Dependent Severe Asthma. *N Engl J Med* 2018; 378: 2475-2485.

276. Menzies-Gow A, Corren J, Bourdin A, Chupp G, Israel E, Wechsler ME, Brightling CE, Griffiths JM, Hellqvist A, Bowen K, Kaur P, Almqvist G, Ponnarambil S, Colice G. Tezepelumab in Adults and Adolescents with Severe, Uncontrolled Asthma. *N Engl J Med* 2021; 384: 1800-1809.
277. Brusselle G, Michils A, Louis R, Dupont L, Van de Maele B, Delobbe A, Pilette C, Lee CS, Gurdain S, Vancayzeele S, Lecomte P, Hermans C, MacDonald K, Song M, Abraham I. "Real-life" effectiveness of omalizumab in patients with severe persistent allergic asthma: The PERSIST study. *Respir Med* 2009; 103: 1633-1642.
278. Molimard M, Buhl R, Niven R, Le Gros V, Thielen A, Thirlwell J, Maykut R, Peachey G. Omalizumab reduces oral corticosteroid use in patients with severe allergic asthma: real-life data. *Respir Med* 2010; 104: 1381-1385.
279. Humbert M, Taille C, Mala L, Le Gros V, Just J, Molimard M. Omalizumab effectiveness in patients with severe allergic asthma according to blood eosinophil count: the STELLAIR study. *Eur Respir J* 2018; 51.
280. Cabon Y, Molinari N, Marin G, Vachier I, Gamez AS, Chanez P, Bourdin A. Comparison of anti-interleukin-5 therapies in patients with severe asthma: global and indirect meta-analyses of randomized placebo-controlled trials. *Clin Exp Allergy* 2016; 47: 129-138.
281. Wenzel S, Castro M, Corren J, Maspero J, Wang L, Zhang B, Pirozzi G, Sutherland ER, Evans RR, Joish VN, Eckert L, Graham NM, Stahl N, Yancopoulos GD, Louis-Tisserand M, Teper A. Dupilumab efficacy and safety in adults with uncontrolled persistent asthma despite use of medium-to-high-dose inhaled corticosteroids plus a long-acting beta2 agonist: a randomised double-blind placebo-controlled pivotal phase 2b dose-ranging trial. *Lancet* 2016; 388: 31-44.
282. Dupin C, Belhadi D, Guilleminault L, Gamez AS, Berger P, De Blay F, Bonniaud P, Leroyer C, Mahay G, Girodet PO, Raheison C, Fry S, Le Bourdelles G, Proust A, Rosencher L, Garcia G, Bourdin A, Chenivresse C, Didier A, Couffignal C, Taille C. Effectiveness and safety of dupilumab for the treatment of severe asthma in a real-life French multi-centre adult cohort. *Clin Exp Allergy* 2020; 50: 789-798.
283. Hanania NA, Korenblat P, Chapman KR, Bateman ED, Kopecky P, Paggiaro P, Yokoyama A, Olsson J, Gray S, Holweg CT, Eisner M, Asare C, Fischer SK, Peng K, Putnam WS, Matthews JG. Efficacy and safety of lebrikizumab in patients with uncontrolled asthma (LAVOLTA I and LAVOLTA II): replicate, phase 3, randomised, double-blind, placebo-controlled trials. *Lancet Respir Med* 2016; 4: 781-796.
284. Panettieri RA, Jr., Sjobring U, Peterffy A, Wessman P, Bowen K, Piper E, Colice G, Brightling CE. Tralokinumab for severe, uncontrolled asthma (STRATOS 1 and STRATOS 2): two randomised, double-blind, placebo-controlled, phase 3 clinical trials. *Lancet Respir Med* 2018; 6: 511-525.
285. Kuna P, Bjermer L, Tornling G. Two Phase II randomized trials on the CRTh2 antagonist AZD1981 in adults with asthma. *Drug Des Devel Ther* 2016; 10: 2759-2770.
286. Bateman ED, O'Brien C, Rugman P, Luke S, Ivanov S, Uddin M. Efficacy and safety of the CRTh2 antagonist AZD1981 as add-on therapy to inhaled corticosteroids and long-acting  $\beta(2)$ -agonists in patients with atopic asthma. *Drug Des Devel Ther* 2018; 12: 1093-1106.
287. Brightling CE, Gaga M, Inoue H, Li J, Maspero J, Wenzel S, Maitra S, Lawrence D, Brockhaus F, Lehmann T, Brindicci C, Knorr B, Bleecker ER. Effectiveness of fevipiprant in reducing exacerbations in patients with severe asthma (LUSTER-1 and LUSTER-2): two phase 3 randomised controlled trials. *The Lancet Respiratory Medicine* 2020.



288. Busse WW, Holgate S, Kerwin E, Chon Y, Feng J, Lin J, Lin SL. Randomized, double-blind, placebo-controlled study of brodalumab, a human anti-IL-17 receptor monoclonal antibody, in moderate to severe asthma. *Am J Respir Crit Care Med* 2013; 188: 1294-1302.
289. Castro M, Rubin AS, Laviolette M, Fiterman J, De Andrade Lima M, Shah PL, Fiss E, Olivenstein R, Thomson NC, Niven RM, Pavord ID, Simoff M, Duhamel DR, McEvoy C, Barbers R, Ten Hacken NH, Wechsler ME, Holmes M, Phillips MJ, Erzurum S, Lunn W, Israel E, Jarjour N, Kraft M, Shargill NS, Quiring J, Berry SM, Cox G, Group AIRTS. Effectiveness and safety of bronchial thermoplasty in the treatment of severe asthma: a multicenter, randomized, double-blind, sham-controlled clinical trial. *Am J Respir Crit Care Med* 2010; 181: 116-124.
290. Wechsler ME, Laviolette M, Rubin AS, Fiterman J, Lapa e Silva JR, Shah PL, Fiss E, Olivenstein R, Thomson NC, Niven RM, Pavord ID, Simoff M, Hales JB, McEvoy C, Slebos DJ, Holmes M, Phillips MJ, Erzurum SC, Hanania NA, Sumino K, Kraft M, Cox G, Serman DH, Hogarth K, Kline JN, Mansur AH, Louie BE, Leeds WM, Barbers RG, Austin JH, Shargill NS, Quiring J, Armstrong B, Castro M, Asthma Intervention Research 2 Trial Study G. Bronchial thermoplasty: Long-term safety and effectiveness in patients with severe persistent asthma. *J Allergy Clin Immunol* 2013; 132: 1295-1302.
291. Thomson NC, Rubin AS, Niven RM, Corris PA, Siersted HC, Olivenstein R, Pavord ID, McCormack D, Laviolette M, Shargill NS, Cox G, Group AIRTS. Long-term (5 year) safety of bronchial thermoplasty: Asthma Intervention Research (AIR) trial. *BMC Pulm Med* 2011; 11: 8.
292. Konietzke P, Weinheimer O, Wielputz MO, Wagner WL, Kaukel P, Eberhardt R, Heussel CP, Kauczor HU, Herth FJ, Schuhmann M. Quantitative CT detects changes in airway dimensions and air-trapping after bronchial thermoplasty for severe asthma. *Eur J Radiol* 2018; 107: 33-38.
293. Langton D, Ing A, Sha J, Bennetts K, Hersch N, Kwok M, Plummer V, Thien F, Farah C. Measuring the effects of bronchial thermoplasty using oscillometry. *Respirology* 2019; 24: 431-436.
294. Lu Y, Xu L, Xia M, Li Y, Cao L. The efficacy and safety of subcutaneous immunotherapy in mite-sensitized subjects with asthma: a meta-analysis. *Respir Care* 2015; 60: 269-278.
295. Xu K, Deng Z, Li D, Yuan H, Liu C, Chen Z, Zhu L. Efficacy of add-on sublingual immunotherapy for adults with asthma: A meta-analysis and systematic review. *Ann Allergy Asthma Immunol* 2018; 121: 186-194.
296. Valovirta E, Petersen TH, Piotrowska T, Laursen MK, Andersen JS, Sorensen HF, Klink R, investigators GAP. Results from the 5-year SQ grass sublingual immunotherapy tablet asthma prevention (GAP) trial in children with grass pollen allergy. *J Allergy Clin Immunol* 2018; 141: 529-538 e513.
297. Pavord ID, Beasley R, Agusti A, Anderson GP, Bel E, Brusselle G, Cullinan P, Custovic A, Ducharme FM, Fahy JV, Frey U, Gibson P, Heaney LG, Holt PG, Humbert M, Lloyd CM, Marks G, Martinez FD, Sly PD, von Mutius E, Wenzel S, Zar HJ, Bush A. After asthma: redefining airways diseases. *The Lancet* 2018; 391: 350-400.
298. McLoughlin RF, McDonald VM. The Management of Extrapulmonary Comorbidities and Treatable Traits; Obesity, Physical Inactivity, Anxiety, and Depression, in Adults With Asthma. *Front Allergy* 2021; 2: 735030.
299. Freitas PD, Xavier RF, McDonald VM, Gibson PG, Cordova-Rivera L, Furlanetto KC, de Oliveira JM, Carvalho-Pinto RM, Cukier A, Stelmach R, Carvalho CRF.

Identification of asthma phenotypes based on extrapulmonary treatable traits. *Eur Respir J* 2021; 57.

300. McDonald VM, Clark VL, Cordova-Rivera L, Wark PAB, Baines KJ, Gibson PG. Targeting treatable traits in severe asthma: a randomised controlled trial. *Eur Respir J* 2020; 55.

301. Colas L, Hassoun D, Magnan A. Needs for Systems Approaches to Better Treat Individuals With Severe Asthma: Predicting Phenotypes and Responses to Treatments. *Front Med (Lausanne)* 2020; 7: 98.

302. Hassoun D, Loirand G, Sauzeau V. [A new pathophysiological element in severe asthma: GTPase Rac]. *Rev Mal Respir* 2022; 39: 100-103.

303. Madaule P, Axel R. A novel ras-related gene family. *Cell* 1985; 41: 31-40.

304. Boureux A, Vignal E, Faure S, Fort P. Evolution of the Rho family of ras-like GTPases in eukaryotes. *Mol Biol Evol* 2007; 24: 203-216.

305. Schaefer A, Reinhard NR, Hordijk PL. Toward understanding RhoGTPase specificity: structure, function and local activation. *Small GTPases* 2014; 5: 6.

306. Vetter IR, Wittinghofer A. The guanine nucleotide-binding switch in three dimensions. *Science* 2001; 294: 1299-1304.

307. Wei Y, Zhang Y, Derewenda U, Liu X, Minor W, Nakamoto RK, Somlyo AV, Somlyo AP, Derewenda ZS. Crystal structure of RhoA-GDP and its functional implications. *Nat Struct Biol* 1997; 4: 699-703.

308. Ihara K, Muraguchi S, Kato M, Shimizu T, Shirakawa M, Kuroda S, Kaibuchi K, Hakoshima T. Crystal structure of human RhoA in a dominantly active form complexed with a GTP analogue. *J Biol Chem* 1998; 273: 9656-9666.

309. Loirand G, Sauzeau V, Pacaud P. Small G proteins in the cardiovascular system: physiological and pathological aspects. *Physiol Rev* 2013; 93: 1659-1720.

310. Cherfils J, Zeghouf M. Regulation of small GTPases by GEFs, GAPs, and GDIs. *Physiol Rev* 2013; 93: 269-309.

311. Schmidt A, Hall A. Guanine nucleotide exchange factors for Rho GTPases: turning on the switch. *Genes Dev* 2002; 16: 1587-1609.

312. Bernardis A, Settleman J. GAP control: regulating the regulators of small GTPases. *Trends Cell Biol* 2004; 14: 377-385.

313. Michaelson D, Silletti J, Murphy G, D'Eustachio P, Rush M, Philips MR. Differential localization of Rho GTPases in live cells: regulation by hypervariable regions and RhoGDI binding. *J Cell Biol* 2001; 152: 111-126.

314. Hodge RG, Ridley AJ. Regulating Rho GTPases and their regulators. *Nat Rev Mol Cell Biol* 2016; 17: 496-510.

315. Ridley AJ. Rho family proteins: coordinating cell responses. *Trends Cell Biol* 2001; 11: 471-477.

316. Etienne-Manneville S, Hall A. Rho GTPases in cell biology. *Nature* 2002; 420: 629-635.

317. Nobes CD, Hall A. Rho, rac, and cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. *Cell* 1995; 81: 53-62.

318. Peng J, Wallar BJ, Flanders A, Swiatek PJ, Alberts AS. Disruption of the Diaphanous-Related Formin Drf1 Gene Encoding mDia1 Reveals a Role for Drf3 as an Effector for Cdc42. *Current Biology* 2003; 13: 534-545.

319. Stradal TE, Scita G. Protein complexes regulating Arp2/3-mediated actin assembly. *Curr Opin Cell Biol* 2006; 18: 4-10.

320. Suetsugu S, Kurisu S, Oikawa T, Yamazaki D, Oda A, Takenawa T. Optimization

- of WAVE2 complex-induced actin polymerization by membrane-bound IRSp53, PIP(3), and Rac. *J Cell Biol* 2006; 173: 571-585.
321. Millard TH, Sharp SJ, Machesky LM. Signalling to actin assembly via the WASP (Wiskott-Aldrich syndrome protein)-family proteins and the Arp2/3 complex. *Biochem J* 2004; 380: 1-17.
322. Goldsmith ZG, Dhanasekaran DN. G protein regulation of MAPK networks. *Oncogene* 2007; 26: 3122-3142.
323. Takeya R, Sumimoto H. Molecular mechanism for activation of superoxide-producing NADPH oxidases. *Mol Cells* 2003; 16: 271-277.
324. Pertz O. Spatio-temporal Rho GTPase signaling - where are we now? *J Cell Sci* 2010; 123: 1841-1850.
325. Machacek M, Hodgson L, Welch C, Elliott H, Pertz O, Nalbant P, Abell A, Johnson GL, Hahn KM, Danuser G. Coordination of Rho GTPase activities during cell protrusion. *Nature* 2009; 461: 99-103.
326. Hoppe AD, Swanson JA. Cdc42, Rac1, and Rac2 display distinct patterns of activation during phagocytosis. *Mol Biol Cell* 2004; 15: 3509-3519.
327. Olayioye MA, Noll B, Hausser A. Spatiotemporal Control of Intracellular Membrane Trafficking by Rho GTPases. *Cells* 2019; 8.
328. Didsbury J, Weber RF, Bokoch GM, Evans T, Snyderman R. rac, a novel ras-related family of proteins that are botulinum toxin substrates. *Journal of Biological Chemistry* 1989; 264: 16378-16382.
329. Ferrandez Y, Zhang W, Peurois F, Akendengue L, Blangy A, Zeghouf M, Cherfils J. Allosteric inhibition of the guanine nucleotide exchange factor DOCK5 by a small molecule. *Sci Rep* 2017; 7: 14409.
330. Lee CF, Carley RE, Butler CA, Morrison AR. Rac GTPase Signaling in Immune-Mediated Mechanisms of Atherosclerosis. *Cells* 2021; 10.
331. Reibel L, Dorseuil O, Stancou R, Bertoglio J, Gacon G. A hemopoietic specific gene encoding a small GTP binding protein is overexpressed during T cell activation. *Biochem Biophys Res Commun* 1991; 175: 451-458.
332. Haataja L, Groffen J, Heisterkamp N. Characterization of RAC3, a novel member of the Rho family. *J Biol Chem* 1997; 272: 20384-20388.
333. Sjostedt E, Zhong W, Fagerberg L, Karlsson M, Mitsios N, Adori C, Oksvold P, Edfors F, Limiszewska A, Hikmet F, Huang J, Du Y, Lin L, Dong Z, Yang L, Liu X, Jiang H, Xu X, Wang J, Yang H, Bolund L, Mardinoglu A, Zhang C, von Feilitzen K, Lindskog C, Ponten F, Luo Y, Hokfelt T, Uhlen M, Mulder J. An atlas of the protein-coding genes in the human, pig, and mouse brain. *Science* 2020; 367.
334. Karlsson M, Zhang C, Mear L, Zhong W, Digre A, Katona B, Sjostedt E, Butler L, Odeberg J, Dusart P, Edfors F, Oksvold P, von Feilitzen K, Zwahlen M, Arif M, Altay O, Li X, Ozcan M, Mardinoglu A, Fagerberg L, Mulder J, Luo Y, Ponten F, Uhlen M, Lindskog C. A single-cell type transcriptomics map of human tissues. *Sci Adv* 2021; 7.
335. Uhlen M, Fagerberg L, Hallstrom BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson A, Kampf C, Sjostedt E, Asplund A, Olsson I, Edlund K, Lundberg E, Navani S, Szigartyo CA, Odeberg J, Djureinovic D, Takanen JO, Hober S, Alm T, Edqvist PH, Berling H, Tegel H, Mulder J, Rockberg J, Nilsson P, Schwenk JM, Hamsten M, von Feilitzen K, Forsberg M, Persson L, Johansson F, Zwahlen M, von Heijne G, Nielsen J, Ponten F. Proteomics. Tissue-based map of the human proteome. *Science* 2015; 347: 1260419.
336. Roberts AW, Kim C, Zhen L, Lowe JB, Kapur R, Petryniak B, Spaetti A, Pollock JD, Borneo JB, Bradford GB, Atkinson SJ, Dinauer MC, Williams DA. Deficiency of the

hematopoietic cell-specific Rho family GTPase Rac2 is characterized by abnormalities in neutrophil function and host defense. *Immunity* 1999; 10: 183-196.

337. Sugihara K, Nakatsuji N, Nakamura K, Nakao K, Hashimoto R, Otani H, Sakagami H, Kondo H, Nozawa S, Aiba A, Katsuki M. Rac1 is required for the formation of three germ layers during gastrulation. *Oncogene* 1998; 17: 3427-3433.

338. Corbetta S, D'Adamo P, Gualdoni S, Braschi C, Berardi N, de Curtis I. Hyperactivity and novelty-induced hyperreactivity in mice lacking Rac3. *Behav Brain Res* 2008; 186: 246-255.

339. Vigorito E, Bell S, Hebeis BJ, Reynolds H, McAdam S, Emson PC, McKenzie A, Turner M. Immunological function in mice lacking the Rac-related GTPase RhoG. *Mol Cell Biol* 2004; 24: 719-729.

340. Rossman KL, Der CJ, Sondek J. GEF means go: turning on RHO GTPases with guanine nucleotide-exchange factors. *Nat Rev Mol Cell Biol* 2005; 6: 167-180.

341. Komander D, Patel M, Laurin M, Fradet N, Pelletier A, Barford D, Cote JF. An alpha-helical extension of the ELMO1 pleckstrin homology domain mediates direct interaction to DOCK180 and is critical in Rac signaling. *Mol Biol Cell* 2008; 19: 4837-4851.

342. Kunimura K, Uruno T, Fukui Y. DOCK family proteins: key players in immune surveillance mechanisms. *Int Immunol* 2020; 32: 5-15.

343. Fukui Y, Hashimoto O, Sanui T, Oono T, Koga H, Abe M, Inayoshi A, Noda M, Oike M, Shirai T, Sasazuki T. Haematopoietic cell-specific CDM family protein DOCK2 is essential for lymphocyte migration. *Nature* 2001; 412: 826-831.

344. Sanui T, Inayoshi A, Noda M, Iwata E, Oike M, Sasazuki T, Fukui Y. DOCK2 Is Essential for Antigen-Induced Translocation of TCR and Lipid Rafts, but Not PKC- $\theta$  and LFA-1, in T Cells. *Immunity* 2003; 19: 119-129.

345. Kunisaki Y, Nishikimi A, Tanaka Y, Takii R, Noda M, Inayoshi A, Watanabe K, Sanematsu F, Sasazuki T, Sasaki T, Fukui Y. DOCK2 is a Rac activator that regulates motility and polarity during neutrophil chemotaxis. *J Cell Biol* 2006; 174: 647-652.

346. Shinohara M, Terada Y, Iwamatsu A, Shinohara A, Mochizuki N, Higuchi M, Gotoh Y, Ihara S, Nagata S, Itoh H, Fukui Y, Jessberger R. SWAP-70 is a guanine-nucleotide-exchange factor that mediates signalling of membrane ruffling. *Nature* 2002; 416: 759-763.

347. Baranov MV, Revelo NH, Dingjan I, Maraspini R, Ter Beest M, Honigmann A, van den Bogaart G. SWAP70 Organizes the Actin Cytoskeleton and Is Essential for Phagocytosis. *Cell Rep* 2016; 17: 1518-1531.

348. Tcherkezian J, Lamarche-Vane N. Current knowledge of the large RhoGAP family of proteins. *Biol Cell* 2007; 99: 67-86.

349. Fidyk NJ, Cerione RA. Understanding the catalytic mechanism of GTPase-activating proteins: demonstration of the importance of switch domain stabilization in the stimulation of GTP hydrolysis. *Biochemistry* 2002; 41: 15644-15653.

350. Amin E, Jaiswal M, Derewenda U, Reis K, Nouri K, Koessmeier KT, Aspenstrom P, Somlyo AV, Dvorsky R, Ahmadian MR. Deciphering the Molecular and Functional Basis of RHOGAP Family Proteins: A SYSTEMATIC APPROACH TOWARD SELECTIVE INACTIVATION OF RHO FAMILY PROTEINS. *J Biol Chem* 2016; 291: 20353-20371.

351. Fukumoto Y, Kaibuchi K, Hori Y, Fujioka H, Araki S, Ueda T, Kikuchi A, Takai Y. Molecular cloning and characterization of a novel type of regulatory protein (GDI) for the rho proteins, ras p21-like small GTP-binding proteins. *Oncogene* 1990; 5: 1321-1328.

352. Lelias JM, Adra CN, Wulf GM, Guillemot JC, Khagad M, Caput D, Lim B. cDNA cloning of a human mRNA preferentially expressed in hematopoietic cells and with homology to a GDP-dissociation inhibitor for the rho GTP-binding proteins. *Proc Natl Acad Sci U S A* 1993; 90: 1479-1483.
353. Zalzman G, Closson V, Camonis J, Honore N, Rousseau-Merck MF, Tavitian A, Olofsson B. RhoGDI-3 is a new GDP dissociation inhibitor (GDI). Identification of a non-cytosolic GDI protein interacting with the small GTP-binding proteins RhoB and RhoG. *J Biol Chem* 1996; 271: 30366-30374.
354. DerMardirossian C, Bokoch GM. GDIs: central regulatory molecules in Rho GTPase activation. *Trends Cell Biol* 2005; 15: 356-363.
355. Olofsson B. Rho guanine dissociation inhibitors: pivotal molecules in cellular signalling. *Cell Signal* 1999; 11: 545-554.
356. Cotteret S, Chernoff J. The evolutionary history of effectors downstream of Cdc42 and Rac. *Genome Biol* 2002; 3: REVIEWS0002.
357. Toliaas KF, Cantley LC, Carpenter CL. Rho family GTPases bind to phosphoinositide kinases. *J Biol Chem* 1995; 270: 17656-17659.
358. Fukata M, Kuroda S, Fujii K, Nakamura T, Shoji I, Matsuura Y, Okawa K, Iwamatsu A, Kikuchi A, Kaibuchi K. Regulation of cross-linking of actin filament by IQGAP1, a target for Cdc42. *J Biol Chem* 1997; 272: 29579-29583.
359. Fukata M, Watanabe T, Noritake J, Nakagawa M, Yamaga M, Kuroda S, Matsuura Y, Iwamatsu A, Perez F, Kaibuchi K. Rac1 and Cdc42 capture microtubules through IQGAP1 and CLIP-170. *Cell* 2002; 109: 873-885.
360. Fanger GR, Johnson NL, Johnson GL. MEK kinases are regulated by EGF and selectively interact with Rac/Cdc42. *EMBO J* 1997; 16: 4961-4972.
361. Nagata K, Puls A, Futter C, Aspenstrom P, Schaefer E, Nakata T, Hirokawa N, Hall A. The MAP kinase kinase kinase MLK2 co-localizes with activated JNK along microtubules and associates with kinesin superfamily motor KIF3. *EMBO J* 1998; 17: 149-158.
362. Teramoto H, Coso OA, Miyata H, Igishi T, Miki T, Gutkind JS. Signaling from the small GTP-binding proteins Rac1 and Cdc42 to the c-Jun N-terminal kinase/stress-activated protein kinase pathway. A role for mixed lineage kinase 3/protein-tyrosine kinase 1, a novel member of the mixed lineage kinase family. *J Biol Chem* 1996; 271: 27225-27228.
363. Diekmann D, Abo A, Johnston C, Segal AW, Hall A. Interaction of Rac with p67phox and regulation of phagocytic NADPH oxidase activity. *Science* 1994; 265: 531-533.
364. Chou MM, Blenis J. The 70 kDa S6 kinase complexes with and is activated by the Rho family G proteins Cdc42 and Rac1. *Cell* 1996; 85: 573-583.
365. Bagrodia S, Cerione RA. Pak to the future. *Trends Cell Biol* 1999; 9: 350-355.
366. Bokoch GM, Vlahos CJ, Wang Y, Knaus UG, Traynor-Kaplan AE. Rac GTPase interacts specifically with phosphatidylinositol 3-kinase. *Biochem J* 1996; 315 ( Pt 3): 775-779.
367. Illenberger D, Schwald F, Pimmer D, Binder W, Maier G, Dietrich A, Gierschik P. Stimulation of phospholipase C-beta2 by the Rho GTPases Cdc42Hs and Rac1. *EMBO J* 1998; 17: 6241-6249.
368. Miki H, Suetsugu S, Takenawa T. WAVE, a novel WASP-family protein involved in actin reorganization induced by Rac. *EMBO J* 1998; 17: 6932-6941.
369. Arana E, Vehlow A, Harwood NE, Vigorito E, Henderson R, Turner M, Tybulewicz VL, Batista FD. Activation of the small GTPase Rac2 via the B cell receptor regulates

- B cell adhesion and immunological-synapse formation. *Immunity* 2008; 28: 88-99.
370. Hajdo-Milasinovic A, van der Kammen RA, Moneva Z, Collard JG. Rac3 inhibits adhesion and differentiation of neuronal cells by modifying GIT1 downstream signaling. *J Cell Sci* 2009; 122: 2127-2136.
371. Mosaddeghzadeh N, Ahmadian MR. The RHO Family GTPases: Mechanisms of Regulation and Signaling. *Cells* 2021; 10.
372. Sells MA, Chernoff J. Emerging from the PAK: The p21-activated protein kinase family. *Trends in Cell Biology* 1997; 7: 162-167.
373. Wang J, Wu JW, Wang ZX. Structural insights into the autoactivation mechanism of p21-activated protein kinase. *Structure* 2011; 19: 1752-1761.
374. Edwards DC, Sanders LC, Bokoch GM, Gill GN. Activation of LIM-kinase by Pak1 couples Rac/Cdc42 GTPase signalling to actin cytoskeletal dynamics. *Nat Cell Biol* 1999; 1: 253-259.
375. Price LS, Leng J, Schwartz MA, Bokoch GM. Activation of Rac and Cdc42 by integrins mediates cell spreading. *Mol Biol Cell* 1998; 9: 1863-1871.
376. Bagrodia S, Derijard B, Davis RJ, Cerione RA. Cdc42 and PAK-mediated signaling leads to Jun kinase and p38 mitogen-activated protein kinase activation. *J Biol Chem* 1995; 270: 27995-27998.
377. Brown JL, Stowers L, Baer M, Trejo J, Coughlin S, Chant J. Human Ste20 homologue hPAK1 links GTPases to the JNK MAP kinase pathway. *Curr Biol* 1996; 6: 598-605.
378. Abo A, Pick E, Hall A, Totty N, Teahan CG, Segal AW. Activation of the NADPH oxidase involves the small GTP-binding protein p21rac1. *Nature* 1991; 353: 668-670.
379. Knaus UG, Heyworth PG, Evans T, Curnutte JT, Bokoch GM. Regulation of phagocyte oxygen radical production by the GTP-binding protein Rac 2. *Science* 1991; 254: 1512-1515.
380. Hordijk PL. Regulation of NADPH oxidases: the role of Rac proteins. *Circ Res* 2006; 98: 453-462.
381. Yamauchi A, Marchal CC, Molitoris J, Pech N, Knaus U, Towe J, Atkinson SJ, Dinauer MC. Rac GTPase isoform-specific regulation of NADPH oxidase and chemotaxis in murine neutrophils in vivo. Role of the C-terminal polybasic domain. *J Biol Chem* 2005; 280: 953-964.
382. Dorseuil O, Reibel L, Bokoch GM, Camonis J, Gacon G. The Rac target NADPH oxidase p67phox interacts preferentially with Rac2 rather than Rac1. *J Biol Chem* 1996; 271: 83-88.
383. Williams DA, Tao W, Yang F, Kim C, Gu Y, Mansfield P, Levine JE, Petryniak B, Derrow CW, Harris C, Jia B, Zheng Y, Ambruso DR, Lowe JB, Atkinson SJ, Dinauer MC, Boxer L. Dominant negative mutation of the hematopoietic-specific Rho GTPase, Rac2, is associated with a human phagocyte immunodeficiency. *Blood* 2000; 96: 1646-1654.
384. Ambruso DR, Knall C, Abell AN, Panepinto J, Kurkchubasche A, Thurman G, Gonzalez-Aller C, Hiester A, deBoer M, Harbeck RJ, Oyer R, Johnson GL, Roos D. Human neutrophil immunodeficiency syndrome is associated with an inhibitory Rac2 mutation. *P Natl Acad Sci USA* 2000; 97: 4654-4659.
385. Gu Y, Jia B, Yang FC, D'Souza M, Harris CE, Derrow CW, Zheng Y, Williams DA. Biochemical and biological characterization of a human Rac2 GTPase mutant associated with phagocytic immunodeficiency. *J Biol Chem* 2001; 276: 15929-15938.
386. Andre-Gregoire G, Dilasser F, Chesne J, Braza F, Magnan A, Loirand G, Sauzeau V. Targeting of Rac1 prevents bronchoconstriction and airway hyperresponsiveness.

*J Allergy Clin Immunol* 2018; 142: 824-833 e823.

387. Kai Y, Motegi M, Suzuki Y, Harada Y, Takeuchi H, Kon R, Ikarashi N, Chiba Y, Kamei J, Sakai H. Increased Rac1 Activation in the Enhanced Carbachol-Induced Bronchial Smooth Muscle Contraction of Repeatedly Antigen-Challenged Mice. *Biol Pharm Bull* 2019; 42: 1605-1607.

388. Sakai H, Kai Y, Sato K, Ikebe M, Chiba Y. Rac1 modulates G-protein-coupled receptor-induced bronchial smooth muscle contraction. *Eur J Pharmacol* 2018; 818: 74-83.

389. Dilasser F, Rose L, Hassoun D, Klein M, Rousselle M, Brosseau C, Guignabert C, Taille C, Dombret MC, Di Candia L, Heddebaut N, Bouchaud G, Pretolani M, Magnan A, Loirand G, Sauzeau V. Essential role of smooth muscle Rac1 in severe asthma-associated airway remodelling. *Thorax* 2021.

390. Page K, Li J, Hodge JA, Liu PT, Vanden Hoek TL, Becker LB, Pestell RG, Rosner MR, Hershenson MB. Characterization of a Rac1 signaling pathway to cyclin D(1) expression in airway smooth muscle cells. *J Biol Chem* 1999; 274: 22065-22071.

391. Juncadella IJ, Kadl A, Sharma AK, Shim YM, Hochreiter-Hufford A, Borish L, Ravichandran KS. Apoptotic cell clearance by bronchial epithelial cells critically influences airway inflammation. *Nature* 2013; 493: 547-551.

392. Gong D, Fei F, Lim M, Yu M, Groffen J, Heisterkamp N. Abr, a negative regulator of Rac, attenuates cockroach allergen-induced asthma in a mouse model. *J Immunol* 2013; 191: 4514-4520.

393. Mulloy JC, Cancelas JA, Filippi MD, Kalfa TA, Guo F, Zheng Y. Rho GTPases in hematopoiesis and hemopathies. *Blood* 2010; 115: 936-947.

394. Gu Y, Filippi MD, Cancelas JA, Siefring JE, Williams EP, Jasti AC, Harris CE, Lee AW, Prabhakar R, Atkinson SJ, Kwiatkowski DJ, Williams DA. Hematopoietic cell regulation by Rac1 and Rac2 guanosine triphosphatases. *Science* 2003; 302: 445-449.

395. Guo F, Cancelas JA, Hildeman D, Williams DA, Zheng Y. Rac GTPase isoforms Rac1 and Rac2 play a redundant and crucial role in T-cell development. *Blood* 2008; 112: 1767-1775.

396. Walmsley MJ, Ooi SK, Reynolds LF, Smith SH, Ruf S, Mathiot A, Vanes L, Williams DA, Cancro MP, Tybulewicz VL. Critical roles for Rac1 and Rac2 GTPases in B cell development and signaling. *Science* 2003; 302: 459-462.

397. Carstanjen D, Yamauchi A, Koornneef A, Zang H, Filippi MD, Harris C, Towe J, Atkinson S, Zheng Y, Dinauer MC, Williams DA. Rac2 regulates neutrophil chemotaxis, superoxide production, and myeloid colony formation through multiple distinct effector pathways. *J Immunol* 2005; 174: 4613-4620.

398. Kuramasu A, Wakabayashi M, Inui M, Yanai K. Distinct Roles of Small GTPases Rac1 and Rac2 in Histamine H4 Receptor-Mediated Chemotaxis of Mast Cells. *J Pharmacol Exp Ther* 2018; 367: 9-19.

399. Benvenuti F, Hugues S, Walmsley M, Ruf S, Fetler L, Popoff M, Tybulewicz VL, Amigorena S. Requirement of Rac1 and Rac2 expression by mature dendritic cells for T cell priming. *Science* 2004; 305: 1150-1153.

400. Caron E, Hall A. Identification of two distinct mechanisms of phagocytosis controlled by different Rho GTPases. *Science* 1998; 282: 1717-1721.

401. Lacy P, Mahmudi-Azer S, Bablitz B, Gilchrist M, Fitzharris P, Cheng D, Man SF, Bokoch GM, Moqbel R. Expression and translocation of Rac2 in eosinophils during superoxide generation. *Immunology* 1999; 98: 244-252.

402. Chae HD, Lee KE, Williams DA, Gu Y. Cross-talk between RhoH and Rac1 in

- regulation of actin cytoskeleton and chemotaxis of hematopoietic progenitor cells. *Blood* 2008; 111: 2597-2605.
403. Cancelas JA, Lee AW, Prabhakar R, Stringer KF, Zheng Y, Williams DA. Rac GTPases differentially integrate signals regulating hematopoietic stem cell localization. *Nat Med* 2005; 11: 886-891.
404. Yang FC, Atkinson SJ, Gu Y, Borneo JB, Roberts AW, Zheng Y, Pennington J, Williams DA. Rac and Cdc42 GTPases control hematopoietic stem cell shape, adhesion, migration, and mobilization. *Proc Natl Acad Sci U S A* 2001; 98: 5614-5618.
405. Dumont C, Corsoni-Tadrzak A, Ruf S, de Boer J, Williams A, Turner M, Kioussis D, Tybulewicz VL. Rac GTPases play critical roles in early T-cell development. *Blood* 2009; 113: 3990-3998.
406. Kurdi AT, Bassil R, Olah M, Wu C, Xiao S, Taga M, Frangieh M, Buttrick T, Orent W, Bradshaw EM, Khoury SJ, Elyaman W. Tiam1/Rac1 complex controls Il17a transcription and autoimmunity. *Nat Commun* 2016; 7: 13048.
407. Arrieumerlou C, Randriamampita C, Bismuth G, Trautmann A. Rac is involved in early TCR signaling. *J Immunol* 2000; 165: 3182-3189.
408. Jacinto E, Werlen G, Karin M. Cooperation between Syk and Rac1 leads to synergistic JNK activation in T lymphocytes. *Immunity* 1998; 8: 31-41.
409. Grill B, Schrader JW. Activation of Rac-1, Rac-2, and Cdc42 by hemopoietic growth factors or cross-linking of the B-lymphocyte receptor for antigen. *Blood* 2002; 100: 3183-3192.
410. Croker BA, Tarlinton DM, Cluse LA, Tuxen AJ, Light A, Yang FC, Williams DA, Roberts AW. The Rac2 guanosine triphosphatase regulates B lymphocyte antigen receptor responses and chemotaxis and is required for establishment of B-1a and marginal zone B lymphocytes. *J Immunol* 2002; 168: 3376-3386.
411. Ushijima M, Uruno T, Nishikimi A, Sanematsu F, Kamikaseda Y, Kunimura K, Sakata D, Okada T, Fukui Y. The Rac Activator DOCK2 Mediates Plasma Cell Differentiation and IgG Antibody Production. *Front Immunol* 2018; 9: 243.
412. Li S, Yamauchi A, Marchal CC, Molitoris JK, Quilliam LA, Dinauer MC. Chemoattractant-stimulated Rac activation in wild-type and Rac2-deficient murine neutrophils: preferential activation of Rac2 and Rac2 gene dosage effect on neutrophil functions. *J Immunol* 2002; 169: 5043-5051.
413. Gomez JC, Soltys J, Okano K, Dinauer MC, Doerschuk CM. The role of Rac2 in regulating neutrophil production in the bone marrow and circulating neutrophil counts. *Am J Pathol* 2008; 173: 507-517.
414. Glogauer M, Marchal CC, Zhu F, Worku A, Clausen BE, Foerster I, Marks P, Downey GP, Dinauer M, Kwiatkowski DJ. Rac1 deletion in mouse neutrophils has selective effects on neutrophil functions. *J Immunol* 2003; 170: 5652-5657.
415. Yamauchi A, Kim C, Li S, Marchal CC, Towe J, Atkinson SJ, Dinauer MC. Rac2-deficient murine macrophages have selective defects in superoxide production and phagocytosis of opsonized particles. *J Immunol* 2004; 173: 5971-5979.
416. Joshi S, Singh AR, Zulcic M, Bao L, Messer K, Ideker T, Dutkowski J, Durden DL. Rac2 controls tumor growth, metastasis and M1-M2 macrophage differentiation in vivo. *PLoS One* 2014; 9: e95893.
417. Joshi S, Singh AR, Wong SS, Zulcic M, Jiang M, Pardo A, Selman M, Hagood JS, Durden DL. Rac2 is required for alternative macrophage activation and bleomycin induced pulmonary fibrosis; a macrophage autonomous phenotype. *PLoS One* 2017; 12: e0182851.
418. Lacy P, Willetts L, Kim JD, Lo AN, Lam B, Maclean EI, Moqbel R, Rothenberg



- ME, Zimmermann N. Agonist activation of f-actin-mediated eosinophil shape change and mediator release is dependent on Rac2. *Int Arch Allergy Immunol* 2011; 156: 137-147.
419. Gu Y, Byrne MC, Paranaivitana NC, Aronow B, Siefiring JE, D'Souza M, Horton HF, Quilliam LA, Williams DA. Rac2, a hematopoiesis-specific Rho GTPase, specifically regulates mast cell protease gene expression in bone marrow-derived mast cells. *Mol Cell Biol* 2002; 22: 7645-7657.
420. Yang FC, Kapur R, King AJ, Tao W, Kim C, Borneo J, Breese R, Marshall M, Dinauer MC, Williams DA. Rac2 stimulates Akt activation affecting BAD/Bcl-XL expression while mediating survival and actin function in primary mast cells. *Immunity* 2000; 12: 557-568.
421. Baier A, Ndoh VN, Lacy P, Eitzen G. Rac1 and Rac2 control distinct events during antigen-stimulated mast cell exocytosis. *J Leukoc Biol* 2014; 95: 763-774.
422. Gotoh K, Tanaka Y, Nishikimi A, Nakamura R, Yamada H, Maeda N, Ishikawa T, Hoshino K, Uruno T, Cao Q, Higashi S, Kawaguchi Y, Enjoji M, Takayanagi R, Kaisho T, Yoshikai Y, Fukui Y. Selective control of type I IFN induction by the Rac activator DOCK2 during TLR-mediated plasmacytoid dendritic cell activation. *J Exp Med* 2010; 207: 721-730.
423. Iwasaki H, Mizuno S, Mayfield R, Shigematsu H, Arinobu Y, Seed B, Gurish MF, Takatsu K, Akashi K. Identification of eosinophil lineage-committed progenitors in the murine bone marrow. *J Exp Med* 2005; 201: 1891-1897.
424. Johnston LK, Hsu CL, Krier-Burris RA, Chhiba KD, Chien KB, McKenzie A, Berdnikovs S, Bryce PJ. IL-33 Precedes IL-5 in Regulating Eosinophil Commitment and Is Required for Eosinophil Homeostasis. *J Immunol* 2016; 197: 3445-3453.
425. Kouro T, Kikuchi Y, Kanazawa H, Hirokawa K, Harada N, Shiiba M, Wakao H, Takaki S, Takatsu K. Critical proline residues of the cytoplasmic domain of the IL-5 receptor alpha chain and its function in IL-5-mediated activation of JAK kinase and STAT5. *Int Immunol* 1996; 8: 237-245.
426. Pazdrak K, Adachi T, Alam R. Src homology 2 protein tyrosine phosphatase (SHPTP2)/Src homology 2 phosphatase 2 (SHP2) tyrosine phosphatase is a positive regulator of the interleukin 5 receptor signal transduction pathways leading to the prolongation of eosinophil survival. *J Exp Med* 1997; 186: 561-568.
427. Burstein ES, Hesterberg DJ, Gutkind JS, Brann MR, Currier EA, Messier TL. The ras-related GTPase rac1 regulates a proliferative pathway selectively utilized by G-protein coupled receptors. *Oncogene* 1998; 17: 1617-1623.
428. Geijsen N, van Delft S, Raaijmakers JAM, Lammers J-WJ, Collard JG, Koenderman L, Coffey PJ. Regulation of p21rac Activation in Human Neutrophils. *Blood* 1999; 94: 1121-1130.
429. Pinto SM, Nirujogi RS, Rojas PL, Patil AH, Manda SS, Subbannayya Y, Roa JC, Chatterjee A, Prasad TS, Pandey A. Quantitative phosphoproteomic analysis of IL-33-mediated signaling. *Proteomics* 2015; 15: 532-544.
430. Shamri R, Young KM, Weller PF. Rho and Rac, but not ROCK, are required for secretion of human and mouse eosinophil-associated RNases. *Clin Exp Allergy* 2019; 49: 190-198.
431. Mitchell T, Lo A, Logan MR, Lacy P, Eitzen G. Primary granule exocytosis in human neutrophils is regulated by Rac-dependent actin remodeling. *Am J Physiol Cell Physiol* 2008; 295: C1354-1365.
432. Fettelet T, Gigon L, Karaulov A, Yousefi S, Simon HU. The Enigma of Eosinophil Degranulation. *Int J Mol Sci* 2021; 22.

433. Knezevic, II, Predescu SA, Neamu RF, Gorovoy MS, Knezevic NM, Easington C, Malik AB, Predescu DN. Tiam1 and Rac1 are required for platelet-activating factor-induced endothelial junctional disassembly and increase in vascular permeability. *J Biol Chem* 2009; 284: 5381-5394.
434. Faroudi M, Hons M, Zachacz A, Dumont C, Lyck R, Stein JV, Tybulewicz VL. Critical roles for Rac GTPases in T-cell migration to and within lymph nodes. *Blood* 2010; 116: 5536-5547.
435. Garcia-Bernal D, Wright N, Sotillo-Mallo E, Nombela-Arrieta C, Stein JV, Bustelo XR, Teixido J. Vav1 and Rac control chemokine-promoted T lymphocyte adhesion mediated by the integrin alpha4beta1. *Mol Biol Cell* 2005; 16: 3223-3235.
436. Yu H, Leitenberg D, Li B, Flavell RA. Deficiency of small GTPase Rac2 affects T cell activation. *J Exp Med* 2001; 194: 915-926.
437. Tarakhovsky A, Turner M, Schaal S, Mee PJ, Duddy LP, Rajewsky K, Tybulewicz VL. Defective antigen receptor-mediated proliferation of B and T cells in the absence of Vav. *Nature* 1995; 374: 467-470.
438. Arrieumerlou C, Donnadieu E, Brennan P, Keryer G, Bismuth G, Cantrell D, Trautmann A. Involvement of phosphoinositide 3-kinase and Rac in membrane ruffling induced by IL-2 in T cells. *Eur J Immunol* 1998; 28: 1877-1885.
439. Llaverro F, Urzelai B, Osinalde N, Galvez P, Lacerda HM, Parada LA, Zugaza JL. Guanine nucleotide exchange factor alphaPIX leads to activation of the Rac 1 GTPase/glycogen phosphorylase pathway in interleukin (IL)-2-stimulated T cells. *J Biol Chem* 2015; 290: 9171-9182.
440. Arrizabalaga O, Lacerda HM, Zubiaga AM, Zugaza JL. Rac1 protein regulates glycogen phosphorylase activation and controls interleukin (IL)-2-dependent T cell proliferation. *J Biol Chem* 2012; 287: 11878-11890.
441. Buttrick T, Khoury SJ, Elyaman W. Opposite functions of STAT3 and Smad3 in regulating Tiam1 expression in Th17 cells. *Small GTPases* 2020; 11: 62-68.
442. Li B, Yu H, Zheng W, Voll R, Na S, Roberts AW, Williams DA, Davis RJ, Ghosh S, Flavell RA. Role of the guanosine triphosphatase Rac2 in T helper 1 cell differentiation. *Science* 2000; 288: 2219-2222.
443. Croker BA, Handman E, Hayball JD, Baldwin TM, Voigt V, Cluse LA, Yang FC, Williams DA, Roberts AW. Rac2-deficient mice display perturbed T-cell distribution and chemotaxis, but only minor abnormalities in T(H)1 responses. *Immunol Cell Biol* 2002; 80: 231-240.
444. Langton D, Ing A, Fielding D, Wang W, Plummer V, Thien F. Bronchodilator responsiveness as a predictor of success for bronchial thermoplasty. *Respirology* 2019; 24: 63-67.
445. Harvey ES, Langton D, Katelaris C, Stevens S, Farah CS, Gillman A, Harrington J, Hew M, Kritikos V, Radhakrishna N, Bardin P, Peters M, Reynolds PN, Upham JW, Baraket M, Bowler S, Bowden J, Chien J, Chung LP, Grainge C, Jenkins C, Katsoulotos GP, Lee J, McDonald VM, Reddel HK, Rimmer J, Wark PAB, Gibson PG. Mepolizumab effectiveness and identification of super-responders in severe asthma. *Eur Respir J* 2020; 55.
446. Goldman M, Hirsch I, Zangrilli JG, Newbold P, Xu X. The association between blood eosinophil count and benralizumab efficacy for patients with severe, uncontrolled asthma: subanalyses of the Phase III SIROCCO and CALIMA studies. *Curr Med Res Opin* 2017; 33: 1605-1613.
447. Corren J, Weinstein S, Janka L, Zangrilli J, Garin M. Phase 3 Study of Reslizumab in Patients With Poorly Controlled Asthma: Effects Across a Broad Range of Eosinophil

Counts. *Chest* 2016; 150: 799-810.

448. Abraham I, Alhossan A, Lee CS, Kutbi H, MacDonald K. 'Real-life' effectiveness studies of omalizumab in adult patients with severe allergic asthma: systematic review. *Allergy* 2016; 71: 593-610.

449. Haldar P, Brightling CE, Singapuri A, Hargadon B, Gupta S, Monteiro W, Bradding P, Green RH, Wardlaw AJ, Ortega H, Pavord ID. Outcomes after cessation of mepolizumab therapy in severe eosinophilic asthma: a 12-month follow-up analysis. *J Allergy Clin Immunol* 2014; 133: 921-923.

450. Slavin RG, Ferioli C, Tannenbaum SJ, Martin C, Blogg M, Lowe PJ. Asthma symptom re-emergence after omalizumab withdrawal correlates well with increasing IgE and decreasing pharmacokinetic concentrations. *J Allergy Clin Immunol* 2009; 123: 107-113 e103.

451. Nopp A, Johansson SG, Adedoyin J, Ankerst J, Palmqvist M, Oman H. After 6 years with Xolair; a 3-year withdrawal follow-up. *Allergy* 2010; 65: 56-60.

452. del Pozo MA, Vicente-Manzanares M, Tejedor R, Serrador JM, Sanchez-Madrid F. Rho GTPases control migration and polarization of adhesion molecules and cytoskeletal ERM components in T lymphocytes. *Eur J Immunol* 1999; 29: 3609-3620.

453. Taylor ML, Metcalfe DD. Kit signal transduction. *Hematol Oncol Clin North Am* 2000; 14: 517-535.

454. Henderson RB, Grys K, Vehlow A, de Bettignies C, Zachacz A, Henley T, Turner M, Batista F, Tybulewicz VL. A novel Rac-dependent checkpoint in B cell development controls entry into the splenic white pulp and cell survival. *J Exp Med* 2010; 207: 837-853.

455. Turner M, Mee PJ, Walters AE, Quinn ME, Mellor AL, Zamoyska R, Tybulewicz VL. A requirement for the Rho-family GTP exchange factor Vav in positive and negative selection of thymocytes. *Immunity* 1997; 7: 451-460.

456. Fischer KD, Zmuldzinas A, Gardner S, Barbacid M, Bernstein A, Guidos C. Defective T-cell receptor signalling and positive selection of Vav-deficient CD4+ CD8+ thymocytes. *Nature* 1995; 374: 474-477.

457. Zhang R, Alt FW, Davidson L, Orkin SH, Swat W. Defective Signaling through the T-Cell and B-Cell Antigen Receptors in Lymphoid Cells Lacking the Vav Protooncogene. *Nature* 1995; 374: 470-473.

458. Song JS, Haleem-Smith H, Arudchandran R, Gomez J, Scott PM, Mill JF, Tan TH, Rivera J. Tyrosine phosphorylation of Vav stimulates IL-6 production in mast cells by a Rac/c-Jun N-terminal kinase-dependent pathway. *J Immunol* 1999; 163: 802-810.

459. Pantarelli C, Welch HCE. Rac-GTPases and Rac-GEFs in neutrophil adhesion, migration and recruitment. *Eur J Clin Invest* 2018; 48 Suppl 2: e12939.

460. Kim C, Marchal CC, Penninger J, Dinauer MC. The hemopoietic Rho/Rac guanine nucleotide exchange factor Vav1 regulates N-formyl-methionyl-leucyl-phenylalanine-activated neutrophil functions. *J Immunol* 2003; 171: 4425-4430.

461. Bahaie NS, Hosseinkhani MR, Ge XN, Kang BN, Ha SG, Blumenthal MS, Jessberger R, Rao SP, Sriramarao P. Regulation of eosinophil trafficking by SWAP-70 and its role in allergic airway inflammation. *J Immunol* 2012; 188: 1479-1490.

462. Gross B, Borggreffe T, Wabl M, Sivalenka RR, Bennett M, Rossi AB, Jessberger R. SWAP-70-deficient mast cells are impaired in development and IgE-mediated degranulation. *European Journal of Immunology* 2002; 32: 1121-1128.

463. Gerard A, van der Kammen RA, Janssen H, Ellenbroek SI, Collard JG. The Rac activator Tiam1 controls efficient T-cell trafficking and route of transendothelial migration. *Blood* 2009; 113: 6138-6147.

## **VI. Annexes**

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# Needs for Systems Approaches to Better Treat Individuals With Severe Asthma: Predicting Phenotypes and Responses to Treatments

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Asthma is a frequent heterogeneous multifactorial chronic disease whose severe forms remain largely uncontrolled despite the availability of many drugs and educational therapy. Several phenotypes and endotypes of severe asthma have been described over the last two decades. Typical type-2-immunity-driven asthma remains the most frequent phenotype, and several targeted therapies have been developed and are now available. On the contrary, non-type-2 immunity-driven severe asthma is less understood and still requires efficient innovative therapies. A personalized approach would allow improving asthma control with the help of robust biomarkers able to predict phenotypes/endotypes, exacerbations, response to targeted treatments and, in the future, possible curative options. Some data from large multicenter cohorts have emerged in recent years, especially in transcriptomics. These data have to be integrated and reproduced longitudinally to provide a systems approach for asthma care. In this focused review, the needs for such an approach and the available data will be reviewed as well as the next steps for achieving personalized medicine in asthma.

**Keywords:** asthma, type 2 inflammation, biologics, system medicine, 4P medicine, omics sciences

## INTRODUCTION

Asthma is a frequent inflammatory chronic disease affecting 240–305 million people worldwide (1), characterized by recurrent episodes of dyspnea, wheezing, chest tightness, and cough. These episodes can evolve to asthma exacerbations, defined as an acute worsening of symptoms that lead to short-acting bronchodilator agonist (SABA) and/or short-course oral corticosteroid use and/or emergency department/general practitioner (GP) unscheduled visits. Severe exacerbations can lead to intensive care unit admission and sometimes to death. Most asthma-related burdens are then due to hospitalizations, days out of work and last but not least altered quality of life (2, 3). Furthermore, asthma incidence has been increasing in industrialized countries for at least three decades, indicating a strong environmental impact (epigenetic pressure) on its pathophysiology and onset that is currently closely related to the “hygiene hypothesis and microbiota” (4, 5). Asthma therefore represents a persistent public health issue in which efforts must focus on optimizing patients’ care and patients’ cure for the decade to come.

Although inhaled steroids, long-acting bronchodilators such as beta-2 agonists (salbutamol, terbutaline as examples) and anticholinergics (ipratropium as an example) have positively

transformed the medical support of asthma, it remains globally uncontrolled in 40–85% of patients (6) complaining of daily symptoms impairing their quality of life (both professional and personal), which leads to exacerbations. Whatever the asthma severity is, poor control remains a critical issue to handle asthma-related burdens and calls for questions: (i) Is the patient adherent to his or her treatment? If not, why? (ii) Should differential diagnosis and/or confounders be considered? (iii) Do any asthma comorbidities remain uncontrolled? (7). Should a pharmacological treatment step-up strategy or a non-pharmacological approach (allergen or occupational avoidance, smoking cessation, anxiety-controlling strategies, respiratory rehabilitation) be considered? (8). Those questions illustrate how complex asthma care is, incorporating many patient life aspects and many care practitioners. This complexity led to chronic disease management programs in which patients were supposed to actively take part in modifying their own disease and environment with the aim of reducing exacerbation rates and improving quality of life (9). This was the first attempt of personalized and participative medicine to better control the disease without modifying its natural course.

Lessons from the clinics are powerful tools for improving our knowledge/paradigm of a disease. Asthma is a multifactorial disease with a huge heterogeneity in clinical presentation, outcomes and response to treatment. The time has come to consider a holistic care and cure approach for asthma thanks to 4P (predictive, preventive, personalized and participative) medicine that can be best fed by new concepts and methods allowing the prediction/prevention of poor asthma control (exacerbations) and response to treatments but also the prediction/prevention of asthma onset. Moreover, such a holistic approach would allow a better understanding of asthma pathophysiology at the patient level, hence improving curative therapies. Among these 4P-related concepts, the systems approach of chronic diseases featured in asthma represents a promising route (10). The improvement in big data management obtained from omics sciences (genomic, transcriptomic, proteomic, lipidomic, glycomomic) has greatly increased the knowledge concerning asthma (Figure 1). The discussion of the state of the art of systems approaches in asthma developed below will thus concentrate on mainstream data and will not detail rapidly outdated data.

## FROM A UNIQUE DISEASE TO MULTIPLE PHENOTYPES

Crosstalk between bench-to-bed and bed-to-bench studies developed during the past 2 decades has led to the emergence of new concepts. In the 1970's Asthma was described as a single disease in which airway hyperresponsiveness (AHR) was the major clinical trait and then treated with SABA. From the 1980's, a clear link between AHR and bronchial inflammation (allergic or not) was established and led to the clinical phenotypes according atopic status and/or the age of onset. To date, asthma is now considered to have a heterogenic broad spectrum of symptoms (11) with multiple phenotypes described according

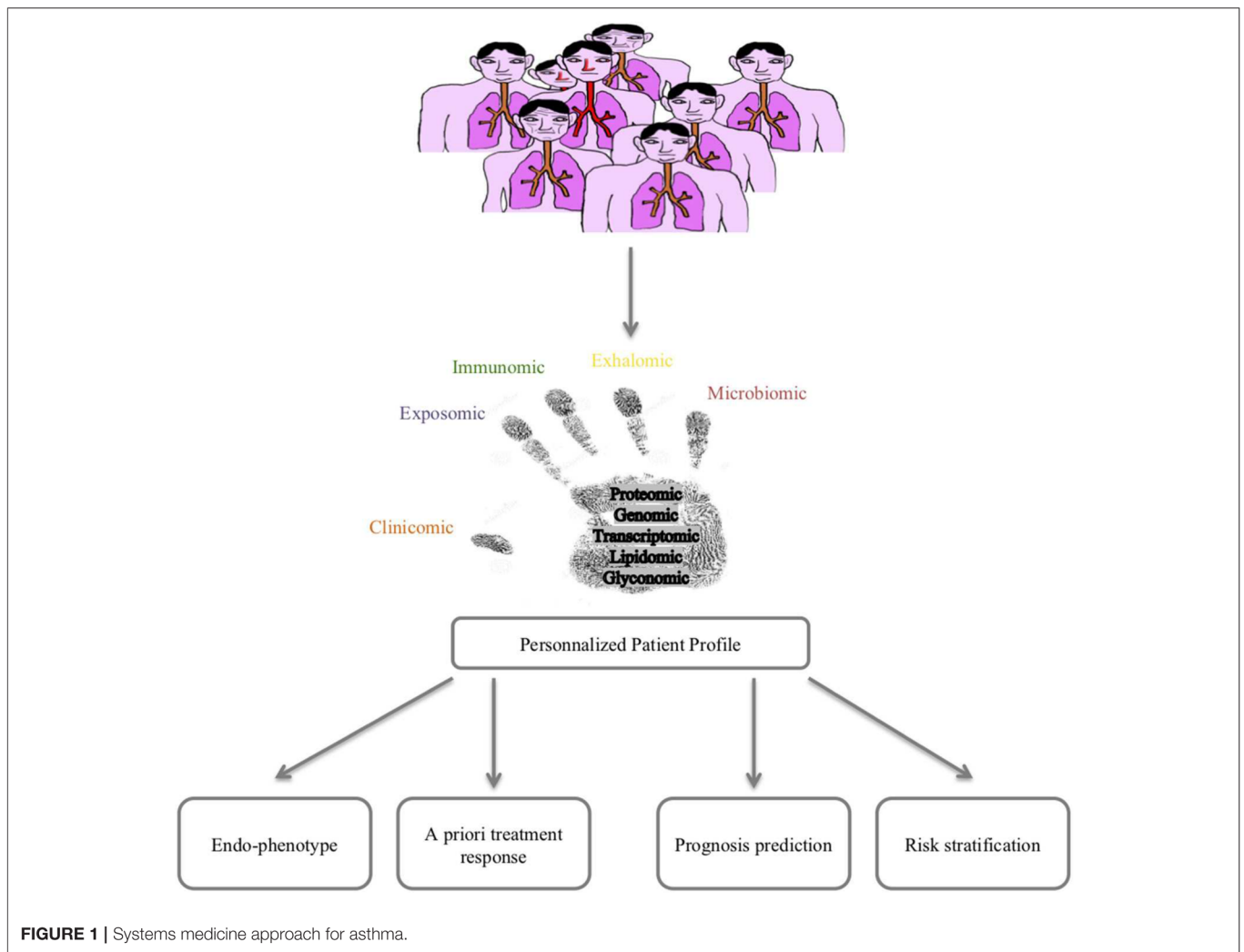
to clinical (age of onset, atopic comorbidities, exacerbation rate, oral corticosteroid and bronchodilator use, and quality of life as examples) and functional parameters (FEV1 and FeNO as examples) as well as multiple cellular and molecular pathways involved, making the story more complex.

## Phenotyping According to Clinical and Functional Parameters

Can clinical, functional and simple biological data be used to achieve a first step in phenotyping? To address this issue, several cohorts were set up: the Severe Asthma Research Program (SARP) in the USA (12), the Leicester cohort in the UK (13), the Airways Disease Endotyping for Personalized Therapeutics (ADEPT) cohort (14), the European Unbiased Biomarkers for the Prediction of Respiratory Disease Outcome Consortium (U-BIOPRED) cohort (15), the pooled European birth cohorts of Mechanisms of the Development of Allergy (MeDALL) (16), the Cohort for Reality and Evolution of Adult Asthma in Korea (COREA) (17), etc. Unbiased methods of clustering led to different phenotypes according to the clinical, functional and biological data chosen. Over time, the stability of those clusters was quite strong overall but varied across phenotypes (18). Although clusters might slightly differ according to the data sets used, some critical characteristics were common, including the age of onset (early vs. late onset of asthma), atopic status, obesity, comorbidities, and eosinophilic inflammation.

Early-onset asthma was mainly represented by type-2-inflammation-allergic asthma, with eosinophilic bronchial infiltration and a moderate blood eosinophil elevation ( $<1,000$  cells/mm<sup>3</sup>), which was a highly stable cluster found in almost every cohort cited above. Allergen-exposure-triggered symptoms and a familial and personal history of atopic diseases (atopic dermatitis, food allergy, and/or allergic rhinitis) were often found. Data from the birth cohort (MeDALL) showed that childhood asthma, rhinitis and eczema were strongly related in symptomatic children, and this finding was interestingly dependent of IgE sensitization to pneumallergens (19). Indeed, there is a frequent association between asthma, regardless of the age of onset and its severity, and aeroallergen sensitization, ranging from 60 to 90% in house dust mite sensitization in many European cohorts (ENFUMOSA, SARP, U-BIOPRED, or TENOR) (20–22). This finding suggested a role for atopy, and, hence, type 2 inflammation, in asthma onset and/or maintenance, which was associated with an increased risk of exacerbation, especially when atopy was established in childhood. In addition, it was suggested that the serum IgE level, which is a non-exclusive biological marker of atopy, was correlated with asthma severity (23).

The role of allergens in asthmatic disease was highlighted through association studies. The most commonly incriminated allergens are house dust mites (*Dermatophagoides farinae* and *pteronyssinus*), house molds and animal dander. House dust mites are associated with a decline in FEV1 and an increase in hospitalization and exacerbation rates (24, 25). Sensitizations to domestic molds of the genera *Aspergillus*

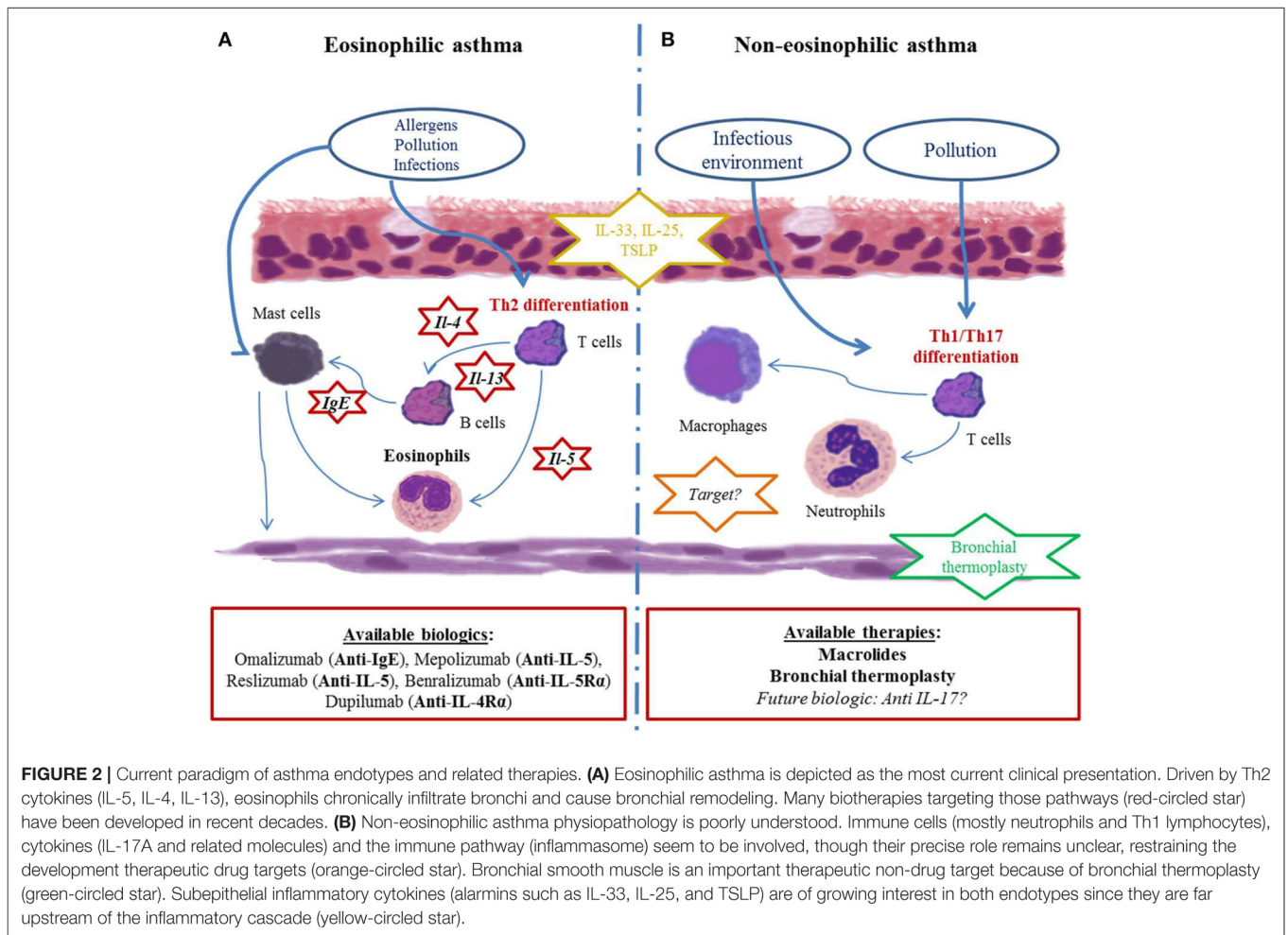


and *Alternaria* are correlated with an increase in exacerbation and hospitalization rates, and this is related to the degree of exposure (high fungal load) (26). Finally, sensitization to animal dander, especially cat dander, is associated with an increase in exacerbation and hospitalization rates in subjects who are highly exposed and with a decline in FEV1 (24, 25). In addition, recent data have evaluated the impact of house dust mite desensitization in a cohort of asthmatic patients ranging from 14 to 70 years of age with mono- or polysensitization to pneumallergens, in which there was a subpopulation of moderate to severe asthmatic individuals. The results showed in patients treated with specific immunotherapy, a significant decrease in inhaled corticosteroid doses and the exacerbation rate, all the more so when asthma was initially poorly controlled and/or moderate to severe (27). Despite these encouraging results, the disease-modifying effect of specific immunotherapy on asthma was not observed in all atopic asthmatic patients, reflecting heterogeneity in bronchial inflammatory patterns and their determinants.

Adult-onset or late-onset asthma is far more heterogeneous, with multiple clinical patterns. Nasal polyposis and aspirin-exacerbated asthma are frequent in this group, which are associated with higher severe airway obstruction and exacerbation rate (28–30).

Exacerbation-prone asthmatic individuals are mainly found among severe asthmatic patients. A recent study using the SARP data set demonstrated that exacerbation-prone asthma was significantly associated with some comorbidities [chronic sinusitis, gastroesophageal reflux disease (GERD) and higher body mass index (BMI)] but also with higher post-albuterol reversibility and blood eosinophils (31).

Obesity-associated asthma is another specific subgroup described in many cohorts. There are many clinical, functional and biological abnormalities found in obese patients to be related to asthma onset and severity (32). Considering functional data, there is a restrictive pattern with airway collapse and a higher airway hyperresponsiveness in obese patients than in non-obese patients (33). Independent of mechanical considerations,



**FIGURE 2 |** Current paradigm of asthma endotypes and related therapies. **(A)** Eosinophilic asthma is depicted as the most current clinical presentation. Driven by Th2 cytokines (IL-5, IL-4, IL-13), eosinophils chronically infiltrate bronchi and cause bronchial remodeling. Many biotherapies targeting those pathways (red-circled star) have been developed in recent decades. **(B)** Non-eosinophilic asthma physiopathology is poorly understood. Immune cells (mostly neutrophils and Th1 lymphocytes), cytokines (IL-17A and related molecules) and the immune pathway (inflammasome) seem to be involved, though their precise role remains unclear, restraining the development therapeutic drug targets (orange-circled star). Bronchial smooth muscle is an important therapeutic non-drug target because of bronchial thermoplasty (green-circled star). Subepithelial inflammatory cytokines (alarmins such as IL-33, IL-25, and TSLP) are of growing interest in both endotypes since they are far upstream of the inflammatory cascade (yellow-circled star).

obesity also leads to significant inflammatory modulation. It can exacerbate eosinophilic inflammation in atopic asthma but also induce and worsen neutrophilic inflammation through TNF $\alpha$ , IL-6, and leptin pathways (34). The diagnosis of asthma in obese patients can be difficult and is often overdiagnosed.

## Phenotyping According to Granulocyte Bronchial Infiltration

In addition to clinical and functional characteristics, blood, and sputum eosinophil levels appear to be critical to define phenotypes of asthma. Since tissue eosinophilia is governed by the production of cytokines such as IL-5 and to a lesser extent by IL-13 and IL-4, it is assumed that eosinophilia either in the blood compartment or in the bronchi of asthmatic patients is a reflect of type 2 inflammation. On the other hand, pathways involved in non-eosinophilic asthma are less clear even though it is thought that non-type-2 immunity, such as type 1 and type 17 inflammation, might play an important role (35) (**Figure 2**). This led to the distinction between type-2- and non-type-2 phenotypes, which also arose from preclinical and clinical development of therapeutic monoclonal antibodies specifically

targeting type 2 inflammation cytokines such as IL-5, IL-4, and/or IL-13 (36).

No clear threshold of blood eosinophil counts can clearly discriminate eosinophilic from non-eosinophilic asthmatic individuals. Although a blood eosinophil count higher than 1,000 cells/mm<sup>3</sup> must encourage the examination of other eosinophil-related systemic diseases, blood eosinophil cell counts between 500 and 1,000 cells/mm<sup>3</sup> are often correlated with eosinophilic airway inflammation. Even with blood eosinophil counts between 300 and 500 cells/mm<sup>3</sup>, it seems that an eosinophilic component might play a role in the pathophysiology of the disease, as shown by the relative efficacy of new biotherapies targeting eosinophils (monoclonal antibodies targeting IL-5 or IL-5R $\alpha$ ) in such patients (37). More recently, a transcriptomic study on airway epithelial cells either from severe asthmatic patients (smoking and non-smoking) or mild-to-moderate asthmatic patients or healthy controls could correlate for the first time a type 2 inflammation predominant gene signature (called “T2-high”) with formerly-assumed biomarkers of type 2 inflammation (blood and sputum eosinophils, FeNO, serum IgE). Blood eosinophil count >115 cells/mm<sup>3</sup> were significantly though weakly correlated with a T2-high signature (38). Despite being



a poor biomarker in determining asthma phenotype, high blood eosinophil counts ( $>1,200$  cells/mm<sup>3</sup>) were recently associated with a higher risk of recurrent exacerbations in patients admitted to the ICU for near-fatal asthma (39).

In induced sputum, a threshold of 3% eosinophils among the recovered cells is commonly used to consider type 2 inflammation-driven asthma. Pavlidis et al., demonstrated that sputum eosinophils  $>1.4\%$  were tightly correlated to type 2 predominant inflammation in severe asthmatic patients (38) rendering this biomarker of great value. However, this useful technique is limited by the complicated clinical procedure that is difficult to apply in clinical practice and is only available in specialized centers (40). The rate of the expired fraction of nitric oxide (FeNO) was also proposed as a biomarker of type 2 inflammation-driven asthma (41). Indeed, FeNO roughly reflects the presence of activated eosinophils in the bronchi and is easy to measure compared to induced-sputum eosinophils. However, FeNO measurements are poorly reproducible and were shown to be poorly correlated to type 2 inflammation in asthmatic patients (38) and are rendering this biomarker of limited value in daily practice. Nevertheless, it is important to identify the type 2 inflammation-driven part of the disease, since it is tightly correlated with the corticosteroid response and the risk of asthma exacerbations.

By contrast, non-eosinophilic and by extension non type 2 asthma poorly responds to corticosteroids. In biopsies from severe asthmatic patients, Wenzel et al. previously reported the existence of neutrophilic and pauci-granulocytic patients (36). An analysis of induced sputum can also distinguish these two patterns of non-eosinophilic patients, although no clear threshold is observed for neutrophils. The same observation was made for the neutrophil blood count. Non-type-2 (neutrophilic/pauci-granulocytic) asthma would therefore appear as an orphan disease, with some drugs such as macrolides being unequally efficient (42), and new drugs being far upstream of any evidence-based interest (anti-IL-17, and drugs targeting epithelial-derived cytokines such as TSLP, IL-33 or IL-25) (43). Recently, Östling et al., on the behalf of U-BIOPRED study group, identified an IL-17-high endotype in the bronchial epithelial cell transcriptome of severe asthmatic patients. The gene signature associated with that endotype was highly similar to the one observed in psoriasis, suggesting anti-IL-17 therapies as a treatment option for those patients (44).

## FROM PHENOTYPES TO TARGETED THERAPIES: TOWARD DECIPHERING MULTIPLES MECHANISMS

From that point of view, asthma could be divided into two main endotypes: type 2 and non-type-2. The former is more frequent, corticosteroid responsive and targeted by several new biologics, whereas the latter is more rare, difficult to treat and comprises obese patients (45). In many patients, it should be considered that type 2 and non-type-2 inflammation are not mutually exclusive. In so-called type 2 asthmatic individuals, a contingent of non-Th2 lymphocytes and neutrophils co-infiltrates the bronchi

together with Th2 lymphocytes and eosinophils (46). Such mixed phenotypes make the story more complex in understanding asthma heterogeneity, though they should be considered in asthma care. Thus, cluster analysis of clinical, functional and basic inflammatory data from large cohorts has permitted the identification of several phenotypes of asthma during the last two decades. These phenotypes are more or less overlapping and are sometimes associated in the same individuals, making it difficult to set up strategies of phenotype-based personalized care (Figure 2).

To understand the pathophysiological pathways involved in the different asthma phenotypes previously described, several molecular studies were run using a modern systems biology unbiased approach with the underlying assumption that discovering associated endotypes would lead to new therapeutic targets.

Woodruff et al. were among the first to identify a panel of 22 differentially expressed genes (mRNA array analysis) in bronchial epithelial cells from mild allergic asthmatic and non-asthmatic patients on the basis of corticosteroid-responsive and tobacco-smoke-induced genes (47, 48). The underlying hypothesis of this novel work was based on epithelial dysfunction driving type 2 inflammation in bronchi due to allergen exposure. These 22 differentially expressed genes could thus reflect new phenotypes and endotypes and new therapeutic targets. Then, the researchers focused on three genes, POSTN (periostin), CLCA1 (chloride channel accessory 1), and SERPINB2 (serpin family B2), which were identified as being upregulated by IL-13 and inhibited by steroids, in a type-2-immunity-driven strategy. Later, high levels of serum periostin proved to be a reliable and promising companion biomarker for identifying a subgroup of severe type-2-immunity-driven asthma patients responding to anti-IL-13 biologics (Figure 2). Though, one of the main limit was the weak number of asthmatic patients under inhaled corticosteroid (ICS). Unfortunately, anti-IL-13 biologics were not as active as expected in severe asthmatic patients to allow further pharmaceutical development (49). It is important to bear in mind that the analysis of serum periostin and anti-IL13 biologics demonstrated that (1) the so-called type-2-immunity-driven phenotype is heterogeneous with an unequal importance of each cytokine pathway in each patient; (2) it is unlikely that a single companion biomarker can provide a specific signature for a specific endotype/phenotype/target; (3) although attractive as a serum assay, serum periostin levels have not proved to be a better discriminant of the type-2-immunity-driven phenotype than blood and sputum eosinophil counts and FeNO, especially when it comes to severe asthma (38, 50); and (4) there is a need for companion markers that are able to predict the response to targeted therapies.

Although conventional drugs, both inhaled and systemic steroids and bronchodilators, are designed to be efficient in any asthmatic patient with significant side effects, new drugs have arisen from the better characterization of the underlying mechanisms mostly in type-2-immunity-driven asthma in the last 20 years. These new drugs are monoclonal biologics targeting specific cytokines or their receptors (IL-5, IL-4R $\alpha$ , IL-5R $\alpha$ ) and are expected to be efficient in selected patients only. The main

limits to date of these novel specific immunomodulatory drugs are their high production cost and the lack of reliable companion biomarkers that allow the prediction of the individual response a priori.

Omalizumab is an anti-IgE monoclonal antibody that appeared on the market more than 10 years ago and was approved for severe asthmatic patients with proven allergic involvement. The efficacy of omalizumab should be assessed after 16 weeks in terms of the improvement of asthma control and the reduction of exacerbation rate. Several *post-hoc* studies showed that higher baseline clinical type 2 immunity biomarkers such as blood and sputum eosinophils and FeNO were positively correlated with anti-IgE monoclonal antibodies responsiveness. Interestingly, no correlation were found between serum IgE and atopic/allergic status (38, 51–54) (**Figure 2**). However, ~25% of severe asthmatic patients remained unresponsive to anti-IgE monoclonal antibodies despite presenting with higher baseline type 2 immunity biomarkers. This suggests that more accurate biomarkers or combinations of markers are needed.

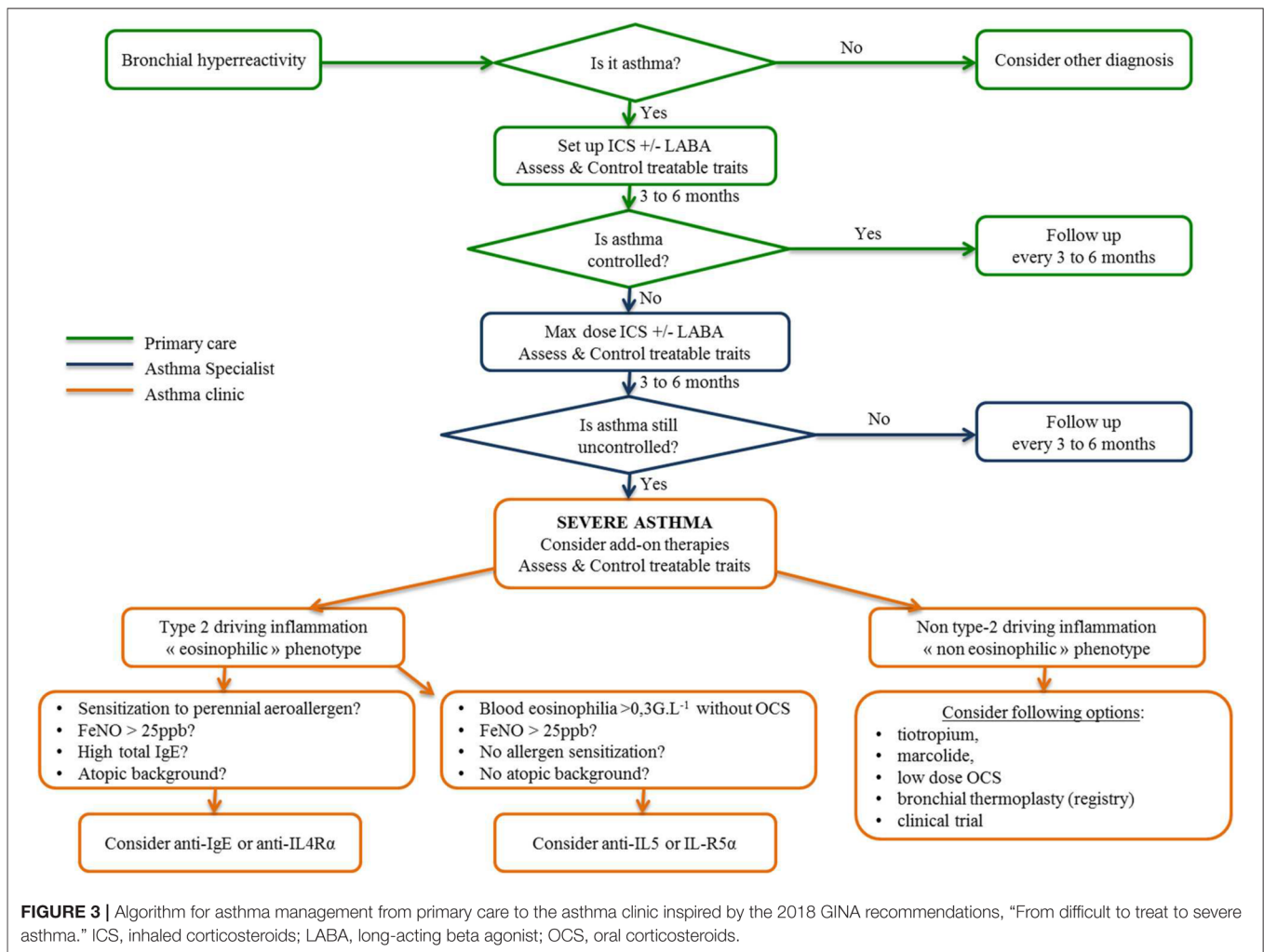
Mepolizumab and reslizumab are anti-IL-5 monoclonal antibodies, albeit benralizumab is an anti-IL-5R $\alpha$  monoclonal antibody, all of which specifically target eosinophils. They have been approved in severe eosinophilic asthma. For these drugs, blood eosinophil count is proposed as a predictive biomarker: the more elevated the blood eosinophil is, the more efficient these drugs are (55). Nevertheless, a proportion of patients, which is yet to be determined in real-life studies, will not respond to these biologics, even with a consequent eosinophil count reduction. Again, this suggests that more accurate biomarkers or combinations of markers are needed (**Figure 2**).

Other biologics targeting type 2 immunity, such as anti-IL-4R $\alpha$  (targeting both IL-4 and IL-13, dupilumab) (56) or anti-TSLP (tezepelumab) (57), are to date in phase II or III trials in type-2- and non-type-2-immunity-driven asthma (**Figure 2**). They are also commercialized in other non-allergic type-2-immunity-driven diseases such as atopic dermatitis (58). These novel drugs are not restricted to eosinophilic asthma or eosinophil-mediated disease. Additionally, IL-33, a cytokine for which a major role was demonstrated in fungus- and virus-induced asthma exacerbations in mouse models but also in moderate asthmatic patients (59, 60), is a target under investigation.

At that stage, it clearly appears that asthma represents a highly heterogeneous entity with many clinical, functional and biological phenotypes intricately involved. Asthma is therefore a chronic disease for which precision medicine must be implemented, especially since targeted expensive drugs are arising. To this aim, reducing asthma to 2 entities, Type 2 inflammation-driven asthma and non-type 2 inflammation-driven asthma, is not satisfactory, and more is needed to further refine asthma phenotypes.

Facing the increasing development of biologics in asthma treatment, international consensus for asthma management has restricted the use of biologics to severe refractory asthma,

whose definition was internationally defined recently (5, 61). Today, the diagnosis and management of asthma is based on defining a personalized and label-free phenotype with *ad hoc* drug and non-drug therapies. To this purpose, Agusti et al. proposed the term “treatable traits,” defined as clinical, functional, biological and psychosocial characteristics classified into three categories (pulmonary, extrapulmonary, and psychosocial), for which therapeutic interventions (drug and non-drug therapies) are possible in theory and linked to an improvement of chronic respiratory disease (62), as depicted in **Figure 3**. First, patients displaying respiratory symptoms concordant with asthma should undergo a thorough clinical examination and pulmonary function tests to exclude all other diagnoses and document bronchial reversibility and/or hyperresponsiveness. When asthma is confirmed, according to up-to-date guidelines, inhaled corticosteroids eventually combined with another controller will be proposed. Treatable traits focusing on extrapulmonary and psychosocial aspects have to be checked and addressed at that stage (primary care). If asthma remains poorly controlled (difficult-to-treat asthma), asthma specialists will be involved to optimize inhaled treatment, reassess differential diagnosis and check and address more treatable traits, especially pulmonary traits (secondary care) (7). When these steps are achieved, if asthma is still uncontrolled, a biologic can be considered as an alternative or add on to a low dose of daily steroids. This requires to determine which type of inflammation is driving the phenotype of these severe asthmatic individuals, according to clinical, functional and biological data as previously described (61, 63). In type 2 eosinophilic allergic patients, anti-IgE or anti-IL4R $\alpha$  should be considered first, whereas in type 2 eosinophilic non-allergic asthmatic patients, anti-IL5 and anti-IL5-R $\alpha$  should be proposed first. To date, no biologic or drug specifically targeting non-type-2 inflammation is available probably due to (1) the relative scarcity of that phenotype compared to type-2 asthma; (2) the ignorance about its physiopathology making it complex to identify therapeutic targets. Low-dose OCS frequently remains the sole option despite long term side effects (diabetes mellitus, increased atherosclerosis, dyslipidemia, high blood pressure, weight gain, osteoporosis, adrenal insufficiency, muscular atrophy, higher frequency of chronic glaucoma and cataract) and variable efficacy in such non-type-2 cases to decrease annual exacerbation rate. OCS related co-morbidities comprise a huge part of the economic and also medical burden in severe asthma. Indeed in a recent study, Barry and al. estimated that non-asthma medication comprised on average from 25 to 30% of the annual cost in severe asthmatic patients (64). It reached up to 80% for diabetes mellitus or osteoporosis as showed in the PACEHR study (65). As a consequence, severe asthmatic patients are more susceptible to be disabled and/or die from OCS related co-morbidities than fatal asthma attacks. As a conclusion, OCS chronic prescription in severe asthmatic patients should be initiated in asthma clinic. The benefit/risk ratio should be followed up at regular intervals or stopped all the more so patients poorly respond. In exacerbation-prone non-eosinophilic asthma, the long-term use of macrolides or bronchial thermoplasty



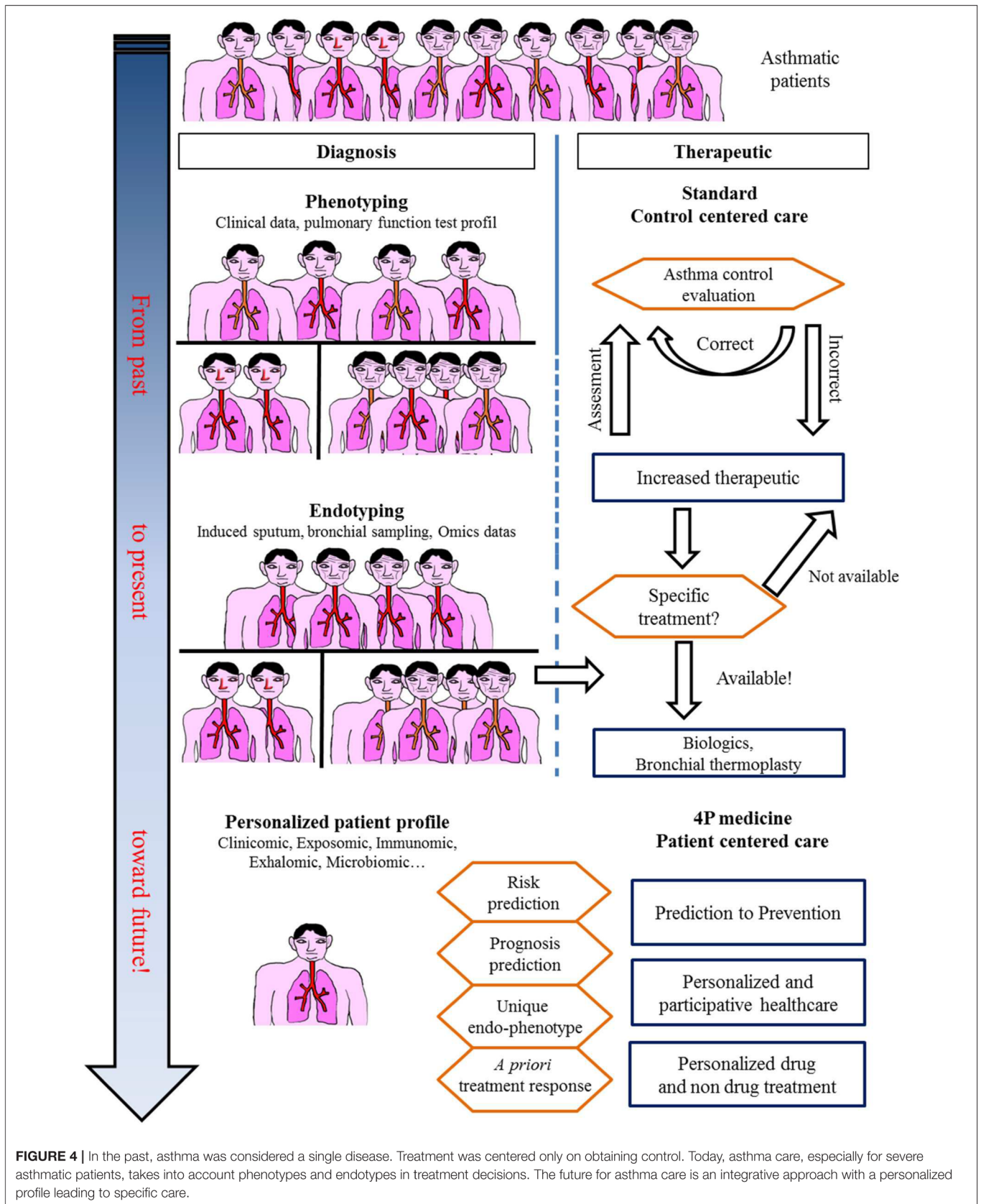
(66) can be discussed. Finally, a clinical trial must be proposed for patients remaining symptomatic despite all available therapies.

## TOWARD A SYSTEMS APPROACH IN ASTHMA: UNBIASED OMICS STUDIES (FIGURE 4)

Whelock et al. nicely reviewed sophisticated large-scale analytical methods to quantify gene expression (transcriptomics), proteins (proteomics), lipids (lipidomics), and metabolites (metabolomics) in various samples, including blood, and as far as pulmonary diseases are concerned, induced sputum, bronchial biopsies, epithelial brushings, and bronchoalveolar lavages (67). Moreover, measurements of volatile organic compounds (VOCs) found in exhaled air and exhaled air condensates (volatolomics, exhalomics) can be addressed as well as the integration of inhaled pollutants and other allergens (exposomics), and more recently characterized pulmonary microbiota (68–70) (Figure 1).

## From Phenotypes to Endotypes Using Unbiased Transcriptomic Approach

To date, omics data in asthma mostly concern mRNA analysis in various compartments (blood, epithelial cells, induced sputum, and bronchial alveolar lavages) obtained from large multicentric cohorts. In the SARP study, bronchial epithelial cell mRNA expression was analyzed in relation to FeNO in 155 individuals with severe asthma and in healthy volunteers (71). On the basis of 549 genes that correlated with FeNO and clinical/functional characteristics, 5 clusters were distinguished, differentiated by 1,384 genes. These genes in turn segregated into 9 gene clusters. Networks of genes related to the type 2 immunity pathway and unknown pathways in asthma pathophysiology were identified. A first Australian report identified upon sputum expression profiling three groups of patients roughly corresponding to what was known from previous works on cellular sputum profiles, i.e., eosinophilic inflammation (group 1), neutrophilic inflammation (group 2), and a pauci-inflammatory profile (group 3) (72). Because of an unbiased approach, this work identified a series of genes related to each phenotype. In a second paper, the same authors validated a six gene pattern



**FIGURE 4 |** In the past, asthma was considered a single disease. Treatment was centered only on obtaining control. Today, asthma care, especially for severe asthmatic patients, takes into account phenotypes and endotypes in treatment decisions. The future for asthma care is an integrative approach with a personalized profile leading to specific care.

that could discriminate eosinophilic from neutrophilic and paucigranulocytic asthma (73). They further explored the extrinsic and intrinsic parameters of this set of genes (i.e., sensitivity, specificity, positive, and negative predictive values) both with blood and sputum eosinophils to predict OCS response (74). The genes were thus chosen from the results of previous studies, with 3 being associated with eosinophilic asthma (CLC, CPA3, and DNASE1L3) and the 3 others being more related to neutrophilic asthma (IL-1 $\beta$ , ALPL, CXCR2). They found one gene (CPA3) to be upregulated before corticosteroid treatment in responder patients, 2 genes for which expression decreased after treatment (CPA3 and CLC), and the 4 other genes remained stable. Importantly, the receiver operating characteristic (ROC) curve showed a better performance of the six gene patterns in predicting OCS response than that of either blood or sputum eosinophils.

A vast amount of transcriptomic data in asthma was published within the last 2 years in the framework of the U-BIOPRED (Unbiased Biomarkers in Prediction of Respiratory Disease Outcomes) European study. In peripheral blood, a severe asthma signature was detected, both in a training and a validation cohort of severe asthmatic subjects compared to non-asthmatic subjects (75). A total of 1,693 differentially expressed genes were identified. Adjustment to the number of circulating cells, which is different between asthmatic and non-asthmatic subjects, reduced the signature to 268 genes. To assess whether the results were OCS dependent (40% of the severe asthmatic subjects included), the analysis repeated in non-OCS-treated patients showed that 30% of the severe asthma signature was independent of OCS. Hierarchical clustering separated severe asthmatic subjects into 2 clusters, one containing 87% of patients with severe asthma and most of the OCS-treated subjects and the other containing 58% of patients with severe asthma and 85% of non-asthmatic patients and mild/moderate asthmatic patients. A series of genes were downregulated in severe asthmatic patients, including B cell-related genes, especially in OCS-treated patients, as expected. T lymphocyte-related genes were in turn of higher expression. The choice of blood compartment to detect signatures specific to bronchial disease is questionable. However, it is widely recognized that blood eosinophils and even serum allergen-specific IgE are suitable biomarkers to consider the eosinophilic or allergic status of asthma and the possibility of using anti-IL5 or anti-IgE monoclonal antibodies, respectively (Figure 3). In addition, as the systems approach is set up to detect predictive biomarkers suitable for daily practice in the clinic, the biomarkers should be non-invasive, reproducible and easy to collect. Finally, asthma can also be considered as part of a systemic disorder, especially when allergic asthma is considered.

Nevertheless, several U-BIOPRED studies reported on transcriptomics from bronchial samples (induced sputum and epithelial cells). Lefaudeux et al. studied the transcriptome and proteome in induced sputum in a training cohort of 266 asthmatic patients and in a validation cohort of 152 patients (76). Four clusters were identified in both cohorts, among which 3 clearly included the most severe patients. Two clusters were related to chronic airflow obstruction, with one associating smokers and ex-smokers with late-onset asthma and

the highest blood eosinophil counts. A third cluster contained non-smokers with OCS therapy and a fourth cluster contained obese females with recurrent asthma exacerbations and normal lung function. Importantly, these clusters are reminiscent of those from previous cluster studies performed in the SARP and Leicester cohorts (12, 13).

Another study of sputum transcriptomics from U-BIOPRED identified 42 genes upregulated in patients with severe asthma compared to mild to moderate asthmatic patients (77). This signature included the IL1 receptor family and inflammasome-associated genes. These genes were also differentially expressed according to the granulocytic status of asthmatic patients (eosinophilic vs. neutrophilic), where IL-13 gene upregulation was associated with eosinophilic asthma. Interestingly, the K Baines group in Australia recently detected inflammasome protein gene expression and notably NLRP3 to be increased in asthmatic sputum and singularly, but not only, in obese asthmatic patients (78). An additional U-BIOPRED paper by Kuo et al. compared mRNA expression in induced sputum reported in 3 transcriptome associated clusters, so-called TACs, with one TAC being characterized by highly type-2-immunity-driven asthma with eosinophilia in the tissue and blood and 2 other non-type-2-immunity-driven TACs characterized by metabolic/mitochondrial and again inflammasome-associated pathways (79). These observations are consistent with two recent studies in which the epithelial IL-6/inflammasome trans-signaling pathway was associated with a severe asthma phenotype prone to a high exacerbation rate, neutrophilic inflammation in sputum and a high BMI index independent of atopic status (80, 81). More precisely, IL-6 trans-signaling was shown to amplify the local inflammatory response and epithelial dysfunction in the lungs, suggesting this mechanism as a potential therapeutic target (81).

Transcriptomics were also applied in induced sputum but also in nasal and bronchial brushings to determine the differential expression of genes in these different compartments within the lungs of asthmatic patients since epithelial dysfunction at asthma onset is widely acknowledged today (82). In this paper, severe adult-onset asthma patients were compared to childhood-onset patients. Differentiating signatures were found in all three compartments, and interestingly, the assignment of gene networks to specific pathophysiological pathways allowed the differentiation of several distinct signatures within each compartment. Specifically, genes related to eosinophilic inflammation, ILC3 and mast cells were upregulated in late-onset asthma. From the same authors, the same study design was used to distinguish patients with persistent airway obstruction. This time, signatures related to the increase in eosinophilic inflammation and IL-13 and to the decrease in IFN- $\gamma$  were identified in persistent obstructive patients, together with genes associated with lung injury and remodeling (50). An additional paper on epithelial brushings and bronchial biopsies determined 4 clusters of patients: 2 were characterized by high expression of type 2 immunity cytokines and a lack of corticosteroid response and differed in the tissue distribution of eosinophils. Another cluster included patients with a higher body mass index. The two other groups were mainly non-eosinophilic (83).

## From Phenotypes to Endotypes Using Unbiased Proteomic/Metabolomic Approach

Beyond transcriptomic analysis, proteomics and metabolomics will also provide new interesting data. For this purpose, the use of exhaled condensates and exhaled air would provide non-invasively obtained lung-derived samples of VOCs (proteins and metabolites) (84). Such samples can easily be taken several times in the same patient and therefore provide longitudinal data that can be integrated with mathematical models, providing for each patient an individual trajectory that can be compared to that of all other patients of a longitudinal cohort (85). Probabilities for exacerbations and response to treatments would then be addressed. Some data have already emerged. Brinkman et al. from the Amsterdam group compared the VOCs at baseline with those of exacerbation and recovery in patients in whom asthma treatments were withdrawn on purpose. eNose correctly classified patients in 21/22 cases of exacerbation compared to baseline and in 17/22 patients of exacerbation compared to recovery (70). The same authors also demonstrated, in an unsupervised approach using exhalomic analysis over an 18-month follow-up, 3 stable clusters of severe asthmatic patients. Two of the clusters referred to the main type of immunity implied (eosinophilic vs. neutrophilic), whereas the other cluster referred to OCS use (69).

A recent unbiased proteomic study on induced sputum supernatant from 200 asthmatic patients (moderate and severe asthma) and 46 healthy volunteers identified 10 subendotypes comprising 247 differential proteins. Eight out of 10 could be meta-classified as eosinophilic (3 subendotypes), neutrophilic (3 subendotypes), or paucigranulocytic but highly atopic (2 subendotypes) according to sputum myeloid cellularity, and the clinical characteristics and transcriptomic pathways were consistent with the 3 TACs described by Kuo et al. (86). The authors also identified predictive biomarkers associated with the 3 main proteotypes. The eosinophilic proteotype was associated with the upregulation of pro-type-2 immunity proteins mostly via an inhibition of neutrophil activation (transthyretin, serotransferrin, alpha1 anti-trypsin) or via the activation of eosinophils (IgG3, C3, histone H4) (86–88). Conversely, the neutrophilic proteotype was associated with the upregulation of proteins secreted by neutrophils (azurocidin, S100, annexin A, neutrophil gelatinase-associated lipocalin, myeloperoxidase) (80). Interestingly, in a hypothesis-based approach, Barbaro et al. detected the exhaled matrix metalloproteinase-9 (MMP9) in different phenotypes of asthma and found it elevated in severe neutrophilic asthma (89), echoing the results of both the aforementioned study by Schofield et al., where MMP9 was associated with neutrophilic inflammation in bronchial mucosa from asthmatic patients (86), and those of a previous study in the field of lung transplantation that showed that the serum MMP9 level was predictive of bronchiolitis obliterans syndrome in lung transplant recipients, a condition also related to airway neutrophilia and bronchial remodeling (90).

## From Phenotypes to Endotypes Using Unbiased Microbiomic and Exposomic Approach

Coupled with biological data coming from tissue- or blood-derived analysis, environmental data and functional microbiomic will provide new insights in underlying mechanisms allowing a better precision medicine approach at individual level. Large databases already exist that are capable of assigning to each individual his or her cumulated exposure to various environmental pollutants. Longitudinal patient data can therefore be coupled to these databases and easily reveal relationships between lung function and pollutant exposure as an example (91). Asthma predisposition factors in early life (parental smoking, RSV and RV infection, asthma in relatives, early sensitization to aeroallergen, atopic dermatitis) are composed of inherited and environmental factors (92–94) where the later seems to play an important role via the hygiene hypothesis and more precisely via microbiota as showed by large epidemiological studies (5, 95, 96). Concerning microbiota, a recent Australian study examined the microbial composition of induced sputum from asthmatic patients and showed that neutrophilic asthma was associated with a different airway microbiology from that seen in other inflammatory phenotypes (97). Further mechanistic studies are needed to better understand the reciprocal interactions and role of the airway microbiota, environmental exposure to pollutants and early life predisposition factors in asthma physiopathology.

## FUTURE DIRECTIONS: FROM OMICS TO “TREATABLE MECHANISMS”

Omics sciences in asthma have started generating a vast amount of data allowing us (1) to better decipher physiopathological pathways and triggers, (2) to better characterize endotypes/phenotypes (clusters of patients), and (3) to better predict exacerbations and/or response to treatments. However, omics data today remain of unequal quality and are unstandardized, rendering comparison and/or extrapolation for modeling the diagnosis or prognosis of asthma difficult. A large effort toward omic data standardization must be considered before defining what the next steps will be, as proposed by the TRIPOD statement (98). Indeed, every unbiased study previously cited naturally attempts to correlate omics-identified patterns (endotype) to well-known phenotypes or characteristics of asthma (eosinophilic vs. non-eosinophilic, severe vs. non-severe, persistent obstructive vs. others, obese vs. non-obese), adding more accurate knowledge to the pathophysiology of asthma and proposing candidate biomarkers for diagnosis and/or prevention. Another perspective from omics sciences is the rising concept of “treatable mechanisms” to replace phenotypes. Indeed, omics sciences have highlighted numerous up- or downregulated pathways that represent potential robust biomarkers bearing enough precision necessary for 4P medicine to better model asthma and provide “care and cure” at the level of a single patient and thus move from

dissociated fingerprints to integrative handprints (Figures 1, 4) (99, 100). Though, one must bear in mind that omic (i.e., molecular phenotype) approach provide association between phenotype and molecular pathways. Mechanistic studies are needed to better decipher/define «treatable mechanisms» and then better define endotypes and therapeutic targets.

Another important question is “How stable are asthma clusters/endotypes/phenotypes and their companion biomarkers in the life span of an asthmatic patient?” A retrospective study based on clinical and functional parameters in 1,325 asthmatic patients with a 20-year follow-up identified 7 baseline clusters of patients. An interesting point is that only 1/5 of the patients moved from a cluster to another during follow-up (18). This question has been addressed by preliminary unbiased studies using omics sciences, such as in the ADEPT cohort, in which a stable and reproducible clustering of patients was found through external validation in the U-BIOPRED cohort over a 12-month follow-up (101). However, these encouraging results need to be confirmed using longitudinal data to achieve an efficient systems approach for modeling asthma “care and cure” in a precision medicine outcome.

In conclusion, recent years have provided a huge amount of data on the heterogeneity of asthma, and the large multicenter initiatives taken are of inestimable value. These data do not provide biomarkers that are ready to use in clinical practice; a great deal of clarification work must be initiated. In addition, longitudinal cohorts aggregating standardized data of various nature for each patient are necessary to achieve a

systems-based approach precision medicine for asthma care and cure (Figures 1, 4).

## AUTHOR CONTRIBUTIONS

AM provided the main concepts of the manuscript (ideas, stratifications, and bibliography). AM, DH, and LC drafted the manuscript. DH and LC conceived the figures and legends and critically revised the manuscript. All of the authors approved the final version of the manuscript.

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## REFERENCES

- GBD 2017 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet*. (2018) 392:1789–858. doi: 10.1016/S0140-6736(18)32279-7
- Papi A, Brightling C, Pedersen SE, Reddel HK. Asthma. *Lancet*. (2018) 391:783–800. doi: 10.1016/S0140-6736(17)33311-1
- Braido F. Failure in asthma control: reasons and consequences. *Scientifica*. (2013) 2013:549252. doi: 10.1155/2013/549252
- Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. *N Engl J Med*. (2002) 347:911–20. doi: 10.1056/NEJMra020100
- Stein MM, Hrusch CL, Gozdz J, Igartua C, Pivniouk V, Murray SE, et al. Innate immunity and asthma risk in amish and hutterite farm children. *N Engl J Med*. (2016) 375:411–21. doi: 10.1056/NEJMoa1508749
- Bousquet J, Khaltaev N. *Global Surveillance, Prevention and Control of Chronic Respiratory Diseases: A Comprehensive Approach*. Geneva: World Health Organization (2007).
- Gherasim A, Dao A, Bernstein JA. Confounders of severe asthma: diagnoses to consider when asthma symptoms persist despite optimal therapy. *World Allergy Organ J*. (2018) 11:29. doi: 10.1186/s40413-018-0207-2
- (IQWiG) IfQaEiHC. *Non-Drug Interventions for Asthma*. Bethesda, MD: Information National Center for Biotechnology; U. S. National Library of Medicine (2017).
- Peytremann-Bridevaux I, Arditi C, Gex G, Bridevaux PO, Burnand B. Chronic disease management programmes for adults with asthma. *Cochrane Database Syst Rev*. (2015) CD007988. doi: 10.1002/14651858.CD007988.pub2
- Hood L, Tian Q. Systems approaches to biology and disease enable translational systems medicine. *Genomics Proteomics Bioinformatics*. (2012) 10:181–5. doi: 10.1016/j.gpb.2012.08.004
- Wenzel SE. Asthma: defining of the persistent adult phenotypes. *Lancet*. (2006) 368:804–13. doi: 10.1016/S0140-6736(06)69290-8
- Moore WC, Bleeker ER, Curran-Everett D, Erzurum SC, Ameredes BT, Bacharier L, et al. Characterization of the severe asthma phenotype by the National heart, lung, and blood institute's severe asthma research program. *J Allergy Clin Immunol*. (2007) 119:405–13. doi: 10.1016/j.jaci.2006.11.639
- Haldar P, Pavord ID, Shaw DE, Berry MA, Thomas M, Brightling CE, et al. Cluster analysis and clinical asthma phenotypes. *Am J Respir Crit Care Med*. (2008) 178:218–24. doi: 10.1164/rccm.200711-1754OC
- Loza MJ, Djukanovic R, Chung KF, Horowitz D, Ma K, Branigan P, et al. Validated and longitudinally stable asthma phenotypes based on cluster analysis of the ADEPT study. *Respir Res*. (2016) 17:165. doi: 10.1186/s12931-016-0482-9
- Shaw DE, Sousa AR, Fowler SJ, Fleming LJ, Roberts G, Corfield J, et al. Clinical and inflammatory characteristics of the European U-BIOPRED adult severe asthma cohort. *Eur Respir J*. (2015) 46:1308–21. doi: 10.1183/13993003.00779-2015
- Anto JM, Bousquet J, Akdis M, Auffray C, Keil T, Momas I, et al. Mechanisms of the Development of Allergy (MeDALL): introducing novel concepts in allergy phenotypes. *J Allergy Clin Immunol*. (2017) 139:388–99. doi: 10.1016/j.jaci.2016.12.940
- Kim TB, Jang AS, Kwon HS, Park JS, Chang YS, Cho SH, et al. Identification of asthma clusters in two independent Korean adult asthma cohorts. *Eur Respir J*. (2013) 41:1308–14. doi: 10.1183/09031936.00100811
- Boudier A, Chanoine S, Accordini S, Anto JM, Basagana X, Bousquet J, et al. Data-driven adult asthma phenotypes based on clinical characteristics are associated with asthma outcomes twenty years later. *Allergy*. (2019) 74:953–63. doi: 10.1111/all.13697
- Garcia-Aymerich J, Benet M, Saey Y, Pinart M, Basagana X, Smit HA, et al. Phenotyping asthma, rhinitis and eczema in MeDALL population-based

- birth cohorts: an allergic comorbidity cluster. *Allergy*. (2015) 70:973–84. doi: 10.1111/all.12640
20. Teague WG, Phillips BR, Fahy JV, Wenzel SE, Fitzpatrick AM, Moore WC, et al. Baseline features of the Severe Asthma Research Program (SARP III) cohort: differences with age. *J Allergy Clin Immunol Pract*. (2018) 6:545–54.e4. doi: 10.1016/j.jaip.2017.05.032
  21. Chipps BE, Zeiger RS, Borish L, Wenzel SE, Yegin A, Hayden ML, et al. Key findings and clinical implications from The Epidemiology and Natural history of asthma: Outcomes and treatment Regimens (TENOR) study. *J Allergy Clin Immunol*. (2012) 130:332–42.e10. doi: 10.1016/j.jaci.2012.04.014
  22. The ENFUMOSA cross-sectional European multicentre study of the clinical phenotype of chronic severe asthma. European network for understanding mechanisms of severe asthma. *Eur Respir J*. (2003) 22:470–7. doi: 10.1183/09031936.03.00261903
  23. Owen CE. Immunoglobulin E: role in asthma and allergic disease: lessons from the clinic. *Pharmacol Ther*. (2007) 113:121–33. doi: 10.1016/j.pharmthera.2006.07.003
  24. Matsui EC, Sampson HA, Bahnson HT, Gruchalla RS, Pongracic JA, Teach SJ, et al. Allergen-specific IgE as a biomarker of exposure plus sensitization in inner-city adolescents with asthma. *Allergy*. (2010) 65:1414–22. doi: 10.1111/j.1398-9995.2010.02412.x
  25. Jaen A, Sunyer J, Basagana X, Chinn S, Zock JP, Anto JM, et al. Specific sensitization to common allergens and pulmonary function in the European community respiratory health survey. *Clin Exp Allergy*. (2002) 32:1713–9. doi: 10.1046/j.1365-2222.2002.01539.x
  26. Sharpe RA, Bearman N, Thornton CR, Husk K, Osborne NJ. Indoor fungal diversity and asthma: a meta-analysis and systematic review of risk factors. *J Allergy Clin Immunol*. (2015) 135:110–22. doi: 10.1016/j.jaci.2014.07.002
  27. Mosbech H, Deckelmann R, de Blay F, Pastorello EA, Trebas-Pietras E, Andres LP, et al. Standardized Quality (SQ) house dust mite sublingual immunotherapy tablet (ALK) reduces inhaled corticosteroid use while maintaining asthma control: a randomized, double-blind, placebo-controlled trial. *J Allergy Clin Immunol*. (2014) 134:568–75.e7. doi: 10.1016/j.jaci.2014.03.019
  28. Mascia K, Haselkorn T, Deniz YM, Miller DP, Bleecker ER, Borish L. Aspirin sensitivity and severity of asthma: evidence for irreversible airway obstruction in patients with severe or difficult-to-treat asthma. *J Allergy Clin Immunol*. (2005) 116:970–5. doi: 10.1016/j.jaci.2005.08.035
  29. White AA, Stevenson DD. Aspirin-exacerbated respiratory disease. *N Engl J Med*. (2018) 379:1060–70. doi: 10.1056/NEJMra1712125
  30. Rajan JP, Wineinger NE, Stevenson DD, White AA. Prevalence of aspirin-exacerbated respiratory disease among asthmatic patients: a meta-analysis of the literature. *J Allergy Clin Immunol*. (2015) 135:676–81.e1. doi: 10.1016/j.jaci.2014.08.020
  31. Denlinger LC, Phillips BR, Ramratnam S, Ross K, Bhakta NR, Cardet JC, et al. Inflammatory and comorbid features of patients with severe asthma and frequent exacerbations. *Am J Respir Crit Care Med*. (2017) 195:302–13. doi: 10.1164/rccm.201602-0419OC
  32. Peters U, Dixon AE, Forno E. Obesity and asthma. *J Allergy Clin Immunol*. (2017) 141:1169–79. doi: 10.1016/j.jaci.2018.02.004
  33. Dixon AE, Peters U. The effect of obesity on lung function. *Expert Rev Respir Med*. (2018) 12:755–67. doi: 10.1080/17476348.2018.1506331
  34. Carr TE, Zeki AA, Kraft M. Eosinophilic and noneosinophilic asthma. *Am J Respir Crit Care Med*. (2018) 197:22–37. doi: 10.1164/rccm.201611-2232PP
  35. Chesne J, Braza F, Mahay G, Brouard S, Aronica M, Magnan A. IL-17 in severe asthma. Where do we stand? *Am J Respir Crit Care Med*. (2014) 190:1094–101. doi: 10.1164/rccm.201405-0859PP
  36. Wenzel SE, Schwartz LB, Langmack EL, Halliday JL, Trudeau JB, Gibbs RL, et al. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. *Am J Respir Crit Care Med*. (1999) 160:1001–8. doi: 10.1164/ajrccm.160.3.9812110
  37. Cabon Y, Molinari N, Marin G, Vachier I, Gamez AS, Chanez P, et al. Comparison of anti-interleukin-5 therapies in patients with severe asthma: global and indirect meta-analyses of randomized placebo-controlled trials. *Clin Exp Allergy*. (2016) 47:129–38. doi: 10.1111/cea.12853
  38. Pavlidis S, Takahashi K, Kwong FNK, Xie J, Hoda U, Sun K, et al. “T2-High” in severe asthma related to blood eosinophil, exhaled nitric oxide and serum periostin. *Eur Respir J*. (2019) 53:1800938. doi: 10.1183/13993003.00938-2018
  39. Yii ACA, Tay TR, Puah SH, Lim HF, Li A, Lau P, et al. Blood eosinophil count correlates with severity of respiratory failure in life-threatening asthma and predicts risk of subsequent exacerbations. *Clin Exp Allergy*. (2019) 49:1578–86. doi: 10.1111/cea.13465
  40. Szefer SJ, Wenzel S, Brown R, Erzurum SC, Fahy JV, Hamilton RG, et al. Asthma outcomes: biomarkers. *J Allergy Clin Immunol*. (2012) 129(3 Suppl.):S9–23. doi: 10.1016/j.jaci.2011.12.979
  41. Lehtimäki L, Csonka P, Mäkinen E, Isojarvi J, Hovi SL, Ahovuo-Saloranta A. Predictive value of exhaled nitric oxide in the management of asthma: a systematic review. *Eur Respir J*. (2016) 48:706–14. doi: 10.1183/13993003.00699-2016
  42. Brusselle GG, Vanderstichele C, Jordens P, Deman R, Slabbynck H, Ringoet V, et al. Azithromycin for prevention of exacerbations in severe asthma (AZISAST): a multicentre randomised double-blind placebo-controlled trial. *Thorax*. (2013) 68:322–9. doi: 10.1136/thoraxjnl-2012-202698
  43. Pepper AN, Renz H, Casale TB, Garn H. Biologic therapy and novel molecular targets of severe asthma. *J Allergy Clin Immunol Pract*. (2017) 5:909–16. doi: 10.1016/j.jaip.2017.04.038
  44. Östling J, van Geest M, Schofield JPR, Jevnikar Z, Wilson S, Ward J, et al. IL-17-high asthma with features of a psoriasis immunophenotype. *J Allergy Clin Immunol*. (2019) 144:1198–213. doi: 10.1016/j.jaci.2019.03.027
  45. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat Med*. (2012) 18:716–25. doi: 10.1038/nm.2678
  46. Simpson JL, Scott R, Boyle MJ, Gibson PG. Inflammatory subtypes in asthma: assessment and identification using induced sputum. *Respirology*. (2006) 11:54–61. doi: 10.1111/j.1440-1843.2006.00784.x
  47. Woodruff PG, Boushey HA, Dolganov GM, Barker CS, Yang YH, Donnelly S, et al. Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids. *Proc Natl Acad Sci USA*. (2007) 104:15858–63. doi: 10.1073/pnas.0707413104
  48. Woodruff PG, Modrek B, Choy DF, Jia G, Abbas AR, Ellwanger A, et al. T-helper type 2-driven inflammation defines major subphenotypes of asthma. *Am J Respir Crit Care Med*. (2009) 180:388–95. doi: 10.1164/rccm.200903-0392OC
  49. Busse WW, Brusselle GG, Korn S, Kuna P, Magnan A, Cohen D, et al. Tralokinumab did not demonstrate oral corticosteroid-sparing effects in severe asthma. *Eur Respir J*. (2018) 53:1800948. doi: 10.1183/13993003.00948-2018
  50. Hekking PP, Loza MJ, Pavlidis S, De Meulder B, Lefaudeux D, Baribaud F, et al. Transcriptomic gene signatures associated with persistent airflow limitation in patients with severe asthma. *Eur Respir J*. (2017) 50:1602298. doi: 10.1183/13993003.02298-2016
  51. Busse W, Spector S, Rosén K, Wang Y, Alpan O. High eosinophil count: a potential biomarker for assessing successful omalizumab treatment effects. *J Allergy Clin Immunol*. (2013) 132:485–6.e11. doi: 10.1016/j.jaci.2013.02.032
  52. Hanania NA, Wenzel S, Rosén K, Hsieh H-J, Mosesova S, Choy DF, et al. Exploring the effects of omalizumab in allergic asthma: an analysis of biomarkers in the EXTRA study. *Am J Respir Crit Care Med*. (2013) 187:804–11. doi: 10.1164/rccm.201208-1414OC
  53. Bousquet J, Wenzel S, Holgate S, Lumry W, Freeman P, Fox H. Predicting response to omalizumab, an anti-IgE antibody, in patients with allergic asthma. *Chest*. (2004) 125:1378–86. doi: 10.1378/chest.125.4.1378
  54. Casale TB, Chipps BE, Rosen K, Trzaskoma B, Haselkorn T, Omachi TA, et al. Response to omalizumab using patient enrichment criteria from trials of novel biologics in asthma. *Allergy*. (2017) 73:490–7. doi: 10.1111/all.13302
  55. Ortega HG, Yancey SW, Mayer B, Gunsoy NB, Keene ON, Bleecker ER, et al. Severe eosinophilic asthma treated with mepolizumab stratified by baseline eosinophil thresholds: a secondary analysis of the DREAM and MENSA studies. *Lancet Respir Med*. (2016) 4:549–56. doi: 10.1016/S2213-2600(16)30031-5
  56. Castro M, Corren J, Pavord ID, Maspero J, Wenzel S, Rabe KF, et al. Dupilumab efficacy and safety in moderate-to-severe uncontrolled asthma. *N Engl J Med*. (2018) 378:2486–96. doi: 10.1056/NEJMoa1804092
  57. Corren J, Parnes JR, Wang L, Mo M, Roseti SL, Griffiths JM, et al. Tezepelumab in adults with uncontrolled asthma. *N Engl J Med*. (2017) 377:936–46. doi: 10.1056/NEJMoa1704064



58. Beck LA, Thaci D, Hamilton JD, Graham NM, Bieber T, Rocklin R, et al. Dupilumab treatment in adults with moderate-to-severe atopic dermatitis. *N Engl J Med*. (2014) 371:130–9. doi: 10.1056/NEJMoa1314768
59. Werder RB, Zhang V, Lynch JP, Snape N, Upham JW, Spann K, et al. Chronic IL-33 expression predisposes to virus-induced asthma exacerbations by increasing type 2 inflammation and dampening antiviral immunity. *J Allergy Clin Immunol*. (2018) 141:1607–19.e9. doi: 10.1016/j.jaci.2017.07.051
60. Castanhinha S, Sherburn R, Walker S, Gupta A, Bossley CJ, Buckley J, et al. Pediatric severe asthma with fungal sensitization is mediated by steroid-resistant IL-33. *J Allergy Clin Immunol*. (2015) 136:312–22.e7. doi: 10.1016/j.jaci.2015.01.016
61. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J*. (2014) 43:343–73. doi: 10.1183/09031936.00202013
62. Agusti A, Bel E, Thomas M, Vogelmeier C, Brusselle G, Holgate S, et al. Treatable traits: toward precision medicine of chronic airway diseases. *Eur Respir J*. (2016) 47:410–9. doi: 10.1183/13993003.01359-2015
63. Bel EH, Sousa A, Fleming L, Bush A, Chung KF, Versnel J, et al. Diagnosis and definition of severe refractory asthma: an international consensus statement from the Innovative Medicine Initiative (IMI). *Thorax*. (2011) 66:910–7. doi: 10.1136/thx.2010.153643
64. Barry LE, Sweeney J, O'Neill C, Price D, Heaney LG. The cost of systemic corticosteroid-induced morbidity in severe asthma: a health economic analysis. *Respir Res*. (2017) 18:129. doi: 10.1186/s12931-017-0614-x
65. Janson C, Lisspers K, Stållberg B, Johansson G, Telg G, Thuresson M, et al. Health care resource utilization and cost for asthma patients regularly treated with oral corticosteroids - a Swedish observational cohort study (PACEHR). *Respir Res*. (2018) 19:168. doi: 10.1186/s12931-018-0855-3
66. Chupp G, Laviolette M, Cohn L, McEvoy C, Bansal S, Shifren A, et al. Long-term outcomes of bronchial thermoplasty in subjects with severe asthma: a comparison of 3-year follow-up results from two prospective multicenter studies. *Eur Respir J*. (2017) 50:1700017. doi: 10.1183/13993003.00017-2017
67. Wheelock CE, Goss VM, Balgoma D, Nicholas B, Brandsma J, Skipp PJ, et al. Application of 'omics technologies to biomarker discovery in inflammatory lung diseases. *Eur Respir J*. (2013) 42:802–25. doi: 10.1183/09031936.00078812
68. Moffatt ME, Cookson WO. The lung microbiome in health and disease. *Clin Med*. (2017) 17:525–9. doi: 10.7861/clinmedicine.17-6-525
69. Brinkman P, Wagener AH, Hekking PP, Bansal AT, Maitland-van der Zee AH, Wang Y, et al. Identification and prospective stability of electronic nose (eNose)-derived inflammatory phenotypes in patients with severe asthma. *J Allergy Clin Immunol*. (2019) 143:1811–20.e7. doi: 10.1016/j.jaci.2018.10.058
70. Brinkman P, van de Pol MA, Gerritsen MG, Bos LD, Dekker T, Smids BS, et al. Exhaled breath profiles in the monitoring of loss of control and clinical recovery in asthma. *Clin Exp Allergy*. (2017) 47:1159–69. doi: 10.1111/cea.12965
71. Modena BD, Tedrow JR, Milosevic J, Bleecker ER, Meyers DA, Wu W, et al. Gene expression in relation to exhaled nitric oxide identifies novel asthma phenotypes with unique biomolecular pathways. *Am J Respir Crit Care Med*. (2014) 190:1363–72. doi: 10.1164/rccm.201406-1099OC
72. Baines KJ, Simpson JL, Wood LG, Scott RJ, Gibson PG. Transcriptional phenotypes of asthma defined by gene expression profiling of induced sputum samples. *J Allergy Clin Immunol*. (2011) 127:153–60; 160.e1–9. doi: 10.1016/j.jaci.2010.10.024
73. Baines KJ, Simpson JL, Wood LG, Scott RJ, Fibbens NL, Powell H, et al. Sputum gene expression signature of 6 biomarkers discriminates asthma inflammatory phenotypes. *J Allergy Clin Immunol*. (2014) 133:997–1007. doi: 10.1016/j.jaci.2013.12.1091
74. Berthon BS, Gibson PG, Wood LG, MacDonald-Wicks LK, Baines KJ. A sputum gene expression signature predicts oral corticosteroid response in asthma. *Eur Respir J*. (2017) 49:1700180. doi: 10.1183/13993003.00180-2017
75. Bigler J, Boedigheimer M, Schofield JPR, Skipp PJ, Corfield J, Rowe A, et al. A severe asthma disease signature from gene expression profiling of peripheral blood from U-BIOPRED cohorts. *Am J Respir Crit Care Med*. (2017) 195:1311–20. doi: 10.1164/rccm.201604-0866OC
76. Lefaudeux D, De Meulder B, Loza MJ, Peffer N, Rowe A, Baribaud F, et al. U-BIOPRED clinical adult asthma clusters linked to a subset of sputum omics. *J Allergy Clin Immunol*. (2017) 139:1797–807. doi: 10.1016/j.jaci.2016.08.048
77. Rossios C, Pavlidis S, Hoda U, Kuo CH, Wiegman C, Russell K, et al. Sputum transcriptomics reveal upregulation of IL-1 receptor family members in patients with severe asthma. *J Allergy Clin Immunol*. (2018) 141:560–70. doi: 10.1016/j.jaci.2017.02.045
78. Wood LG, Li Q, Scott HA, Rutting S, Berthon BS, Gibson PG, et al. Saturated fatty acids, obesity, and the nucleotide oligomerization domain-like receptor protein 3 (NLRP3) inflammasome in asthmatic patients. *J Allergy Clin Immunol*. (2019) 143:305–15. doi: 10.1016/j.jaci.2018.04.037
79. Kuo CS, Pavlidis S, Loza M, Baribaud F, Rowe A, Pandis I, et al. T-helper cell type 2 (Th2) and non-Th2 molecular phenotypes of asthma using sputum transcriptomics in U-BIOPRED. *Eur Respir J*. (2017) 49:1602135. doi: 10.1183/13993003.02135-2016
80. Turan N, Edwards MJ, Bates S, Shaw D, Chung KF, Loza MJ, et al. IL-6 pathway upregulation in subgroup of severe asthma is associated with neutrophilia and poor lung function. *Clin Exp Allergy*. (2018) 48:475–8. doi: 10.1111/cea.13085
81. Jevnikar Z, Östling J, Ax E, Calvén J, Thörn K, Israelsson E, et al. Epithelial IL-6 trans-signaling defines a new asthma phenotype with increased airway inflammation. *J Allergy Clin Immunol*. (2019) 143:577–90. doi: 10.1016/j.jaci.2018.05.026
82. Hekking PP, Loza MJ, Pavlidis S, de Meulder B, Lefaudeux D, Baribaud F, et al. Pathway discovery using transcriptomic profiles in adult-onset severe asthma. *J Allergy Clin Immunol*. (2018) 141:1280–90. doi: 10.1016/j.jaci.2017.06.037
83. Kuo CS, Pavlidis S, Loza M, Baribaud F, Rowe A, Pandis I, et al. A transcriptome-driven analysis of epithelial brushings and bronchial biopsies to define asthma phenotypes in U-BIOPRED. *Am J Respir Crit Care Med*. (2017) 195:443–55. doi: 10.1164/rccm.201512-2452OC
84. Horvath I, Barnes PJ, Loukides S, Sterk PJ, Hogman M, Olin AC, et al. A European respiratory society technical standard: exhaled biomarkers in lung disease. *Eur Respir J*. (2017) 49:17E4904. doi: 10.1183/13993003.E4904-2017
85. Gourraud PA, Henry RG, Cree BA, Crane JC, Lizee A, Olson MP, et al. Precision medicine in chronic disease management: the multiple sclerosis BioScreen. *Ann Neurol*. (2014) 76:633–42. doi: 10.1002/ana.24282
86. Schofield JPR, Burg D, Nicholas B, Strazzeri F, Brandsma J, Staykova D, et al. Stratification of asthma phenotypes by airway proteomic signatures. *J Allergy Clin Immunol*. (2019) 144:70–82. doi: 10.1016/j.jaci.2019.03.013
87. Kaneko M, Swanson MC, Gleich GJ, Kita H. Allergen-specific IgG1 and IgG3 through Fc gamma RII induce eosinophil degranulation. *J Clin Invest*. (1995) 95:2813–21. doi: 10.1172/JCI117986
88. Ishihara K, Hong J, Zee O, Ohuchi K. Possible mechanism of action of the histone deacetylase inhibitors for the induction of differentiation of HL-60 clone 15 cells into eosinophils. *Br J Pharmacol*. (2004) 142:1020–30. doi: 10.1038/sj.bjp.0705869
89. Barbaro MP, Spanevello A, Palladino GP, Salerno FG, Lacedonia D, Carpagnano GE. Exhaled matrix metalloproteinase-9 (MMP-9) in different biological phenotypes of asthma. *Eur J Intern Med*. (2014) 25:92–6. doi: 10.1016/j.ejim.2013.08.705
90. Pain M, Royer PJ, Loy J, Girardeau A, Tissot A, Lacoste P, et al. T cells promote bronchial epithelial cell secretion of matrix metalloproteinase-9 via a C-C chemokine receptor type 2 pathway: implications for chronic lung allograft dysfunction. *Am J Transplant*. (2017) 17:1502–14. doi: 10.1111/ajt.14166
91. Benmerad M, Slama R, Botturi K, Claustre J, Roux A, Sage E, et al. Chronic effects of air pollution on lung function after lung transplantation in the Systems prediction of Chronic Lung Allograft Dysfunction (SysCLAD) study. *Eur Respir J*. (2017) 49:1600206. doi: 10.1183/13993003.00206-2016
92. Rhodes HL, Sporik R, Thomas P, Holgate ST, Cogswell JJ. Early life risk factors for adult asthma: a birth cohort study of subjects at risk. *J Allergy Clin Immunol*. (2001) 108:720–5. doi: 10.1067/mai.2001.119151

93. Arshad SH, Kurukulaaratchy RJ, Fenn M, Matthews S. Early life risk factors for current wheeze, asthma, and bronchial hyperresponsiveness at 10 years of age. *Chest*. (2005)127:502–8. doi: 10.1378/chest.127.2.502
94. Feldman AS, He Y, Moore ML, Hershenson MB, Hartert TV. Toward primary prevention of asthma. Reviewing the evidence for early-life respiratory viral infections as modifiable risk factors to prevent childhood asthma. *Am J Respir Crit Care Med*. (2015) 191:34–44. doi: 10.1164/rccm.201405-0901PP
95. von Mutius E, Illi S, Hirsch T, Leupold W, Keil U, Weiland SK. Frequency of infections and risk of asthma, atopy and airway hyperresponsiveness in children. *Eur Respir J*. (1999) 14:4–11. doi: 10.1034/j.1399-3003.1999.14a03.x
96. von Mutius E, Vercelli D. Farm living: effects on childhood asthma and allergy. *Nat Rev Immunol*. (2010) 10:861–8. doi: 10.1038/nri2871
97. Taylor SL, Leong LEX, Choo JM, Wesselingh S, Yang IA, Upham JW, et al. Inflammatory phenotypes in patients with severe asthma are associated with distinct airway microbiology. *J Allergy Clin Immunol*. (2018) 141:94–103.e15. doi: 10.1016/j.jaci.2017.03.044
98. Collins GS, Reitsma JB, Altman DG, Moons KG. Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): the TRIPOD statement. *BMC Med*. (2015) 13:1. doi: 10.1186/s12916-014-0241-z
99. Chung KF, Adcock IM. Precision medicine for the discovery of treatable mechanisms in severe asthma. *Allergy*. (2019) 74:1649–59. doi: 10.1111/all.13771
100. De Meulder B, Lefaudeux D, Bansal AT, Mazein A, Chaiboonchoe A, Ahmed H, et al. A computational framework for complex disease stratification from multiple large-scale datasets. *BMC Syst Biol*. (2018) 12:60. doi: 10.1186/s12918-018-0556-z
101. Silkoff PE, Laviolette M, Singh D, FitzGerald JM, Kelsen S, Backer V, et al. Longitudinal stability of asthma characteristics and biomarkers from the Airways Disease Endotyping for Personalized Therapeutics (ADEPT) study. *Respir Res*. (2016) 17:43. doi: 10.1186/s12931-016-0360-5

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Original research

# Essential role of smooth muscle Rac1 in severe asthma-associated airway remodelling

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## ABSTRACT

**Background** Severe asthma is a chronic lung disease characterised by inflammation, airway hyperresponsiveness (AHR) and airway remodelling. The molecular mechanisms underlying uncontrolled airway smooth muscle cell (aSMC) proliferation involved in pulmonary remodelling are still largely unknown. Small G proteins of the Rho family (RhoA, Rac1 and Cdc42) are key regulators of smooth muscle functions and we recently demonstrated that Rac1 is activated in aSMC from allergic mice. The objective of this study was to assess the role of Rac1 in severe asthma-associated airway remodelling.

**Methods and results** Immunofluorescence analysis in human bronchial biopsies revealed an increased Rac1 activity in aSMC from patients with severe asthma compared with control subjects. Inhibition of Rac1 by EHT1864 showed that Rac1 signalling controlled human aSMC proliferation induced by mitogenic stimuli through the signal transducer and activator of transcription 3 (STAT3) signalling pathway. In vivo, specific deletion of Rac1 in SMC or pharmacological inhibition of Rac1 by nebulisation of NSC23766 prevented AHR and aSMC hyperplasia in a mouse model of severe asthma. Moreover, the Rac1 inhibitor prevented goblet cell hyperplasia and epithelial cell hypertrophy whereas treatment with corticosteroids had less effect. Nebulisation of NSC23766 also decreased eosinophil accumulation in the bronchoalveolar lavage of asthmatic mice.

**Conclusion** This study demonstrates that Rac1 is overactive in the airways of patients with severe asthma and is essential for aSMC proliferation. It also provides evidence that Rac1 is causally involved in AHR and airway remodelling. Rac1 may represent as an interesting target for treating both AHR and airway remodelling of patients with severe asthma.

## INTRODUCTION

Asthma is a heterogenous and complex disease that affects more than 300 million people worldwide.<sup>1,2</sup> The disease expression includes wheezy dyspnoea, expiration blocking, cough and thoracic oppression in a context of chronic bronchial inflammation.<sup>3</sup> Current treatments are based on anti-inflammatory therapies (inhaled and oral corticosteroids) and inhaled bronchodilators. Severe asthma is defined as asthma that is not improved by standard treatment

## key message

### What is the key question?

► The objective of this study was to assess the role of Rac1 in severe asthma-associated airway remodelling.

### What is the bottom line?

► The current study unveils an overactivation of smooth muscle Rac1 in bronchi from severe asthmatics, and highlights a leading role of Rac1 in airway remodeling.

### Why read on?

► Rac1 appears as a new attractive therapeutic target in severe asthma.

and that requires a combination of high doses of inhaled corticosteroids with an add-on therapy to be controlled, or that remains uncontrolled or even worsens despite these treatments.<sup>4</sup> Severe asthma leads to a poor quality of life and important health-care expenses due to direct (care visits and treatments) and indirect (day off work) costs.<sup>5,6</sup> Thus, improving the therapeutic management of these patients represents a major public health challenge.

In addition to chronic inflammation, severe asthma is characterised by airway hyperresponsiveness (AHR) and structural changes of the airway wall. This airway remodelling includes goblet cell hyperplasia, thickening of the basal membrane, angiogenesis and airway smooth muscle cells (aSMCs) hypertrophy and hyperplasia.<sup>7–9</sup> The extent of this remodelling correlates with the severity of asthma and the degree of airflow obstruction.<sup>10</sup> Currently, the only available treatment targeting airway remodelling is bronchial thermoplasty, a bronchoscopy procedure that consists in reducing the aSMC mass through the local deliver of controlled radiofrequency energy. Although bronchial thermoplasty has been shown to be effective in controlling asthma in severe asthmatics,<sup>11–13</sup> its long-term effects are not known and the selection of patients who could benefit from this invasive procedure remains challenging. Nevertheless, the use of this procedure demonstrated the therapeutic value of targeting aSMCs in severe asthma and the need of developing new pharmacologic strategies for

limiting aSMC proliferation and airway remodelling in severely affected patients.

It has been widely demonstrated in vitro that the small G protein Rac1 activity controls aSMC proliferation.<sup>14 15</sup> We thus hypothesised that Rac1 may be implicated in the proliferation of aSMC and consequently in the deleterious airway remodelling associated with severe asthma. By using human bronchial biopsies, human aSMC cultures and a mice model of severe allergic asthma sensitised to house dust mite (HDM) that closely mimics human pathology, we demonstrated that activation of Rac1 is causally involved in aSMCs proliferation and airway remodelling associated with severe asthma. We also show that in vivo pharmacological inhibition of Rac1 prevents asthma-associated airway remodelling thus confirming Rac1 as an alternative potential target for the treatment of severe asthma.

## METHODS

### Human bronchial biopsies

Bronchial biopsies were obtained by bronchial endoscopy from severe asthmatics as previously described.<sup>16</sup> All enrolled patients gave written approval. Control samples were obtained from donor lung transplants under NaRacAS (expression and activity of Rac1 in bronchial smooth muscle cells from from asthmatic patients) protocol (NCT03325088). Clinical protocols were previously approved by relevant ethic committees.

### Analysis of Rac1 activity

Human pulmonary biopsies paraffin-embedded sections were deparaffinised and permeabilised (phosphate buffered saline (PBS)+0.1% Triton-X100) before incubation with anti-Rac-GTP antibody (26903, NewEast Biosciences, King of Prussia, Pennsylvania) (1/1000) overnight at room temperature (RT). After three washes in PBS, sections were incubated for 1 hour at RT with the secondary Alexa568-labelled anti-rabbit antibody (1/1000). Anti-SM22 $\alpha$  antibody (Abcam) (1/500 O/N at RT) with Alexa488-labelled anti-mouse antibody (1/1000 1 hour at RT) were used to localise smooth muscle. To quantify Rac-GTP levels within the smooth muscle, Rac-GTP fluorescence intensities were measured inside a mask delimited by SM22a-positive cells and normalised to the control condition.

### aSMCs proliferation

Primary aSMCs were isolated from human bronchial biopsies. Additional detail on the method is provided in an online data supplement. Human aSMCs were seeded into 24-well plates (10 000 aSMCs/well) and allowed to adhere during 6 hour before serum starvation during 24 hours. When indicated, human aSMCs were treated with the Rac inhibitor, EHT1864 ( $10^{-5}$  M; Tocris Bioscience), P21-activated kinases (Pak) inhibitor IPA3 ( $10^{-5}$  M; Tocris Bioscience), Akt inhibitor VIII ( $10^{-5}$  M; Calbiochem), MEK inhibitor PD98059 ( $10^{-5}$  M; ThermoFisher), JAK inhibitor ruxolitinib ( $10^{-5}$  M; InvivoGen) added 30 min before stimulation with bFGF (25 ng/mL; Miltenyi Biotech), PDGF-bb (25 ng/mL; Miltenyi Biotech), interleukins (IL)-13 (10 ng/mL; Miltenyi Biotech), IL-33 (10 ng/mL; Miltenyi Biotech), IL-17 (20 ng/mL; Miltenyi Biotech), IL-9 (10 ng/mL; Miltenyi Biotech) or TSLP (10 ng/mL; Miltenyi Biotech) for 48 hours. Cells were stained with EdU for 12 hours at  $10^{-5}$  M according to the manufacturer's indications (EdU Staining Proliferation Kit iFluor 488, ID: ab219801, Abcam). Proliferation was quantified by the ratio of EdU-positive cells over the total number of cells. Proliferative signalling pathways were analysed by immunoblotting detailed in an online data supplement.

### Allergic asthma models

C57Bl/6 Rac1<sup>lox/lox</sup> and SMMHC-Rac1<sup>lox/lox</sup> mice were obtained as previously described.<sup>17</sup> Rac1 deletion in smooth muscle cells (SMCs) was induced in 8-week-old SMMHC-Rac1<sup>lox/lox</sup> males by intraperitoneal injection of tamoxifen (1 mg/day in sunflower oil) for five consecutive days during 2 weeks. Tamoxifen-treated Rac1<sup>lox/lox</sup> mice were used as control. Allergic asthma was induced in mice using a total HDM extract (*Dermatophagoides farinae*, from Stallergenes Greer, Antony, France), as described previously.<sup>18</sup> Additional details on the method for experimental models and the analyses of bronchoalveolar lavage fluid and airways reactivity are provided in an online data supplement.

### Histology

To assess smooth muscle hypertrophy/hyperplasia, lung sections were stained by immunohistochemistry with SM22a antibody (Abcam). Hyperplasia was expressed as the ratio of SM22a-positive area to the total bronchial area and normalised to the control condition. Additional detail on the method for making pulmonary sections is provided in an online data supplement.

### Statistics

Mann-Whitney test was performed for two-group comparisons. For multiple comparisons, the non-parametric Kruskal-Wallis test was used followed by Dunns' post-test to specifically compare indicated groups. The two-way ANOVA test was used for multiple comparisons of bronchial contractility studies. Data analysis was performed using the GraphPad Prism software. The threshold for statistical significance was set at  $p < 0.05$ .

## RESULTS

### Rac1 activity is increased in aSMC from patients with severe asthma

To validate the potential role of Rac1 in airway remodelling associated with severe asthma, we determined the level of Rac1 activity in bronchial biopsies from healthy subjects and from patients with severe asthma (table 1). Asthmatic donors of bronchial biopsies were mainly 50-year-old female patients with adult asthma onset. They suffered from hyper eosinophilic exacerbation-prone asthma for a mean duration of 24.7 years, with pulmonary function tests moderately altered but predominantly characterised by fixed airflow obstruction. These patients were uncontrolled (mean values of asthma control test=8.5) and they showed poor quality of life (mean values of Asthma Quality-of-Life Questionnaire=2.3) (table 1), despite an optimal treatment with high doses of inhaled corticosteroids associated with another controller, sometimes in combination with oral corticosteroids. Immunofluorescence measurements of Rac1 activity, using an antibody that specifically recognises the active GTP-bound form of Rac1, revealed a greater intensity in bronchial biopsies from asthmatic patients than from controls (figure 1A,B). Labelling of aSMC by anti-SM22a antibody showed that the aSMC area was larger in bronchial biopsies of severe asthmatics than in control subjects (figure 1A,B). Furthermore, the high level of Rac1-GTP detected in biopsies of patients with severe asthma is mainly localised in SM22a-positive area indicating that it results from an increase in Rac1 activity in aSMC (figure 1A,B). The activation of Rac1 observed within the aSMC is thus consistent with our hypothesis of a potential role of Rac1 signalling in airway remodelling and in aSMC hyperplasia in severe asthmatics.

**Table 1** Clinical and paraclinical data of asthmatic and control donors.

	Asthma control p value (n=11) (n=4)
Age (years)	50.4±12.4 50.8±11.4 NS
Gender (F/H)	7/4 3/1 NS
BMI (kg.m <sup>-2</sup> )	29.2±9.4 24.0±5 0.34
Atopy (Y/N)	8/3 0/4 0.025
Total IgE (IU/mL)	333±274
Asthma duration (years)	24.7±20.3
Eosinophils (mm <sup>-3</sup> )	294±177
Pulmonary function test	
FEV1 (% predicted)	59±16
FEV1/FVC	0.58±0.12
Reversibility (Y/N)	2/
Treatment	
Daily OCS (Y/N)	6/5
OCS posology (mg/day)	23±20
ICS (µg/day)	2091±831
LABA (Y/N)	10/1
Daily nebulisation (Y/N)	7/4
Exacerbation annual rate	9.3±7.1
Hospitalisation annual rate	2.2±4
Control and quality of life score	
ACT Score	8.5±3
<20 (Y/N)	11/0
AQLQ Score	2.3±0.7

ACT, asthma control test; AQLQ, Asthma Quality-of-Life Questionnaire; BMI, body mass index; FEV1, Forced Expiratory Volume in one second; FVC, forced vital capacity; ICS, inhaled cortico steroids; IgE, immunoglobulin E; LABA, long acting beta2 agonists; OCS, oral cortico steroids. SEM). Fisher statistical test was used to compare Control and Asthmatic patients.

### aSMCs proliferation depends on Rac1 activity

Several growth factors, cytokines and chemokines have been proposed to participate in airway remodelling in severe asthma by promoting aSMC proliferation.<sup>19–24</sup> We therefore assessed the role of Rac1 in primary human aSMC proliferation in response to different mitogenic stimuli. Among the mitogen factors used, only bFGF and PDGFbb induced a significant proliferation of control human aSMC (online supplemental figure E1). Interestingly, both in basal condition and on exposure to bFGF, the proliferation rate of aSMC from severe asthma patients was significantly higher than that of aSMC from control subjects (figure 2A,B). These differences are abolished by the Rac inhibitor, EHT1864, that prevented both spontaneous and bFGF- and PDGFbb-induced proliferation of aSMC from control and severe asthmatics (figure 2A,C). These results suggest that Rac1 activity is involved in the mitogenic effect of bFGF and PDGFbb on human aSMC and participates to the high proliferation rate of aSMC of asthmatic patients. To validate this hypothesis, we assessed the activation of the Rac1 signalling pathway, by measuring the phosphorylation of Pak, one of the main downstream targets of Rac1, by western blot (figure 3A). Stimulation of control aSMC with bFGF and PDGFbb indeed increased Pak phosphorylation and this response was

prevented by EHT1864, thereby confirming the activation of Rac1 (figure 3A).

### Rac1/Pak1/STAT3 signaling pathway is involved in growth factor-induced aSMCs proliferation

Transduction pathways activated by mitogens involved in asthmatic airway remodelling have been shown to converge to a relatively limited number of intracellular signalling modules, mainly P44/42 mitogen-activated protein kinases (MAPK), phosphoinositide 3-kinase (PI3K)/Akt and JAK/STAT.<sup>24–28</sup> Indeed, we confirm that bFGF and PDGFbb rapidly increased the phosphorylation levels of P44/42, Akt, and signal transducer and activator of transcription 3 (STAT3) in control human aSMCs (figure 3A and online supplemental figure E2). Inhibition of these signalling pathways by PD98059, Akt VIII inhibitor and ruxolitinib, respectively, reduced the proliferation of control human aSMCs at baseline and after bFGF and PDGFbb stimulation, attesting the role of these signalling pathways in this process (figure 3B). Phosphorylation of STAT3 induced by bFGF and PDGFbb was prevented by the Rac1 inhibitor EHT1864 (figure 3A), which had no effect on P44/42 activation in response to the two mitogenic factors (online supplemental figure E2). EHT1864 also downregulated Akt phosphorylation in bFGF-treated human aSMC, but not in aSMC stimulated by PDGFbb (online supplemental figure E2). These results demonstrated the essential role of Rac1 in the activation of the STAT3 pathway by mitogenic stimuli in human aSMC.

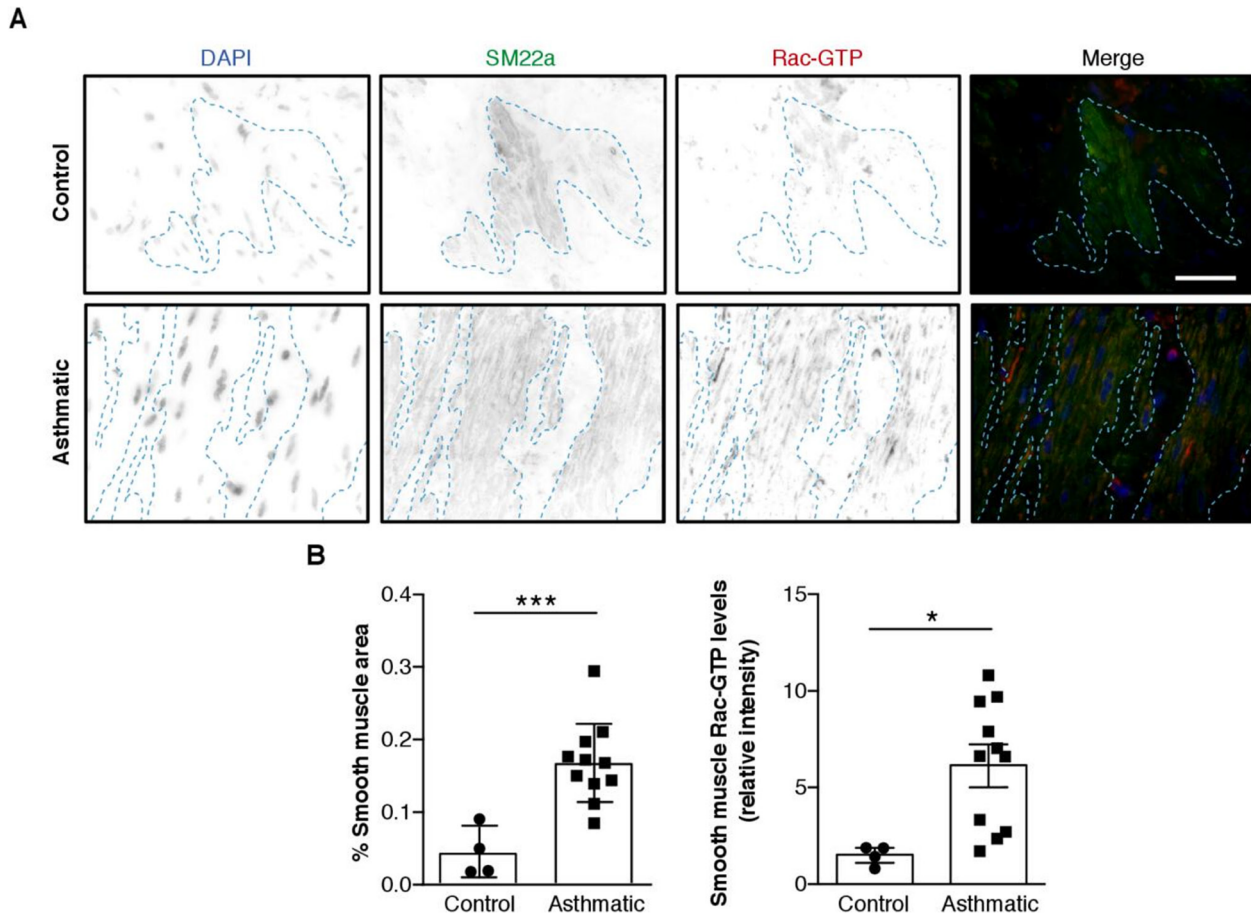
### SM Rac1 deletion prevents airway remodeling in a mouse model of severe asthma

To establish the role of Rac1 in airway remodelling in vivo, we developed a murine model of severe allergic asthma induced by a percutaneous sensitisation and repeated intranasal challenges with HDM. This model allows the observation of major changes of the airway wall including aSMCs hyperplasia (figure 4A,B), AHR of bronchial rings to methacholine (figure 4C), and mixed inflammation with eosinophil and neutrophil accumulation in bronchoalveolar lavage (BAL) fluid (figure 4D). Nebulisation of the reference corticosteroid, beclomethasone at 150 µg/kg<sup>29</sup> had no significant effect on airway inflammation in this severe asthma model, whereas it prevented BAL eosinophilia and neutrophilia in an acute allergic asthma model (online supplemental figure E3). These results show that this severe allergic asthma model closely mimics the main features of severe asthma in humans, including the corticosteroid resistance.

We next submitted tamoxifen-inducible SM-Rac1-KO mice<sup>14</sup> to the severe allergic asthma protocol. ASM area and AHR were significantly reduced in SM-Rac1-KO mice compared with SM-Rac1<sup>lox/lox</sup> (figure 4B,C), whereas the histological grade, inflammatory cells accumulation in BAL and mucus production (figure 4A,D) remained unchanged. These results suggest a causal role of Rac1 in SMC hyperplasia and the resulting airway remodelling associated with severe allergic asthma.

### Inhalation of a Rac1 inhibitor prevents aSMC hyperplasia, AHR, and pulmonary inflammation in a murine allergic severe asthma model

As a proof of concept to demonstrate the therapeutic value of pharmacological inhibition of Rac1 to limit airway remodelling associated with severe asthma, the Rac1 inhibitor NSC23766 was administered by repeated nebulisations before each HDM challenge. NSC23766 abrogated SMC hyperplasia and AHR of bronchial rings in response to methacholine (figure 5A-C),



**Figure 1** Rac activity is increased in airway smooth muscle contained in bronchial biopsies from asthmatics. (A) Representative images of Rac-GTP immunofluorescence (red) in biopsies sections from control (n=4) and patients with severe asthma (n=11). Nuclei were detected by 4',6'-diamidino-2-phenylindole staining (blue) and smooth muscle by SM22a immunofluorescence (green). Scale bar, 100  $\mu$ m. (B) Quantification of smooth muscle area, mean fluorescence intensity of the Rac1-GTP signal within the biopsy and within the smooth muscle layer. Data are presented as mean $\pm$ SEM. Mann-Whitney statistical test was used to compare control and asthmatic groups. \*P<0.05, \*\*\*p<0.001.

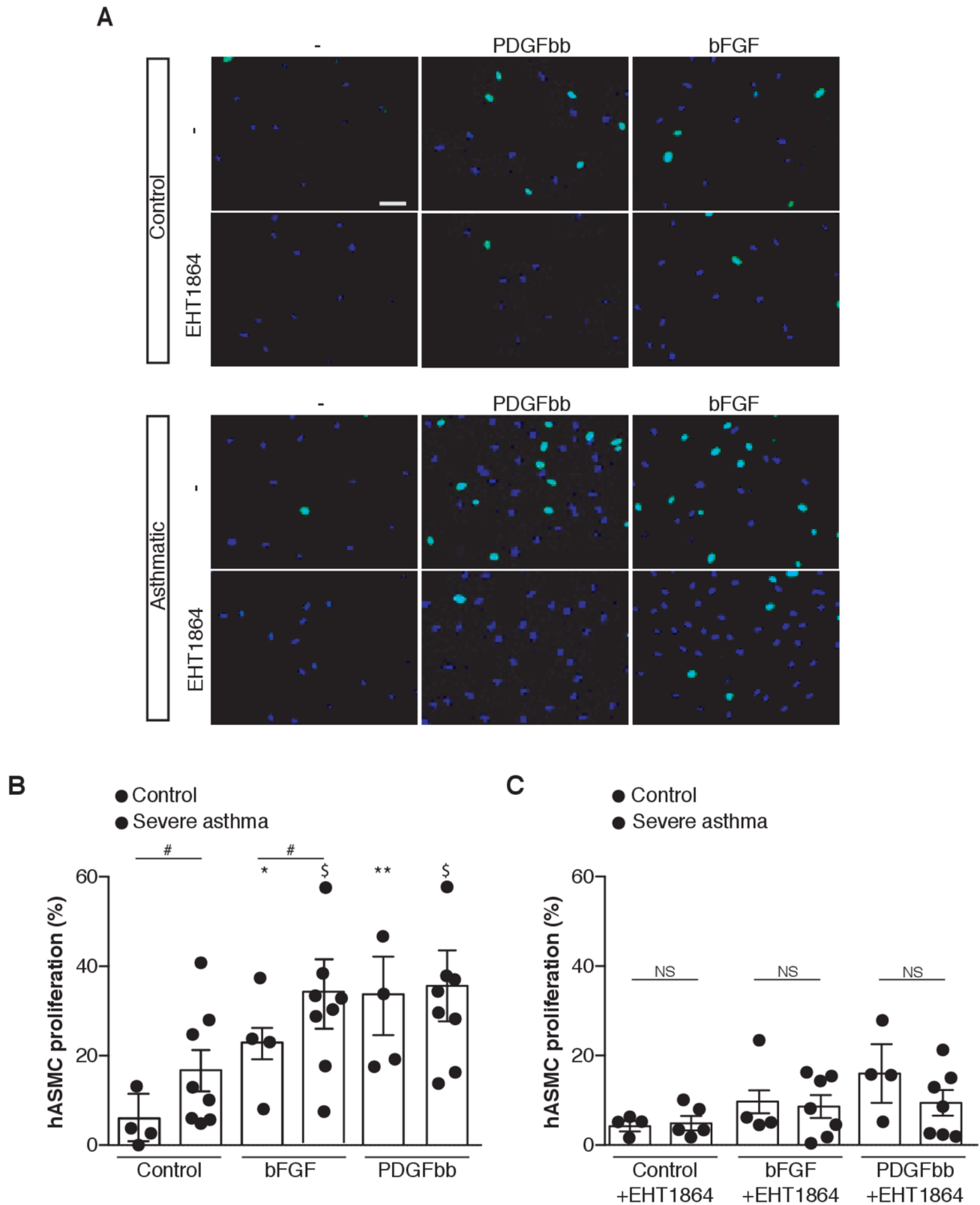
but also peri-bronchial/vascular infiltrates of inflammatory cells (figure 5A,B). This effect of NSC23766 on inflammatory cell infiltration was confirmed by the significant decrease of the number of macrophages and eosinophils in BAL fluid of NSC23766-treated mice, as compared with vehicle-treated mice (figure 5D). The efficiency of NSC23766 on airway remodelling and pulmonary inflammation was shown to be higher than that of current reference treatments such as repeated high doses of beclomethasone inhalations (1500  $\mu$ g/kg), or the long-lasting  $\beta$ 2-agonist, formoterol (figure 5A,B,D). Despite a significant reduction of inflammatory cell infiltrate, beclomethasone failed to prevent aSMC hyperplasia, and formoterol had no effect on lung inflammation and remodelling.

## DISCUSSION

Our current study revealed a leading role of Rac1 in airway remodelling and in aSMC hyperplasia associated with severe asthma, by promoting STAT3-dependent aSMC proliferation. This involvement of Rac1 in the pathological remodelling in the human disease is consistent with its overactivation observed in bronchi from severe asthmatics.

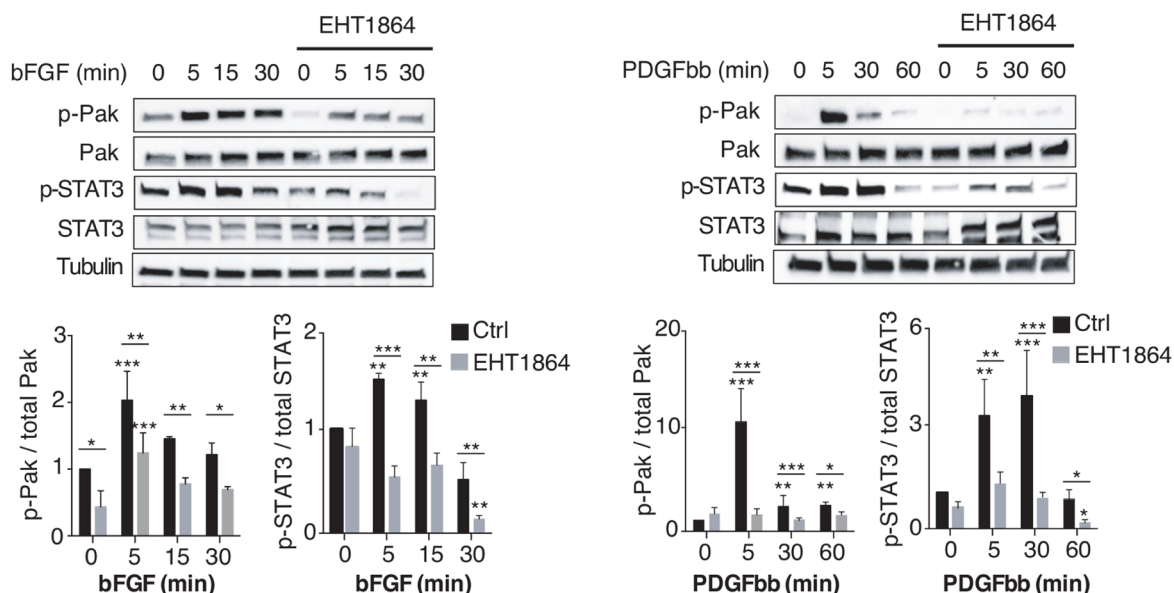
The increase of aSMC mass is one of the main features of airway remodelling associated with asthma and is considered as a marker of disease severity.<sup>12,19</sup> It relies on a high proliferation rate of aSMC in patients with severe asthma compared with mild

and moderate asthma, or control subjects.<sup>30</sup> A number of mediators can operate in concert to stimulate aSMC proliferation, including growth factors (PDGFbb and bFGF), cytokines and chemokines, which are produced by inflammatory and airway structural cells, and by products of mast cells infiltrating the aSMC bundles, such as histamine, tryptase, and leukotrienes.<sup>22,23</sup> Although initially described as the main intracellular signalling pathway mediating cytokine responses, the JAK/STAT signalling pathway has been shown to be activated by many different ligands and receptors, including growth factor/tyrosine kinase receptors and G protein coupled receptors.<sup>31</sup> Consistent with our results, Rac1 has been shown to be required for growth factor receptor-mediated and G protein coupled-receptor-mediated activation of the JAK/STAT pathway, thus defining Rac1 as a hub in signalling networks that control human aSMC proliferation.<sup>24,32,33</sup> This role of Rac1 is in agreement with the strong activity of Rac1 systematically observed in the remodelled airway wall of patients with severe asthma compared with control subjects, even though the number of samples analysed was limited. Recently, P-Rex1, a Rac1 exchange factor, has been shown to be aberrantly upregulated in lung tissue from patients with asthma and to potentiate growth factor-induced human aSMC proliferation.<sup>34</sup> Therefore, it can be hypothesised that the activation of aSMC Rac1 observed in severe asthma patients could be related, at least in part, to this aberrant upregulation of

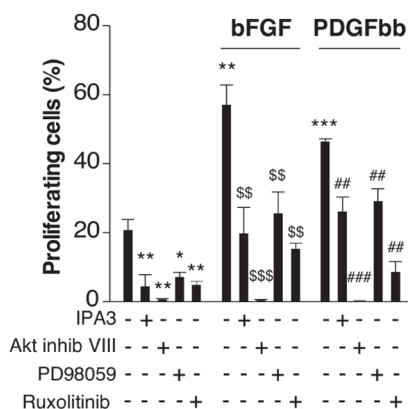
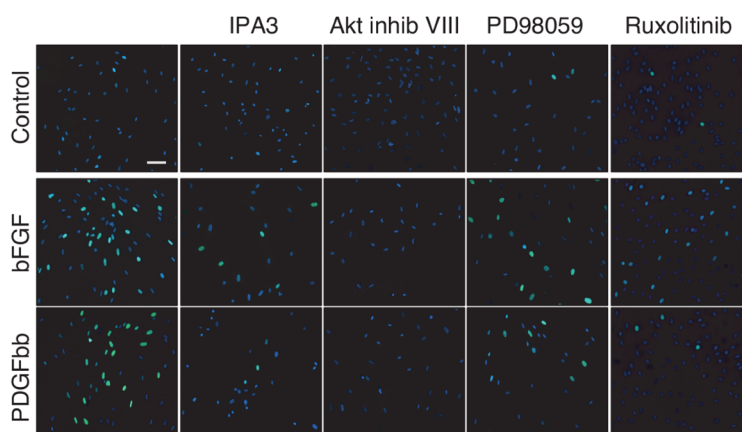


**Figure 2** Rac1 inhibition reduces bFGF-induced and PDGFbb-induced aSMCs proliferation. (A) Representative images of airway smooth muscle cell (aSMC) proliferation from control and severe asthmatics induced by bFGF and PDGFbb, in the absence and in the presence of the Rac1 inhibitor, EHT1864. Nuclei are detected by 4',6-diamidino-2-phenylindole staining (blue) and aSMC proliferation by EdU staining (green). Scale bar, 25  $\mu$ m. (B,C) Quantification of aSMC proliferation by EdU staining in the absence (B) and in the presence (C) of EHT1864. The results are expressed as the percentage of cell proliferation (EdU-positive cells) (mean $\pm$ SEM of n=3 independent experiments). Kruskal-Wallis test followed by Dunns' post-test were used. \*P<0.05, \*\*p<0.01 versus untreated cells from control subjects;  $^{\$}$ P<0.05 versus untreated cells from patients with severe asthma;  $^{\#}$ p<0.05 versus cells from control subjects.

A

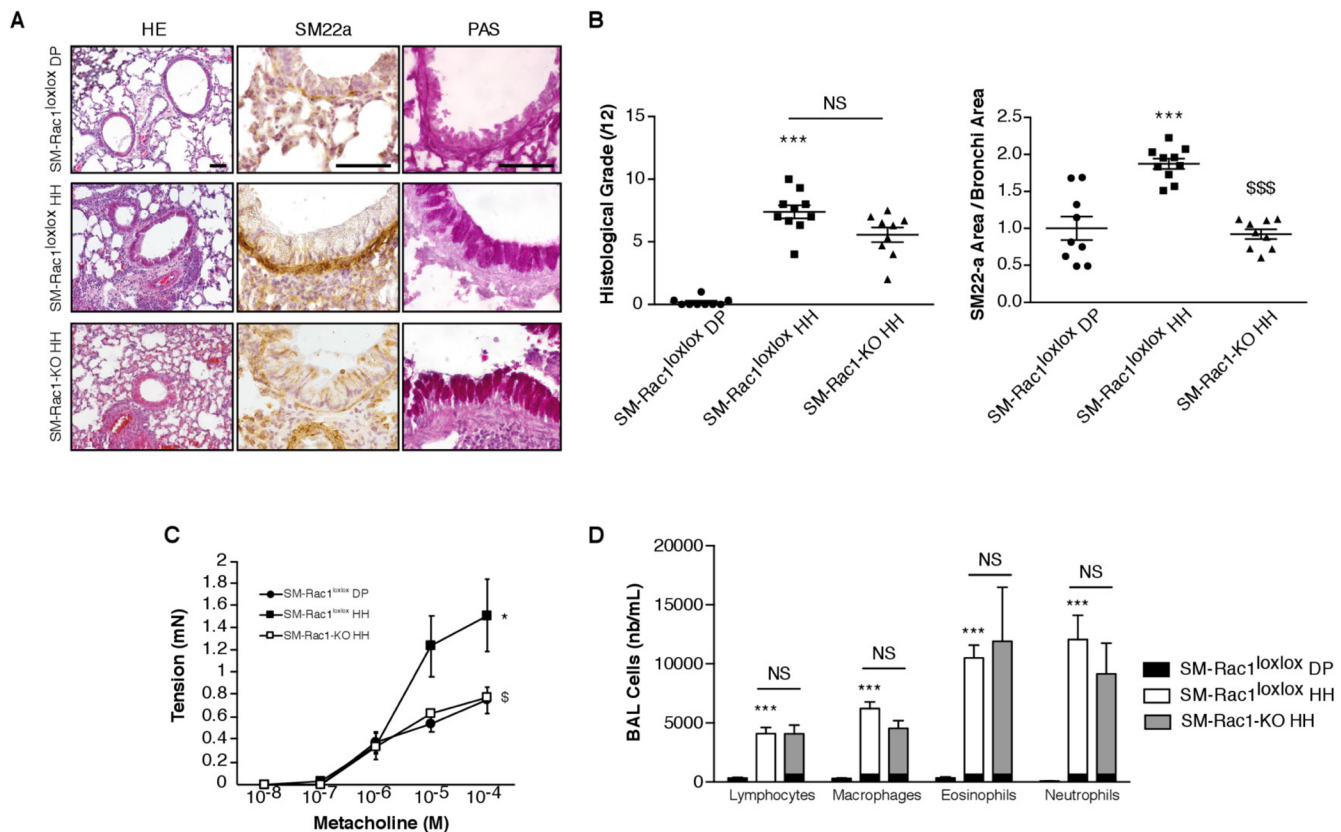


B



**Figure 3** Role of Rac1/P21-activated kinases (Pak1) in bFGF-induced activation of Akt-dependent signalling pathway. (A) Immunoblot analysis and corresponding quantification of Pak and STAT3 expression and phosphorylation in control airway smooth muscle cell (aSMCs) stimulated with bFGF or PDGFbb at different time points, in the absence, or in the presence of EHT1864 (n=4–5 independent experiments). (B) Control aSMC proliferation induced by bFGF and PDGFbb, in the absence and in the presence of inhibitors of PAK (IPA3), Akt (Akt Inhib VIII), P44/42 (PD98059) or JaAK2 (ruxolitinib). Nuclei are detected by 4',6-diamidino-2-phenylindole staining (blue) and haSMC proliferation by EdU staining (green). Scale bar, 25  $\mu$ m. Quantification of aSMC proliferation by EdU staining. The results are expressed as the percentage of EdU-positive cells. (n=3–4 independent experiments). Data are presented as mean $\pm$ SEM. Kruskal-Wallis test followed by Dunns' post-test were used. \*P<0.05, \*\*p<0.01, \*\*\*p<0.001 versus untreated cells; <sup>ss</sup>p<0.01, <sup>sss</sup>p<0.001 versus bFGF treated cells; #p<0.01, ###p<0.001 versus PDGFbb treated cells.





**Figure 4** SM-Rac1 deletion prevents smooth muscle hyperplasia associated in an experimental model of severe allergic asthma. (A) Hematoxylin/Eosin (HE staining, SM22a immunohistochemistry and Periodic acid–Schiff (PAS) staining of lung sections from naïve (DP) or house dust mite-sensitised (HH) mice of the indicated genotypes. Images are representative of 9–10 mice in each experimental condition. Scale bars, 100  $\mu$ m. (B) Histological grade and smooth muscle hyperplasia quantification on lung sections from DP and HH mice of the indicated genotypes ( $n=9-10$  mice). (C) Contractile responses to increasing concentration of methacholine of bronchial rings from DP and HH mice of the indicated genotypes ( $n=3-4$ ). (D) Infiltrating cells in bronchoalveolar lavage fluid from DP and HH mice of the indicated genotypes ( $n=8-14$ ). Data are expressed as mean  $\pm$  SEM. Kruskal-Wallis test followed by Dunn's post-test were used for (B) and (D). Two-way analysis of variance test was used for (C). NS, not significant. \* $P<0.05$ , \*\*\* $p<0.001$  versus SM-Rac1<sup>lox/lox</sup> DP mice;  $^{\$}$  $p<0.05$ ,  $^{\$ \$ \$}$  $p<0.001$  versus SM-Rac1<sup>lox/lox</sup> HH mice.

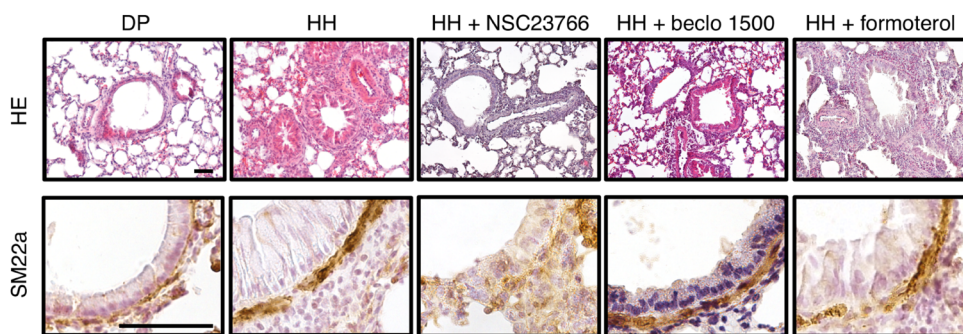
P-Rex1 expression, and would be responsible for the increased aSMC proliferation in asthma.

The increase in aSMC mass in asthma patients was associated with airflow obstruction.<sup>9 10</sup> Indeed, aSMC are not only responsible for AHR through their contractile activity, but also contribute to the inflammatory process by modifying the extracellular matrix and producing mediators that act on inflammatory cells. Pharmacological targeting of aSMC thus appears as an attractive strategy for novel asthma therapies. Current therapeutic strategies remain based on the chronic use of high dose inhaled or oral corticosteroids, resulting in various and harmful long-term side effects.<sup>35 36</sup> New therapeutic strategies such as monoclonal antibodies anti-IgE (omalizumab), anti-IL-5 (mepolizumab, reslizumab), anti-IL-5 receptor (benralizumab) and anti-IL-4/IL-13 receptor (dupilumab) significantly improve exacerbations rate and asthma control, and lower oral corticosteroid use in patients with severe asthma.<sup>37-41</sup> However, these strategies focus on specific inflammatory endotypes,<sup>39 42-44</sup> and the potential impacts of these new strategies on airway remodelling are still missing. In this context, our demonstration that Rac1 is a node in signalling pathways that plays a major role in the contraction and proliferation of aSMC makes this protein a pharmacological target of choice for severe asthma. The efficiency of SMC Rac1 deletion and repeated inhalations of the Rac1 inhibitor NSC23766 to prevent AHR and aSMC hyperplasia

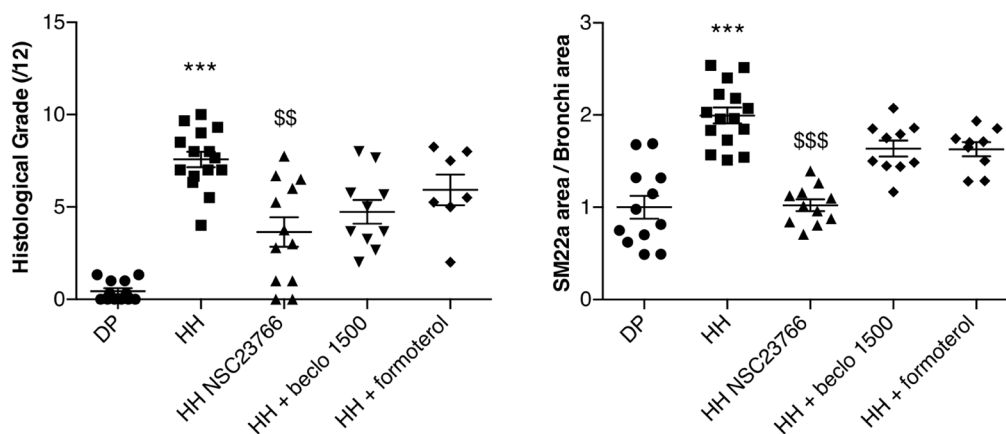
validate this hypothesis in a severe allergic asthma model in mice that recapitulate the human disease. Moreover, in addition to these expected effects, Rac1 inhibition also reduced eosinophilic inflammation, thus demonstrating that the anti-inflammatory action of Rac1 inhibitor already described in an acute murine model of allergic asthma,<sup>14</sup> is also effective in severe chronic asthma. To our knowledge, this is the first demonstration of a drug able to combine all the desired effects for the treatment of severe asthma, that is, limiting aSMC contraction and proliferation and reducing inflammation. Since Rac1 is known to have ubiquitous expression and multiple functions,<sup>45</sup> an open and important question that remains to be addressed is the possible side effects of Rac1 inhibitor. However, in asthma, the opportunity of administrating the treatment locally might be an effective way to limit potential adverse effects. Indeed, repeated administration of NSC23766 by nebulisation failed to alter blood pressure, whereas SMC Rac1 deletion has been shown to elicit this effect.<sup>46</sup>

In conclusion, we suggest that Rac1 may represent a relevant target to develop new drugs of clinical interest for the treatment of severe asthma. Our data support the concept that inhibition of Rac1-dependent signalling pathway may simultaneously limit aSMC hyperplasia, AHR and inflammation, thereby providing a novel approach to reverse airway remodelling and restore airway function in patients with severe asthma.

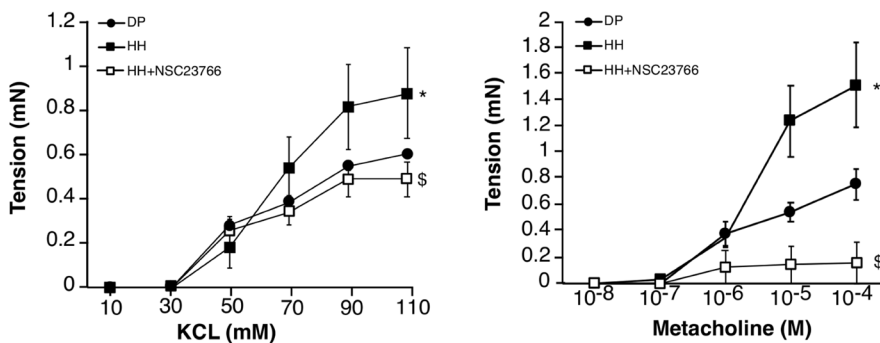
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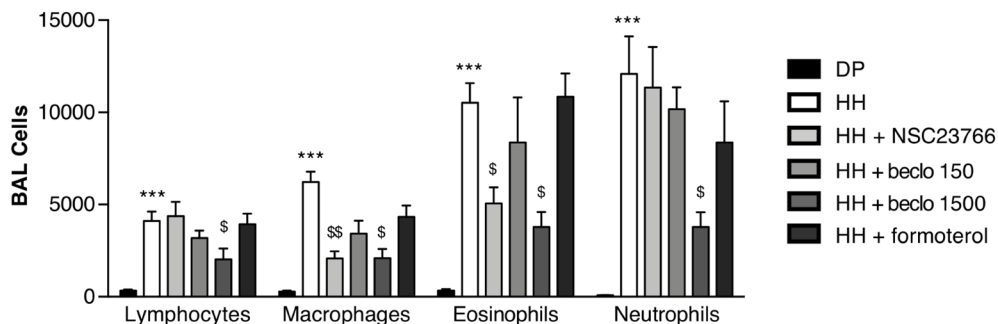
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**Figure 5** NSC23766 inhalations reduce pulmonary remodelling and smooth muscle hyperplasia associated with severe allergic asthma. (A) Hematoxylin/Eosin (HE) staining and SM22a immunohistochemistry of lung sections from naive (DP) or house dust mite-sensitised (HH) mice treated with NaCl, NSC23766, beclomethasone (1500 μg/kg) or formoterol. Images are representative of 9–15 mice in each experimental condition. Scale bars, 100 μm. (B) Histological grade and smooth muscle hyperplasia measured on lung sections from DP and HH mice treated with NaCl, NSC23766, beclomethasone or formoterol (n=9–15 mice). (C) Contractile responses to increasing concentrations of KCl and methacholine of bronchi rings from DP and HH mice treated with NaCl or NSC23766 (n=4–5). (D) Infiltrating cells in bronchoalveolar lavage fluid from DP and HH mice treated with NaCl, NSC23766, beclomethasone (150 or 1500 μg/kg), or formoterol (n=9–14 mice). Data are represented as mean±SEM. Kruskal-Wallis test followed by Dunn's post-test were used for (B) and (D). Two-way analysis of variance test was used for (c). \*P<0.05, \*\*\*p<0.001 versus DP NaCl; \$p<0.05, \$\$\$p<0.01 and \$\$\$\$p<0.001 versus HH NaCl.

The development of Rac1 inhibitors may thus offer a new therapeutic option for patients who are refractory to current treatments.

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## REFERENCES

- Variations in the prevalence of respiratory symptoms, self-reported asthma attacks, and use of asthma medication in the European community respiratory health survey (ECRHS). *Eur Respir J* 1996;9:687–95.
- Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: Isaac. The International study of asthma and allergies in childhood (Isaac) Steering Committee. *Lancet Lond. Engl* 1998;351:1225–32.
- Reddel HK, Bateman ED, Becker A, et al. A summary of the new GINA strategy: a roadmap to asthma control. *Eur Respir J* 2015;46:622–39.
- Chung KF, Wenzel SE, Brozek JL, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J* 2014;43:343–73.
- Lane S, Molina J, Plusa T. An international observational prospective study to determine the cost of asthma exacerbations (COAX). *Respir Med* 2006;100:434–50.
- Moore WC, Bleecker ER, Curran-Everett D, et al. Characterization of the severe asthma phenotype by the National heart, lung, and blood Institute's severe asthma research program. *J Allergy Clin Immunol* 2007;119:405–13.
- Bento AM, Hershenson MB. Airway remodeling: potential contributions of subepithelial fibrosis and airway smooth muscle hypertrophy/hyperplasia to airway narrowing in asthma. *Allergy Asthma Proc* 1998;19:353–8.
- Jeffery PK. Remodeling in asthma and chronic obstructive lung disease. *Am J Respir Crit Care Med* 2001;164:S28–38.
- Prakash YS. Emerging concepts in smooth muscle contributions to airway structure and function: implications for health and disease. *Am J Physiol Lung Cell Mol Physiol* 2016;311:L1113–40.
- James AL, Bai TR, Maud T, et al. Airway smooth muscle thickness in asthma is related to severity but not duration of asthma. *Eur Respir J* 2009;34:1040–5.
- Castro M, Rubin A, Laviolette M, et al. Persistence of effectiveness of bronchial thermoplasty in patients with severe asthma. *Ann Allergy Asthma Immunol* 2011;107:65–70.
- Castro M, Rubin AS, Laviolette M, et al. Effectiveness and safety of bronchial thermoplasty in the treatment of severe asthma: a multicenter, randomized, double-blind, sham-controlled clinical trial. *Am J Respir Crit Care Med* 2010;181:116–24.
- Thomson NC, Rubin AS, Niven RM, et al. Long-Term (5 year) safety of bronchial thermoplasty: asthma intervention research (air) trial. *BMC Pulm Med* 2011;11:8.
- Liu Y, Li W, Ye C, et al. Gambogic acid induces G0/G1 cell cycle arrest and cell migration inhibition via suppressing PDGF receptor  $\beta$  tyrosine phosphorylation and Rac1 activity in rat aortic smooth muscle cells. *J Atheroscler Thromb* 2010;17:901–13.
- Page K, Li J, Hodge JA, et al. Characterization of a Rac<sub>1</sub> Signaling Pathway to Cyclin D<sub>1</sub> Expression in Airway Smooth Muscle Cells. *J Biol Chem* 1999;274:22065–71.
- Aubier M, Thabut G, Hamidi F, et al. Airway smooth muscle enlargement is associated with protease-activated receptor 2/ligand overexpression in patients with difficult-to-control severe asthma. *J Allergy Clin Immunol* 2016;138:729–39.
- André-Grégoire G, Dilasser F, Chesné J, et al. Targeting of Rac1 prevents bronchoconstriction and airway hyperresponsiveness. *J Allergy Clin Immunol* 2018;142:824–33.
- Chesné J, Braza F, Chadeuf G, et al. Prime role of IL-17A in neutrophilia and airway smooth muscle contraction in a house dust mite-induced allergic asthma model. *J Allergy Clin Immunol* 2015;135:1643–5.
- Hashimoto M, Tanaka H, Abe S. Quantitative analysis of bronchial wall vascularity in the medium and small airways of patients with asthma and COPD. *Chest* 2005;127:965–72.
- Hoshino M, Takahashi M, Aoike N. Expression of vascular endothelial growth factor, basic fibroblast growth factor, and angiogenin immunoreactivity in asthmatic airways and its relationship to angiogenesis. *J Allergy Clin Immunol* 2001;107:295–301.
- Redington AE, Roche WR, Madden J, et al. Basic fibroblast growth factor in asthma: measurement in bronchoalveolar lavage fluid basally and following allergen challenge. *J Allergy Clin Immunol* 2001;107:384–7.
- Vignola AM, Chanez P, Chiappara G, et al. Transforming growth factor-beta expression in mucosal biopsies in asthma and chronic bronchitis. *Am J Respir Crit Care Med* 1997;156:591–9.
- Bossé Y, Rola-Pleszczynski M. Fgf2 in asthmatic airway-smooth-muscle-cell hyperplasia. *Trends Mol Med* 2008;14:3–11.
- Simeone-Penney MC, Severgnini M, Rozo L, et al. Pdgf-Induced human airway smooth muscle cell proliferation requires STAT3 and the small GTPase Rac1. *Am J Physiol Lung Cell Mol Physiol* 2008;294:L698–704.
- Zhou L, Hershenson MB. Mitogenic signaling pathways in airway smooth muscle. *Respir Physiol Neurobiol* 2003;137:295–308.
- Yap HM, Israf DA, Harith HH, et al. Crosstalk between signaling pathways involved in the regulation of airway smooth muscle cell hyperplasia. *Front Pharmacol* 2019;10:1148.
- Jee S-H, Chu C-Y, Chiu H-C, et al. Interleukin-6 induced basic fibroblast growth factor-dependent angiogenesis in basal cell carcinoma cell line via JAK/STAT3 and PI3-kinase/Akt pathways. *J Invest Dermatol* 2004;123:1169–75.
- Shi H, Lin B, Huang Y, et al. Basic fibroblast growth factor promotes melanocyte migration via activating PI3K/Akt-Rac1-FAK-JNK and ERK signaling pathways. *IUBMB Life* 2016;68:735–47.
- Hrvacic B, Bosnjak B, Tudja M, et al. Applicability of an ultrasonic nebulization system for the airways delivery of beclomethasone dipropionate in a murine model of asthma. *Pharm Res* 2006;23:1765–75.
- Johnson PR, Roth M, Tamm M, et al. Airway smooth muscle cell proliferation is increased in asthma. *Am J Respir Crit Care Med* 2001;164:474–7.
- Bousoik E, Montazeri Aliabadi H. "Do We Know Jack" About JAK? A Closer Look at JAK/STAT Signaling Pathway. *Front Oncol* 2018;8:287.
- Pelletier S, Duhamel F, Coulombe P, et al. Rho family GTPases are required for activation of JAK/STAT signaling by G protein-coupled receptors. *Mol Cell Biol* 2003;23:1316–33.
- Movassagh H, Shan L, Halayko AJ, et al. Neuronal chemorepellent semaphorin 3E inhibits human airway smooth muscle cell proliferation and migration. *J Allergy Clin Immunol* 2014;133:560–7.
- Huang Y, Xie Y, Jiang H, et al. Upregulated P-Rex1 exacerbates human airway smooth muscle hyperplasia in asthma. *J Allergy Clin Immunol* 2019;143:778–81.
- Buchman AL. Side effects of corticosteroid therapy. *J Clin Gastroenterol* 2001;33:289–94.
- Volmer T, Effenberger T, Trautner C, et al. Consequences of long-term oral corticosteroid therapy and its side-effects in severe asthma in adults: a focused review of the impact data in the literature. *Eur Respir J* 2018;52. doi:10.1183/13993003.00703-2018. [Epub ahead of print: 25 10 2018].
- Normansell R, Walker S, Milan SJ, et al. Omalizumab for asthma in adults and children. *Cochrane Database Syst Rev* 2014:CD003559.
- Pavord ID, Korn S, Howarth P, et al. Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo-controlled trial. *Lancet* 2012;380:651–9.
- Bleecker ER, FitzGerald JM, Chanez P, et al. Efficacy and safety of benralizumab for patients with severe asthma uncontrolled with high-dosage inhaled corticosteroids and long-acting  $\beta_2$ -agonists (SIROCCO): a randomised, multicentre, placebo-controlled phase 3 trial. *The Lancet* 2016;388:2115–27.
- Castro M, Zangrilli J, Wechsler ME, et al. Reslizumab for inadequately controlled asthma with elevated blood eosinophil counts: results from two multicentre, parallel, double-blind, randomised, placebo-controlled, phase 3 trials. *Lancet Respir Med* 2015;3:355–66.
- FitzGerald JM, Bleecker ER, Nair P, et al. Benralizumab, an anti-interleukin-5 receptor  $\alpha$  monoclonal antibody, as add-on treatment for patients with severe, uncontrolled,

- eosinophilic asthma (CALIMA): a randomised, double-blind, placebo-controlled phase 3 trial. *The Lancet* 2016;388:2128–41.
- 42 FitzGerald JM, Bleecker ER, Menzies-Gow A, *et al.* Predictors of enhanced response with benralizumab for patients with severe asthma: pooled analysis of the SIROCCO and CALIMA studies. *Lancet Respir Med* 2018;6:51–64.
- 43 Haldar P, Brightling CE, Singapuri A, *et al.* Outcomes after cessation of mepolizumab therapy in severe eosinophilic asthma: a 12-month follow-up analysis. *J Allergy Clin Immunol* 2014;133:921–3.
- 44 Hanania NA, Wenzel S, Rosén K, *et al.* Exploring the effects of omalizumab in allergic asthma: an analysis of biomarkers in the extra study. *Am J Respir Crit Care Med* 2013;187:804–11.
- 45 Jaffe AB, Hall A. Rho GTPases: biochemistry and biology. *Annu Rev Cell Dev Biol* 2005;21:247–69.
- 46 André G, Sandoval JE, Retailleau K, *et al.* Smooth muscle specific Rac1 deficiency induces hypertension by preventing p116RIP3-dependent RhoA inhibition. *J Am Heart Assoc* 2014;3:e000852.

# Type 2 immunity-driven diseases: Towards a multidisciplinary approach

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## Abstract

Asthma, atopic dermatitis and chronic rhinoconjunctivitis are highly heterogeneous. However, epidemiologic associations exist between phenotypic groups of patients. Atopic march is one such association but is not the only common point. Indeed, beyond such phenotypes, hallmarks of type 2 immunity have been found in these diseases involving immune dysregulation as well as environmental triggers and epithelial dysfunction. From the canonical Th2 cytokines (IL-4, IL-5, IL-13), new cellular and molecular actors arise, from the epithelium's alarmins to new innate immune cells. Their interactions are now better understood across the different environmental barriers, and slight differences appeared. In parallel, the development of type 2-targeting biotherapies not only raised hope to treat those diseases but also raised new questions regarding their true pathophysiological involvement. Here, we review the place of type 2 immunity in the different phenotypes of asthma, chronic rhinitis, chronic rhinosinusitis and atopic dermatitis, highlighting nuances between them. New hypotheses rising from the use of biotherapies will be discussed along with the uncertainties and unmet needs of this field.

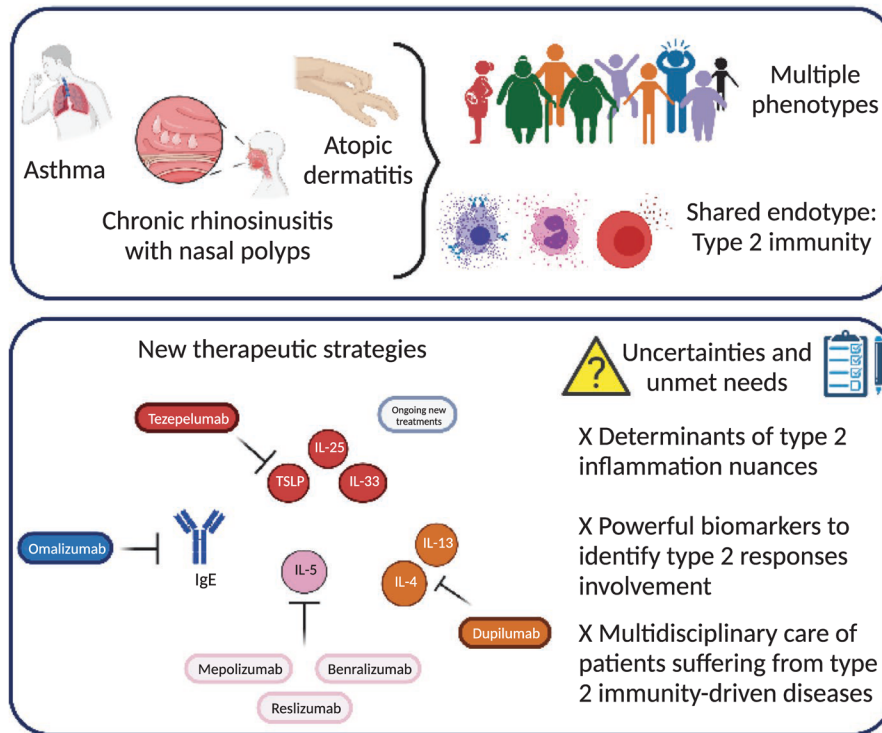
## 1 | INTRODUCTION

Atopic dermatitis (AD), allergic rhinitis (AR), chronic rhinosinusitis with (CRSwNP) or without (CRSsNP) nasal polyps and asthma share a complex interplay between a genetic background, a polarized

immune response and the environment. Their increasing prevalence in urban areas in comparison with rural areas further highlights the role of environmental factors in their development.<sup>1</sup> Interestingly, they can succeed themselves or coexist at the same time in a single individual throughout life. For example, atopic march describes the

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## GRAPHICAL ABSTRACT

Heterogenic diseases as asthma, atopic dermatitis and chronic rhinosinusitis with nasal polyposis can be divided into multiple phenotypes. Such clinical presentations share common pathophysiological paths among with type 2 immunity play an important role. Better understanding of the mechanisms underlying type 2 responses allowed the successful development of specific therapeutic strategies. Indeed, biotherapies are now available to target key actors like the immunoglobulin E, the interleukin (IL) 5, the IL-4 and IL-13 pathway and more recently, the thymic stromal lymphopoietin. Even though those innovations improve the healthcare of type-2 mediated diseases, unmet needs remain to be resolved. Graphic abstract created with BioRender.com

successive development of AD, food allergy, AR and allergic asthma during childhood.<sup>2</sup> Nevertheless, such an association is not absolute, and if common pathophysiological mechanisms are shared, they are insufficient to explain the whole pattern's heterogeneity.

To address such heterogeneity, phenotypes, defined by the association of specific observable characteristics, were identified. Cluster analyses of large cohorts of patients demonstrated that clinical phenotypes could be isolated with different clinical presentations, clinical courses and responses to treatment.<sup>3</sup> From those considerations, the concept arises that, behind such variable phenotypes, different physiopathological pathways could be deciphered. Such specific pathways, called endotype, were developed from omics data analysed by innovative bioinformatic tools.<sup>4</sup>

Type 2 inflammation is a particular endotype that plays an important dual role in environment-related responses.<sup>5</sup> On the one hand, it is involved in physiological responses against venom and helminths and in tissue repair.<sup>6</sup> On the other hand, allergic and/or atopic diseases are prototypical examples of sustained and uncontrolled type 2 immunity.<sup>7</sup> Data from studies of allergic diseases and animal models highlighted the critical roles of key cytokines, namely, IL-4, IL-5 and IL-13, the production of specific IgEs and key cellular actors, principally, eosinophils and mast cells.<sup>8</sup> In parallel with the

### Key Messages

- Specific phenotypes of cutaneous and respiratory diseases share a common endotype: type 2 inflammation.
- Better understanding of type 2 inflammation leads to common innovative therapeutic strategies among such diseases.
- Uncertainties remain and critical unmet needs should be resolved to improve healthcare of such diseases.

endotype concept, the treatable mechanisms theory gave rise to specific biologics (monoclonal antibodies and small molecules) targeting such key pathways.

Here, we will discuss how type 2 inflammation is differentially implicated in several respiratory and cutaneous diseases. Next, an update of the new therapeutic strategies available will be presented along with the new hypotheses that come from its use in practice. Finally, we will highlight the uncertainties of this field and the unmet needs.

## 2 | TYPE 2 INFLAMMATION: WHERE DOES IT FIT?

### 2.1 | From phenotypes to endotypes

AD, AR, CRSwNP or CRSsNP and asthma have a broad definition, which highlights their heterogeneity. AR requires the demonstration of a causal link between atopy, allergen sensitization and allergen-driven nasal symptoms. Conversely, CRS, a frequent upper airway condition (11% of the adult population), is defined by evidence of rhinosinusitis on computed tomography (CT) and/or by nasofibros-copy along with chronic nasal symptoms ( $\geq 12$  weeks).<sup>9,10</sup> The same observation can be made with asthma, which is determined by respiratory symptoms related to variable airflow limitation in a variable bronchial inflammation setting.<sup>11</sup> AD is clinically defined by chronic or relapsing eczematous skin lesions associated with pruritus.<sup>12</sup> To reduce heterogeneity and help decipher the critical physiopathological pathways involved, clinical studies of such diseases have been performed to identify homogeneous phenotypic groups (Table 1).

Several clinical characteristics to be considered, including age of onset, are crucial. Indeed, infancy onset of AD is strongly associated with the early onset of AR and asthma.<sup>13</sup> In adulthood, a comparable association between AD and asthma can be found.<sup>14</sup> Atopy, another critical phenotypic trait, is defined by the propensity of an individual to develop a specific IgE response against harmless antigens. As a part of AR definition, nasal symptoms need to be consistent with specific IgE production and/or cutaneous prick tests. Conversely, atopy and evidence of IgE-related cutaneous symptoms are necessary to define extrinsic AD in exclusion of intrinsic AD. It is then mainly associated with other atopic diseases such as AD and allergic asthma.

Ethnic background has a differential impact on the expression of those diseases. For example, in AD, the clinical manifestation can be completely different among Asian, African American and European patients.<sup>15</sup> In CRS, a multi-centre study in Europe, Asia and Oceania demonstrated that lower predominance of eosinophils in nasal polyps and mixed cytokines pattern (Th1/Th2/TH17) could be found in Chinese population.<sup>16</sup> In contrast, in asthma and AR such consideration does not seem to be critical. In parallel, some phenotypic characteristics are specific to the disease. In CRS, two phenotypes are described following nasal endoscopy: one with (CRSwNP) and one without (CRSsNP) nasal polyps with a ratio of approximately 1 to 4.<sup>17</sup> In asthma, obesity is frequently associated with a specific severe presentation responding to personalized care (loss of weight, bariatric surgery when necessary, etc.).<sup>18</sup>

Beyond such phenotypes, endotypes have been discovered over the years thanks to cluster analyses and physiopathological assessments (Figure 1).

It has been highlighted that AD can be divided into several physiopathological pathways according to serum measures.<sup>19</sup> Two high type 2 cytokine family clusters could be described depending on high levels of thymic stromal lymphopoietin (TSLP) or pulmonary and activation-regulated chemokine (PARC). Interestingly, such clusters

were differently associated with AD severity, the latter being associated with more severe disease.

In parallel, cluster analysis performed on a European cohort of patients suffering from chronic rhinosinusitis demonstrated that 2 main endotypes could be distinguished: an IL-5-high (eosinophilic) endotype linked to type 2 immunity and an IL-5-low (noneosinophilic)-driven disease.<sup>20,21</sup> Eosinophilic chronic rhinosinusitis (ECRS) was then defined by an eosinophil count in nasal mucosa greater than or equal to 70 eosinophils/HPF (magnification,  $\times 400$ ). A strong association between CRSwNP and eosinophilic signatures has been described, consistent with that in other studies.<sup>22</sup> Nasal polyps also have higher total and specific local IgEs associated with eosinophilic inflammation.<sup>23</sup> Up-regulation of the coagulation cascade and down-regulation of fibrinolysis strongly induce abnormal fibrin deposition in nasal mucosa, and type 2 inflammation plays a central role in the imbalance of coagulation and fibrinolysis, further highlighting its role in polyp formation.<sup>21</sup>

In asthma, the natural course of the disease mainly depends on the involvement or not of an eosinophilic inflammation.<sup>3,24</sup> Eosinophilic asthma is defined by significant bronchial infiltration by eosinophils evaluated by sputum induction ( $> 3\%$  of cells). Blood hypereosinophilia ( $> 500 \text{ mm}^{-3}$ ) can be observed in approximately 10% of the overall population of asthmatic patients, and significant sputum eosinophilia can be observed in approximately 50% of the severe asthma population.<sup>25,26</sup> Allergic asthma and nonallergic asthma associated with CRS are examples of eosinophilic asthma.<sup>27-29</sup> In those subpopulations, eosinophils are closely linked to exacerbation rate, control of the disease and quality of life. Noneosinophilic asthma includes neutrophilic asthma and pauci-granulocytic asthma, which are less understood.<sup>30,31</sup>

Some pathological paths that seem specific could be important in several fields. Filaggrin gene mutations (R501X and 2282del4) are closely linked to AD susceptibility, particularly in those with elevated serum total IgE.<sup>32</sup> Interestingly, such mutations are also linked with asthma predisposition and severity.<sup>33,34</sup> Similar observations could be made between filaggrin gene mutations and sensitization and AR in meta-analyses.<sup>35</sup> Another example is the link between the microbiome and disease course. *Staphylococcus aureus* colonization and their specific IgE are associated with CRSwNP pathophysiology.<sup>36</sup> A comparable observation can be made in AD lesion type- and severity-linked *S. aureus* colonization.<sup>37,38</sup> It is also correlated with high IgE production and type 2 responses in AD.<sup>39,40</sup>

Even though respiratory and cutaneous diseases seem to be completely different, epidemiologic associations of specific phenotypes can be found, and type 2 immunity hallmarks can be found.

### 2.2 | Type 2 inflammation: Similarities and differences among diseases.

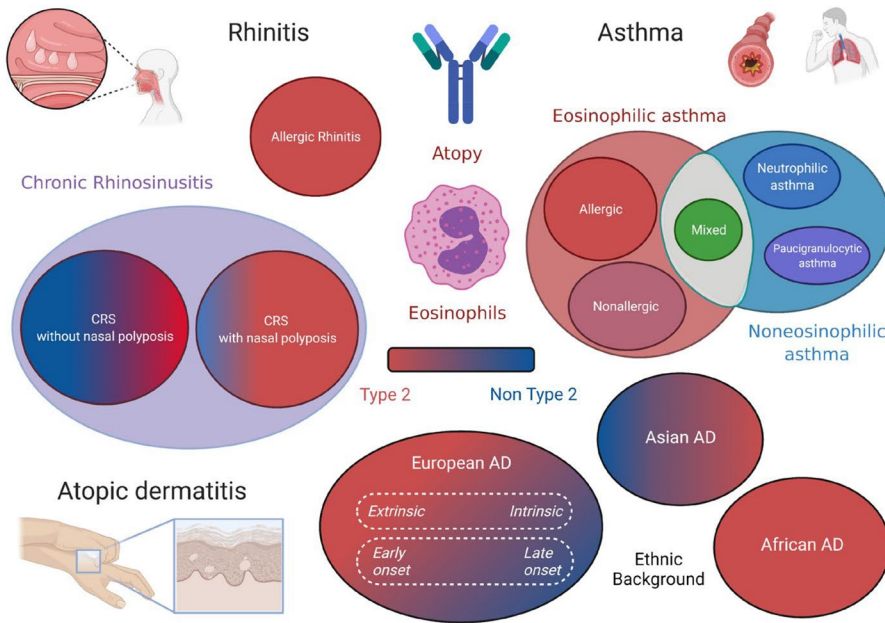
Though type 2 inflammation can be found in several diseases, complex interactions between the environment and cellular actors drive the observed nuances depending on the barrier (Figure 2).

TABLE 1 Main phenotypes of environment-driven diseases implicating type 2 immunity

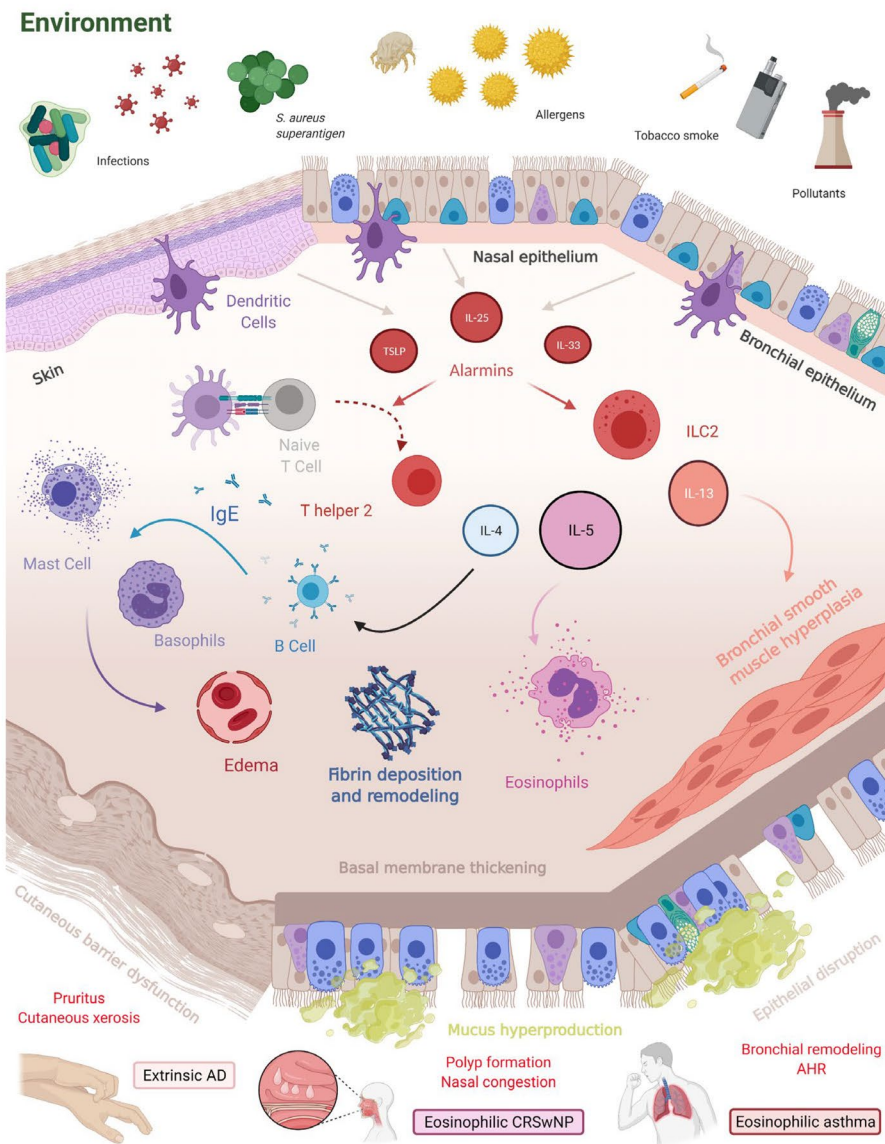
Barrier	Disease	Phenotype	Clinical characteristics	Biological characteristics	Associated comorbidities
Cutaneous	AD	<b>Extrinsic AD</b>	Early onset Atopic background Chronic relapsing course	High total and allergen-specific IgE High rate of filaggrin mutations	<b>AR</b> <b>Allergic asthma</b> Food allergy
		<b>Intrinsic AD</b>	No atopic background	Normal total IgE levels and no specific IgE	NA
Respiratory	Asthma	<b>Allergic asthma</b>	Early onset Atopic background	High total and allergen-specific IgE	<b>ARAD</b>
		<b>Nonallergic eosinophilic asthma</b>	Late onset Severe asthma	High blood eosinophilia High sputum eosinophilia	<b>CRSwNP</b>
		<b>Obesity-related asthma</b>	Adult onset Obesity	Variable inflammatory components	Dysmetabolic syndrome
		<b>Noneosinophilic asthma</b>	Late onset	Paucigranulocytic or neutrophilic infiltrates	NA
	Rhinitis	<b>AR</b>	Early onset Atopic background	High total and allergen-specific IgE	<b>AD</b> Allergic conjunctivitis
	CRS	<b>CRSwNP</b>	Adult onset High recurrence post polypectomy	High blood eosinophilia High mucosal eosinophilia IgE formation against <i>S. aureus</i> enterotoxins	<b>Severe eosinophilic asthma</b>
		<b>CRSsNP</b>	Early adult onset Less severe sinus lesions	Low eosinophilia Neutrophilic infiltration Overexpression of TGFβ	NA

Abbreviations: AD, atopic dermatitis; AR, allergic rhinitis; CRSsNP, chronic rhinosinusitis without nasal polyposis; CRSwNP, chronic rhinosinusitis with nasal polyposis. The bold text indicates the most common phenotypes of disease and their most common associated comorbidities.





**FIGURE 1** Phenotypic traits and main endotypes of environment-driven diseases. Type 2 immunity is implicated only in a small proportion of patients affected by chronic rhinitis, asthma and atopic dermatitis. Created with BioRender.com



**FIGURE 2** Type 2 inflammation in environment-driven diseases. Diverse environmental aggression activates innate and adaptive immunity towards type 2 polarization. The epithelium plays an important role not only as an activator but also as a collateral target of inflammation. Eosinophilic infiltration and basophil and mast cell degranulation upon renewed stimulation induce oedema and critical tissue remodelling. Overall, tissue inflammation and epithelial dysfunction lead to hallmarks of cutaneous and respiratory symptoms. Abbreviations: AD, atopic dermatitis; AHR, airway hyperresponsiveness; CRSwNP, chronic rhinosinusitis with nasal polyposis; IgE, Immunoglobulin E, IL, interleukin; ILC2, group 2 innate lymphoid cell; TSLP, thymic stromal lymphopoietin. Created with BioRender.com

Epithelial dysfunction is shared across type 2-driven diseases. In AD, it is a multifactorial core characteristic including genetic factors such as filaggrin mutations.<sup>41</sup> A dysfunctional cutaneous barrier leads to overstimulated damaging immune responses that weaken the epithelium, creating a vicious cycle.<sup>42</sup> Recently, it has been demonstrated that IL-13 alters the tight junctions of cutaneous and bronchial epithelia in AD and asthma, respectively.<sup>43,44</sup> Mature polyps from CRSwNP patients, defined by end-stage remodelled polyps (stromal oedema, fibrin deposition, loss of epithelial cells), also present a down-regulation of adhesion molecules in comparison with healthy mucosa.<sup>45</sup> However, the epithelium is not only a passive physical barrier but also a potent modulator of immune responses through the secretion of alarmins.

Thymic stromal lymphopoietin from the IL-7 family is able to activate Langerhans cells and skew them to a pro-Th2 phenotype in AD.<sup>46,47</sup> Conversely, stimulation of the TSLP receptor increases IL-4 secretion by CD4<sup>+</sup> T cells from AD.<sup>48</sup> TSLP from nasal polyps induces higher IL-5 secretion by mast cells *ex vivo*, highlighting its important role in the upper airways.<sup>49</sup> A similar consideration was made with higher TSLP epithelial expression found in bronchial biopsies from asthmatic patients in comparison with healthy volunteers.<sup>50</sup> In parallel, autocrine type 2 processes can be found: stimulation by type 2 cytokines and TLR3 increases the ability of cultured human bronchial epithelial cells to be stimulated by TSLP.<sup>51</sup>

Another cytokine mainly secreted by the epithelium is IL-25 aka IL-17E, an IL-17 family cytokine. IL-25 is overexpressed in bronchial mucosa and the dermis after epithelial exposure to relevant allergens and is up-regulated in nasal polyps from patients with CRSwNP.<sup>52,53</sup> Notably, increased IL-25 production in nasal polyps is associated with corticosteroid sensitivity.<sup>54,55</sup> Considering asthmatic patients, high expression of IL-25 evaluated in bronchial biopsies is associated with higher eosinophil infiltration, sputum eosinophils and blood eosinophils.<sup>56</sup>

IL-33, a member of the IL-1 cytokine family, is also implicated in type 2 inflammatory responses. It is produced not only by epithelial cells but also by bronchial smooth muscle cells.<sup>57</sup> IL-33 strongly elicits the activation of innate type 2 airway immunity, which leads to eosinophil infiltration.<sup>58</sup> In a mouse model of asthma using an evaluation of bronchial contraction *ex vivo*, IL-33 stimulation was correlated with airway hyperresponsiveness.<sup>59</sup> Increased production of IL-33 by keratinocytes leads to the expansion of various innate cells in mouse models of AD.<sup>60,61</sup> Genetic studies in CRSwNP also highlighted the association between IL-33 and nasal polyposis.<sup>62</sup>

The activation of the immune cascade depends not only on epithelial stimuli but also on professional antigen-presenting cells such as dendritic cells (DCs). In asthma, airway dendritic cells are essential for inducing naïve T cell commitment towards T helper 2 polarization and subsequent proliferation.<sup>63</sup> In allergic mouse models of asthma, dendritic cells are critical for inducing robust T helper 2 immunity against house dust mites.<sup>64</sup> Comparable observations can be made with skin dendritic cells (Langerhans cells), which act as environmental sensors and drive type 2 responses in AD skin.<sup>65</sup>

Type 2 adaptative immunity involves CD4<sup>+</sup> T helper cells secreting IL-4, IL-5 and IL-13. In asthma, these cytokines can discriminate 2 types of asthma inflammatory profiles: type 2 (or eosinophilic) and non-type 2.<sup>66</sup> It has been demonstrated that IL-13<sup>+</sup> skin homing Th2 cells were higher in AD patients, particularly those with high IgE phenotypes.<sup>67</sup> In AD, IL-4 and IL-13 lead to skin inflammation, itch and skin hyperpermeability by down-regulating filaggrin production by keratinocytes.<sup>68</sup> However, CD4<sup>+</sup> T helper cells are not the only source of cardinal type 2 cytokines. Group 2 innate lymphoid cells (ILC2s) are also important producers of IL-5, IL-9 and IL-13 depending on the transcription factors GATA-3 and ROR $\alpha$  and the cytokine environment.<sup>69-72</sup> Activated ILC2s have been found in greater numbers in the airways of severe eosinophilic asthmatic patients than in mild asthmatic patients and healthy volunteers.<sup>73</sup> More recently, human studies demonstrated that local allergen stimulation leads to a local infiltration of activated ILC2-secreting pro-T2 cytokines.<sup>74,75</sup> Cooperation between ILC-2 and T helper 2 cells is sufficient to induce strong type 2 immunity in an adoptive transfer mouse model of asthma.<sup>76</sup> Moreover, ILC-2 stimulated by TSLP demonstrates corticosteroid resistance, allowing the sustained secretion of type 2 cytokines despite treatment.<sup>77</sup> Comparable data are found in eosinophilic nasal polyposis with a higher rate of ILC2s in the mucosa and corticoid sensitivity in these cells.<sup>78,79</sup> Finally, skin lesions from patients suffering from AD demonstrate a high number of ILC-2 along with basophils in comparison with healthy skin.<sup>80</sup>

In parallel, in response to IL-4, specific B cells switch their antibody class towards IgE production. These IgEs are essential to sensitize basophils and mast cells to a specific allergen. In allergy-driven type 2 immunity, specific IgE is secreted and fixed to high affinity Fc receptors present on mast cells and basophils. Upon a second stimulation, the cross-linking of fixed IgE leads to the activation of mast cells and basophils and the secretion of presynthesized cytokines. Histamine increases vessel permeability, which leads to tissue oedema. Tryptase, prostaglandins and leukotrienes also increase blood permeability, which favours inflammatory cell migration in tissues. Interestingly, a specific environment can stimulate the production of IgE: in DA, *S. aureus* can trigger it by inducing IL-36 production.<sup>65</sup> In parallel, specific IgEs also play important role in sensing small amount of allergen, activating and polarizing (type 2 immunity) of the adaptative immune system. Indeed, IgE-allergen complexes can be internalized by antigen-presenting cells, like DCs, B cells or even basophils, through IgE high (Fc $\epsilon$ R1) and low (CD23) affinity receptors and then presented to T cells.<sup>81-83</sup> This facilitated antigen presentation has been implicated in the effector phases allergic rhinitis and allergic asthma and in the immunopathology of atopic dermatitis.<sup>84,85</sup>

Eosinophils are important effectors involved in type 2 immunity. Their production by the bone marrow and their ability to infiltrate tissue are tightly regulated by GM-CSF, IL-5, and eotaxin-1.<sup>86</sup> In asthma, IL-5 and eotaxin are critical to eosinophil trafficking to the lung.<sup>87,88</sup> Along with body mass index and bronchodilator responsiveness, the blood eosinophil count was significantly associated with the exacerbation rate in an exacerbation-prone severe asthmatic subpopulation.<sup>89</sup> Conversely, eosinophilic inflammation also

TABLE 2 Biotherapies of clinical trials in type 2-driven airways and cutaneous diseases

Type 2-related pathway	Targeted therapy	AD	CRSwNP	Asthma	Ref
<b>IgE</b>	Omalizumab	No effect	Improved symptoms	Improved symptoms	9798,101,102
<b>IL-5</b>	Mepolizumab	No effect	Reduced need for surgery	Improved symptoms	103,104,114,115
	Benralizumab	Ongoing NCT03563066	Ongoing NCT044605094	Improved symptoms	106–108
	Reslizumab	No data available	Withdrawn	Improved symptoms	105
<b>IL-4/IL-13</b>	Dupilumab	Improved symptoms	Improved symptoms	Improved symptoms	116–119
	Tralokinumab	Improved symptoms	Improved symptoms	Improved symptoms	124–125–128
<b>IL-13</b>	Lebrikizumab	Ongoing NCT04146363	Ongoing NCT04178967	No effect	123
<b>CRTh2</b>	Fevipirant	No effect	No data available	No effect	132
	AZD1981	No data available	No data available	No effect	130
<b>TSLP</b>	Tezepelumab	No data available	No data available	No effect	136
<b>IL-33</b>	REGN3500	Ongoing Phase II	Ongoing combination with dupilumab	Improved symptoms	NA

Abbreviations: AD, atopic dermatitis; CRSwNP, chronic rhinosinusitis with nasal polyposis; IgE, Immunoglobulin E, IL, Interleukin; OCS, oral corticosteroids. The bold text indicates the most common phenotypes of disease and their most common associated comorbidities.

correlates with airway obstruction and airway hyperresponsiveness to methacholine.<sup>90</sup> The production and release of eosinophil basic protein is mainly associated with tissue remodelling and also key features of type 2 disease, such as airway hyperresponsiveness and polyp formation.<sup>91</sup> However, eosinophilic infiltrates of nasal mucosa and polyps are incompletely associated with symptoms and quality of life.<sup>92,93</sup> On the other hand, mucosal eosinophils and blood hyper-eosinophilia are significantly correlated with polyposis recurrence after nasal polypectomy.<sup>94</sup> In AD, eosinophils are classically elevated in patients' serum and infiltrate lesional skin.<sup>95</sup> Eosinophils can be detected in cutaneous biopsies from acute and chronic lesions of AD.<sup>96</sup> However, cutaneous eosinophilia does not seem to correlate with the severity of the disease. Eosinophils seem to be differently associated with severity depending on the involved organ.

Type 2 immunity develops across different environmental barriers following common paths. However, the stimuli and interaction slightly differ from one another. Effector cells and tissue damage are somehow different, and further studies are needed to better understand why.

### 2.3 | Targeting type 2 immunity: What have we learned?

Precision medicine has developed in the last 2 decades and aims to personalize therapeutic strategies. Specific biotherapies targeting key pathways of type 2 immunity have been developed and used in the aforementioned diseases, providing interesting insights in real-life settings (Table 2).

IgE was the first type 2-related pathway to be targeted with omalizumab, a humanized monoclonal antibody. A phase III study demonstrated that omalizumab significantly improves the asthma exacerbation rate in that population compared with placebo.<sup>97,98</sup> Routine clinical practice studies have confirmed its positive impact on the control of the disease, quality of life and ability to lower oral corticosteroid therapy.<sup>99,100</sup> Recent phase III trials of omalizumab in nasal polyposis found significant improvements of symptoms and endoscopic findings under treatment.<sup>101</sup> Strikingly, even though IgE seemed to be important in descriptive studies, omalizumab failed to show clear efficiency in AD until now, raising the question of its true implication in the disease course.<sup>102</sup> Whether omalizumab could be efficient in specific AD phenotypes remains to be demonstrated. Indeed, former RCTs used large inclusion criteria without taking into account the different AD endotypes. It cannot be excluded that some AD subpopulations may benefit from omalizumab treatment.

The IL-5 pathway, mainly associated with eosinophilic involvement, is targeted by the monoclonal antibodies mepolizumab, reslizumab and benralizumab. These drugs are now recommended in severe eosinophilic asthma to reduce the asthma exacerbation rate and oral corticosteroid background treatment.<sup>103–109</sup> Such treatments remain efficient in the long term (1 year and beyond), but some data are in favour of a loss of effect after discontinuation, for

example, considering mepolizumab.<sup>110–113</sup> The IL-5 pathway seems to be important in symptom development, but sustained untargeted parallel stimuli may explain such recrudescence after discontinuation. In CRSwNP, a phase III study (SYNAPSE) recently demonstrated that mepolizumab significantly improved the total nasal polyp score and nasal obstruction in comparison with placebo.<sup>114</sup> A randomized clinical trial to assess the efficacy and safety of benralizumab in eosinophilic CRSwNP is ongoing (NCT04157335). In contrast, mepolizumab failed to demonstrate significant clinical effects on AD at 2 weeks in a small RCT.<sup>115</sup> Benralizumab phase 2 clinical trials are recruiting patients with moderate to severe AD (NCT04605094, NCT03563066). The results from such trials would help define the relevance of targeting eosinophils in AD.

Another approved biologic that can be used to focus on type 2 inflammation is dupilumab. This fully human monoclonal antibody targets IL-4 receptor  $\alpha$ , blocking IL-4 and IL-13 signalling. Phase III clinical trials demonstrated a significant reduction in the exacerbation rate and efficient tapering of oral corticosteroid treatment in moderate to severe asthma.<sup>116,117</sup> Comparable results were observed in CRSwNP, with a significant reduction in nasal symptoms and endoscopic scores and improved quality of life under treatment compared to placebo.<sup>118</sup> Likewise, in uncontrolled moderate-to-severe AD, dupilumab significantly increased the proportion of patients with an Investigator General Assessment (IGA) score of 0 or 1 or a reduction  $\geq 2$  under treatment compared to placebo.<sup>119</sup> Whether targeting the IL-4/IL-13 pathway has sustained effects over time and truly changes the disease course remains to be demonstrated. Interestingly, a main side effect of dupilumab is a transient increase in blood eosinophilia independent of the treatment response. Strikingly, data from phase 3 studies demonstrated that a high rate of conjunctivitis could be observed under treatment in AD patients, whereas such side effects seem to be less frequent in patients treated for asthma or CRSwNP.<sup>120</sup> Few and small series have been published in that subject with the more frequent presentations described are tarsal and bulbar conjunctivitis, blepharitis and limbitis.<sup>121,122</sup> Small studies suggest that AD severity at baseline and history of conjunctivitis may be risk factors of developing such side effect under dupilumab. Treatment associates artificial tears, topical steroids topical immunosuppressive treatment (tacrolimus ointment, ciclosporine drops). In most severe cases with weak therapeutic response, dupilumab cessation is needed. Until now, to our knowledge, the underlying immune mechanisms remains poorly understood.

Moreover, biologics attempting to directly target IL-13 (tralokinumab and lebrikizumab) failed to show significant efficiency in asthma.<sup>123–125</sup> Strategies to find suitable biomarkers (blood eosinophils, FeNO, serum periostin) to select potential responders were designed but did not succeed.<sup>126</sup> In contrast, phase 3 clinical trials recently demonstrated a significant improvement of AD under treatment with tralokinumab with or without topical steroids compared with topical steroids alone or placebo.<sup>127,128</sup> Promising results from a phase II study considering lebrikizumab in moderate-to-severe AD led to the recent onset of 2 parallel phase III clinical trials (ADVOCATE 1 and 2, NCT04146363, NCT04178967).<sup>129</sup> These

different response profiles highlight that despite common pathophysiological pathways, it is still difficult to predict how individual responses occur both between type 2 inflammatory diseases and within a specific disease.

Beyond canonical type 2 cytokines, promising new targets have been discovered. Chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTh2) is activated by prostaglandin 2, promoting the chemotaxis and activation of Th2 lymphocytes and eosinophils. In a proof-of-concept study, AZD1981, an oral CRTh2 antagonist, failed to show a significant increase in peak expiratory flow (primary endpoint), but trends towards an improvement in asthma control score were seen in moderate-to-severe asthma patients.<sup>130</sup> Other data from atopic asthmatic patients display a trend towards an increase in FEV1 under treatment.<sup>131</sup> In parallel, fevipiprant, another oral CRTh2 antagonist, failed to achieve a significant improvement in severe asthma considering the rate of exacerbation.<sup>132</sup> Comparable failure was observed considering the targeting of CRTh2 in AD (NCT01785602).<sup>133</sup> In AD, an innovative strategy has been explored with the nemolizumab, a monoclonal antibody directed against IL-31 Receptor  $\alpha$  subunit, that demonstrated a significant symptomatic improvement of pruritus and cutaneous inflammation compared to placebo plus topical agents.<sup>134</sup> Indeed, IL-31, an IL-6 family cytokine member mainly secreted by Th2 cells, acts not only on peripheral nerve cells (pruritus) but also on immune cells (mast cells, granulocytes) by enhancing their ability to secrete pro-inflammatory cytokines such as IL-4 and IL-13.<sup>135</sup>

Finally, trials assessing the ability of anti-alarmins to restrict the initiation of type 2 responses are recruiting. In severe asthma, tezepelumab, an anti-TSLP antibody significantly reduced the annualized rate of asthma exacerbation.<sup>136</sup> Interestingly, such improvement was observed independently of blood eosinophils ( $\geq$  or  $<300/\text{mm}^3$ ). In parallel, an antibody targeting IL-33, REGN3500, is currently being tested in AD and asthma as monotherapy or in combination with dupilumab (NCT03736967, NCT03112577, NCT03736967). Moreover, astegolimab, a monoclonal antibody targeting IL-33 receptor ST2, recently demonstrated in a phase 2b clinical trial a reduction of annualized asthma exacerbation rate in treated severe asthmatics compared to placebo.<sup>137</sup> Nevertheless, to our knowledge, no anti-IL-25 antibodies are currently being tested in clinical trials. The results from biotherapies targeting alarmins are awaited to evaluate the efficiency of stopping epithelial upstream signals to prevent type 2 immunity sustainment.

### 3 | TYPE 2 INFLAMMATION: UNCERTAINTIES AND UNMET NEEDS

Although its pathophysiology is now better understood, some aspects of type 2 inflammation remain misunderstood (Table 3). First, some discrepancies exist between observational data and results from clinical trials. For example, IgE seems to be important in AD, but its targeting has no effect in clinical settings.<sup>102</sup> In parallel, the true pathophysiological role of eosinophils in the aforementioned diseases

can be discussed in anti-IL5 trials.<sup>138-140</sup> Why decreasing eosinophils in type 2 respiratory diseases is efficient in reducing symptoms but not in cutaneous counterparts is an interesting question to be answered. Conversely, targeting both IL-4 and IL-13 is efficient in improving symptoms in AD and CRSwNP.<sup>141,142</sup> Nevertheless, conflicting results between asthma and AD while aiming specifically at IL-13 may raise the question of potential IL-4-driven and IL-13-driven diseases.

Another issue to be settled is the relevance of assessing type 2 inflammation involvement with biomarkers. A biomarker is a molecule, a gene or a characteristic that is linked to a specific diagnosis, prognosis or response to treatment. To be useful, a biomarker must be not only relevant and consistent but also specific and easy to use. Dealing with type 2 inflammation assessment in clinical practice, 2 situations must be emphasized. First, a clinician should be able to recognize type 2 involvement in individual settings. Second, tools should be available to help the clinician choose the best specific treatment for the patient.

In asthma, type 2 extended evaluation is principally done in rare severe forms of the disease (4% of the adult asthma population) due to the complex procedure involved and the cost of the biotherapies.<sup>143,144</sup> However, such consideration must be tempered, as research on an allergic component has long been part of asthma healthcare strategies (avoidance of allergens, AIT). Phenotyping and endotyping of asthma of any severity is an important objective to improve our knowledge of the natural course of the disease and also its treatment. Consensual eosinophilic severe asthma definition was proposed by a European Respiratory Society taskforce based on a combination of major and minor criteria.<sup>145</sup> This definition is based mainly on 3 biomarkers: sputum induction, blood eosinophilia and  $F_{ENO}$  (exhaled nitric fraction).<sup>146</sup> Sputum induction is considered to be the gold standard of inflammation evaluation in asthma.<sup>143</sup> However, this technique requires trained operators and pathologists, lacks standardization and is also restricted to specialized expert centres. Recent guidelines from the European Respiratory Society and American Thoracic Society recommend assessing blood eosinophilia and  $F_{ENO}$  at the expense of sputum induction.<sup>109</sup> Nevertheless, it should be bear in mind that the correlations among blood eosinophilia,  $F_{ENO}$ , eosinophilic infiltration and symptoms are far from perfect.<sup>147,148</sup> Conversely, with such obstacles to ensure the strong endotyping of

patients, robust biomarkers for the prediction of the response to biotherapies are still lacking. Even though correlations between blood eosinophilia and the response to anti-IL5 strategies have been separately reported, there are still unexpected non-responders.<sup>149-151</sup>

Considering chronic rhinosinusitis, the main characteristic associated with eosinophilic inflammation is the presence of nasal polyps explored by nasal endoscopy. However, with new efficient therapies available against type 2 immunity pathways, biomarkers and clear strategies are needed. The European Position Paper on Rhinosinusitis and Nasal Polyps 2020 (EPOS 2020) recently positions treatable trait checking, including type 2 inflammation, at the core of secondary and tertiary care of chronic rhinosinusitis.<sup>152</sup> The use of biotherapy, namely, dupilumab, the only biological currently validated, is restricted to patients with bilateral polyposis who already had endoscopic sinus surgery with a combination of type 2 inflammation evidence, significant symptoms, an inadequate response to medical therapies and/or asthma comorbidity. Along with non-invasive biomarkers, such as blood eosinophils ( $\geq 0.25$  G/L) and total IgE ( $\geq 100$  UI/ml), direct sampling from former surgery allows the direct evaluation of eosinophil involvement in an individual patient (significant if  $\geq 10$ /high power field) at the cost of relative invasiveness, similar to sputum induction in asthma. Studies are needed to assess the potential of biotherapies to avoid sinus surgery, and robust biomarkers will be needed.

In AD, the use of biomarkers, especially IgE level assessments, is not recommended in routine practice.<sup>12</sup> Assessing eosinophilic infiltration in skin does not seem to be relevant, as anti-IL5 biological strategies failed to show sufficient efficacy in AD, questioning the real implication of eosinophils in the disease. Moreover, the efficacy of dupilumab in moderate-to-severe extrinsic and intrinsic AD does not argue in favour of type 2 inflammation involvement assessment. However, in clinical research settings, analysis of serum biomarkers allowed the description of four different clusters relative to different inflammatory subtypes (IL-1R1, Th1/Th2/Th17, Th2/Th22, low Th2/eosinophils).<sup>153</sup> Conversely, it has been demonstrated that proteomic analysis of tape strips of lesional and non-lesional skin could draw immune profiles modified by treatment with dupilumab.<sup>154</sup> Whether further pretreatment exploration of immunity in AD would lead to better healthcare remains to be demonstrated.<sup>155</sup>

TABLE 3 Unmet needs in type 2-driven diseases

Category	Unmet need	Needed research	Expected outcome
Pathophysiology	Identification of determinants of the nuances of type 2 inflammation	Direct comparison of pathophysiology between type 2-driven diseases	Identification of new targets. Identification of critical disease-modifying traits
Diagnosis	Clear and consensual tools and biomarkers to assess type 2 inflammation	Large-scale clinical and omics studies	Clear and consensual guidelines for type 2 inflammation assessment
Treatment	Clear biomarker to guide biotherapy choice Global assessment of response of type 2 diseases to biotherapies	Predicting real-life clinical studies involving multiple biotherapies Multiple type 2 disease studies involving multiple biotherapies	Multiparametric score predicting response to biotherapy Phenotype-guided treatment Global care of patients suffering from type 2 diseases

Promising results were obtained for fezakinumab, a monoclonal antibody directed against IL-22, a member of IL-10 family cytokine whose receptor is mainly expressed on epithelial cells and implicated in proliferation and tissue repair. Indeed, it demonstrated stronger clinical effects and better transcriptomic improvement in severe AD patients with high IL-22 expression than in those with low IL-22 expression.<sup>156</sup> Moreover, interesting results have been obtained using janus kinase (JAK) inhibitors (upadacitinib and baricitinib) in AD by targeting JAK1/2-STAT6 signalling pathways.<sup>157</sup>

As we presented, there are clear associations between type 2-driven diseases. They can be successive in time or even concomitant, especially considering chronic rhinosinusitis and asthma. Assuming that some pathophysiological mechanisms are shared, the question of whether a unique treatment targeting type 2 immunity could lead to an overall improvement is interesting. Data already exist with concomitant improvement of asthma and chronic rhinosinusitis. Sinus surgery in patients suffering from chronic rhinosinusitis associated with asthma interestingly improves asthma-related quality of life.<sup>158</sup> Asthma control is negatively correlated with the rate of acute exacerbation and recurrence of chronic rhinosinusitis with nasal polyps.<sup>159,160</sup>

Another interesting unsolved question is the potential of treating AD during infancy using type 2-specific treatment to lower the incidence of respiratory type 2 diseases in adulthood. Due to the incomplete association between those 2 events and the complexity of long-term longitudinal interventional studies, questions may remain unanswered for a long time. Until now, there is no data supporting a long-term disease-modifying effect of biologics in type 2-driven diseases. A potential explanation of such constatation may be the targeting of specific path without an action in the overall immune response. Such overall sustained action on immune responses has been demonstrated considering allergic immunotherapy (AIT) in allergic asthma and AR.<sup>161</sup> Besides, first data to be further develop seem to be in favour of preventive effect of AIT in the development of new sensitization and diseases.<sup>162</sup>

Personalized healthcare strategies and biological tools are now shared between respiratory and cutaneous type 2-driven diseases. Improvements in the evaluation of pathophysiological mechanisms and prediction of treatment responses are needed. Conversely, the opportunity to treat several diseases by targeting common traits is an appealing objective to achieve in the future. However, we still lack evidence to support such strategies. Multidisciplinary evaluation of patients to improve the correct recognition and care of the different type 2 mediated diseases is a first step to achieve such objective. Randomized clinical trials and also pragmatic trials assessing multidisciplinary approach in comparison to standard care are needed.

## 4 | CONCLUSION

Respiratory and cutaneous diseases involving type 2 immunity share common clinical and pathophysiological characteristics.

While some are concomitant, such as CRSwNP and asthma, consistent with the concept of unified airways, the association between AD and respiratory diseases seems to principally differ. In parallel, the development of new biotherapies targeting specific paths related to type 2 immunity raised new questions. Indeed, discrepancies in clinical responses between the different biotherapies were observed considering the targeting of eosinophils (anti-IL5) and IgE, raising the question of their true implication in the specific pathophysiology of respiratory and cutaneous diseases. In contrast, the broad positive effect of dupilumab in type 2-driven pathologies makes us wonder whether some hierarchy in the development of immune dysregulation exists. Studies targeting epithelium upstream signals are ongoing, and their results may contribute to answering this question.

Our conception of the global healthcare of these conditions changed with the rise of personalized and precision medicine in our routine practice. The specific evaluation of relevant targets is now mandatory, but robust biomarkers are still needed. The demonstration of type 2 immunity implications in several other diseases, such as food allergies, eosinophilic esophagitis, chronic urticaria, and atopic keratoconjunctivitis, is now leading to the extension of trials of type 2-targeting biotherapies. Whether a multidisciplinary approach could possibly improve the overall care of patients suffering from multiple type 2 diseases is a critical question. However, specific interventional studies are needed to obtain strong evidence in favour of this hypothesis.

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## CONFLICT OF INTERESTS

DH declares the following conflict of interest: Support for attending meetings and/or travel from SANOFI, Novartis, GSK and AstraZeneca. OM declares the following link of interest: Adviser, and/or clinical study investigator for Medtronic, Sanofi-Genzyme, GSK, Novartis, MSD, ALK. SB declares the following conflict of interest: Scientific adviser, consultant, and/or clinical study investigator for Almirall, Sanofi-Genzyme, Abbvie, Novartis, Janssen, Leo-Pharma, Pfizer, Eli Lilly, UCB Pharma. AM declares the following conflict of interest: Consultant or investigator for Novartis, GSK, SANOFI & Astra Zeneca. LC declares the following link of interest: Support for a PhD.

## AUTHOR CONTRIBUTIONS

DH and LC drafted the manuscript. DH made the figures and tables. OM, SB, AM and LC critically revised the manuscript.

## DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

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## REFERENCES

- Lambrecht BN, Hammad H. The immunology of the allergy epidemic and the hygiene hypothesis. *Nat Immunol*. 2017;18(10):1076-1083.
- Spergel JM, Paller AS. Atopic dermatitis and the atopic march. *J Allergy Clin Immunol*. 2003;112(6 Suppl):S118-S127.
- Haldar P, Pavord ID, Shaw DE, et al. Cluster analysis and clinical asthma phenotypes. *Am J Respir Crit Care Med*. 2008;178(3):218-224.
- Colas L, Hassoun D, Magnan A. Needs for systems approaches to better treat individuals with severe asthma: predicting phenotypes and responses to treatments. *Front Med (Lausanne)*. 2020;7:98.
- Pulendran B, Artis D. New paradigms in type 2 immunity. *Science*. 2012;337(6093):431-435.
- Gieseck RL 3rd, Wilson MS, Wynn TA. Type 2 immunity in tissue repair and fibrosis. *Nat Rev Immunol*. 2018;18(1):62-76.
- Akdis CA, Arkwright PD, Bruggen MC, et al. Type 2 immunity in the skin and lungs. *Allergy*. 2020;75(7):1582-1605.
- Lambrecht BN, Hammad H, Fahy JV. The cytokines of asthma. *Immunity*. 2019;50(4):975-991.
- Meltzer EO, Hamilos DL, Hadley JA, et al. Rhinosinusitis: developing guidance for clinical trials. *J Allergy Clin Immunol*. 2006;118(5 Suppl):S17-S61.
- Hastan D, Fokkens WJ, Bachert C, et al. Chronic rhinosinusitis in Europe—an underestimated disease. A GA(2)LEN study. *Allergy*. 2011;66(9):1216-1223.
- Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention, 2021. Available from: [www.ginasthma.org](http://www.ginasthma.org)
- Eichenfield LF, Tom WL, Chamlin SL, et al. Guidelines of care for the management of atopic dermatitis: section 1. Diagnosis and assessment of atopic dermatitis. *J Am Acad Dermatol*. 2014;70(2):338-351.
- von Kobyletzki LB, Bornehag CG, Hasselgren M, Larsson M, Lindstrom CB, Svensson A. Eczema in early childhood is strongly associated with the development of asthma and rhinitis in a prospective cohort. *BMC Dermatol*. 2012;12:11.
- Smirnova J, Montgomery S, Lindberg M, Svensson A, von Kobyletzki L. Associations of self-reported atopic dermatitis with comorbid conditions in adults: a population-based cross-sectional study. *BMC Dermatol*. 2020;20(1):23.
- Czarnowicki T, He H, Krueger JG, Guttman-Yassky E. Atopic dermatitis endotypes and implications for targeted therapeutics. *J Allergy Clin Immunol*. 2019;143(1):1-11.
- Wang X, Zhang N, Bo M, et al. Diversity of TH cytokine profiles in patients with chronic rhinosinusitis: a multicenter study in Europe, Asia, and Oceania. *J Allergy Clin Immunol*. 2016;138(5):1344-1353.
- Benjamin MR, Stevens WW, Li N, et al. Clinical characteristics of patients with chronic rhinosinusitis without nasal polyps in an academic setting. *J Allergy Clin Immunol Pract*. 2019;7(3):1010-1016.
- Moore WC, Meyers DA, Wenzel SE, et al. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. *Am J Respir Crit Care Med*. 2010;181(4):315-323.
- Thijs JL, Strickland I, Bruijnzeel-Koomen C, et al. Moving toward endotypes in atopic dermatitis: identification of patient clusters based on serum biomarker analysis. *J Allergy Clin Immunol*. 2017;140(3):730-737.
- Tomassen P, Vandeplass G, Van Zele T, et al. Inflammatory endotypes of chronic rhinosinusitis based on cluster analysis of biomarkers. *J Allergy Clin Immunol*. 2016;137(5):1449-1456 e4.
- Koennecke M, Klimek L, Mullol J, Gevaert P, Wollenberg B. Subtyping of polyposis nasi: phenotypes, endotypes and comorbidities. *Allergo J Int*. 2018;27(2):56-65.
- Van Zele T, Claeys S, Gevaert P, et al. Differentiation of chronic sinus diseases by measurement of inflammatory mediators. *Allergy*. 2006;61(11):1280-1289.
- Bachert C, Gevaert P, Holtappels G, Johansson SG, van Cauwenberge P. Total and specific IgE in nasal polyps is related to local eosinophilic inflammation. *J Allergy Clin Immunol*. 2001;107(4):607-614.
- Wenzel SE, Schwartz LB, Langmack EL, et al. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. *Am J Respir Crit Care Med*. 1999;160(3):1001-1008.
- Schleich F, Brusselle G, Louis R, et al. Heterogeneity of phenotypes in severe asthmatics. the Belgian Severe Asthma Registry (BSAR). *Respir Med*. 2014;108(12):1723-1732.
- Price DB, Rigazio A, Campbell JD, et al. Blood eosinophil count and prospective annual asthma disease burden: a UK cohort study. *Lancet Respir Med*. 2015;3(11):849-858.
- Lambrecht BN, De Veerman M, Coyle AJ, Gutierrez-Ramos JC, Thielemans K, Pauwels RA. Myeloid dendritic cells induce Th2 responses to inhaled antigen, leading to eosinophilic airway inflammation. *J Clin Invest*. 2000;106(4):551-559.
- de Groot JC, Storm H, Amelink M, et al. Clinical profile of patients with adult-onset eosinophilic asthma. *ERJ Open Res*. 2016;2(2):00100-2015.
- Shaw DE, Sousa AR, Fowler SJ, et al. Clinical and inflammatory characteristics of the European U-BIOPRED adult severe asthma cohort. *Eur Respir J*. 2015;46(5):1308-1321.
- Tliba O, Panettieri RA Jr. Paucigranulocytic asthma: uncoupling of airway obstruction from inflammation. *J Allergy Clin Immunol*. 2019;143(4):1287-1294.
- Moore WC, Hastie AT, Li X, et al. Sputum neutrophil counts are associated with more severe asthma phenotypes using cluster analysis. *J Allergy Clin Immunol*. 2014;133(6):1557-1563. e5.
- Weidinger S, Illig T, Baurecht H, et al. Loss-of-function variations within the filaggrin gene predispose for atopic dermatitis with allergic sensitizations. *J Allergy Clin Immunol*. 2006;118(1):214-219.
- Palmer CN, Ismail T, Lee SP, et al. Filaggrin null mutations are associated with increased asthma severity in children and young adults. *J Allergy Clin Immunol*. 2007;120(1):64-68.
- Rodriguez E, Baurecht H, Herberich E, et al. Meta-analysis of filaggrin polymorphisms in eczema and asthma: robust risk factors in atopic disease. *J Allergy Clin Immunol*. 2009;123(6):1361-1370. e7.
- van den Oord RA, Sheikh A. Filaggrin gene defects and risk of developing allergic sensitisation and allergic disorders: systematic review and meta-analysis. *BMJ*. 2009;339:b2433.
- Van Zele T, Gevaert P, Watelet JB, et al. *Staphylococcus aureus* colonization and IgE antibody formation to enterotoxins is increased in nasal polyposis. *J Allergy Clin Immunol*. 2004;114(4):981-983.
- Tauber M, Balica S, Hsu CY, et al. *Staphylococcus aureus* density on lesional and nonlesional skin is strongly associated with disease severity in atopic dermatitis. *J Allergy Clin Immunol*. 2016;137(4):1272-1274.
- Guzik TJ, Bzowska M, Kasprovicz A, et al. Persistent skin colonization with *Staphylococcus aureus* in atopic dermatitis: relationship to clinical and immunological parameters. *Clin Exp Allergy*. 2005;35(4):448-455.
- Simpson EL, Villarreal M, Jepson B, et al. Patients with atopic dermatitis colonized with *staphylococcus aureus* have a distinct phenotype and endotype. *J Invest Dermatol*. 2018;138(10):2224-2233.

40. Park HY, Kim CR, Huh IS, et al. *Staphylococcus aureus* colonization in acute and chronic skin lesions of patients with atopic dermatitis. *Ann Dermatol*. 2013;25(4):410-416.
41. Jungersted JM, Scheer H, Mempel M, et al. Stratum corneum lipids, skin barrier function and filaggrin mutations in patients with atopic eczema. *Allergy*. 2010;65(7):911-918.
42. Kezic S, O'Regan GM, Yau N, et al. Levels of filaggrin degradation products are influenced by both filaggrin genotype and atopic dermatitis severity. *Allergy*. 2011;66(7):934-940.
43. Sugita K, Altunbulakli C, Morita H, et al. Human type 2 innate lymphoid cells disrupt skin keratinocyte tight junction barrier by IL-13. *Allergy*. 2019;74(12):2534-2537.
44. Sugita K, Steer CA, Martinez-Gonzalez I, et al. Type 2 innate lymphoid cells disrupt bronchial epithelial barrier integrity by targeting tight junctions through IL-13 in asthmatic patients. *J Allergy Clin Immunol*. 2018;141(1):300-310. e11.
45. Meng J, Zhou P, Liu Y, et al. The development of nasal polyp disease involves early nasal mucosal inflammation and remodelling. *PLoS One*. 2013;8(12):e82373.
46. Soumelis V, Reche PA, Kanzler H, et al. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol*. 2002;3(7):673-680.
47. Nakajima S, Igyarto BZ, Honda T, et al. Langerhans cells are critical in epicutaneous sensitization with protein antigen via thymic stromal lymphopoietin receptor signaling. *J Allergy Clin Immunol*. 2012;129(4):1048-1055. e6.
48. Tatsuno K, Fujiyama T, Yamaguchi H, Waki M, Tokura Y. TSLP directly interacts with skin-homing Th2 cells highly expressing its receptor to enhance IL-4 production in atopic dermatitis. *J Invest Dermatol*. 2015;135(12):3017-3024.
49. Nagarkar DR, Poposki JA, Tan BK, et al. Thymic stromal lymphopoietin activity is increased in nasal polyps of patients with chronic rhinosinusitis. *J Allergy Clin Immunol*. 2013;132(3):593-600. e12.
50. Shikotra A, Choy DF, Ohri CM, et al. Increased expression of immunoreactive thymic stromal lymphopoietin in patients with severe asthma. *J Allergy Clin Immunol*. 2012;129(1): 104-111. e9.
51. Kato A, Favoreto S Jr, Avila PC, Schleimer RP. TLR3- and Th2 cytokine-dependent production of thymic stromal lymphopoietin in human airway epithelial cells. *J Immunol*. 2007;179(2): 1080-1087.
52. Corrigan CJ, Wang W, Meng Q, et al. Allergen-induced expression of IL-25 and IL-25 receptor in atopic asthmatic airways and late-phase cutaneous responses. *J Allergy Clin Immunol*. 2011;128(1):116-124.
53. Shin HW, Kim DK, Park MH, et al. IL-25 as a novel therapeutic target in nasal polyps of patients with chronic rhinosinusitis. *J Allergy Clin Immunol*. 2015;135(6):1476-1485. e7.
54. Hong HY, Chen FH, Sun YQ, et al. Local IL-25 contributes to Th2-biased inflammatory profiles in nasal polyps. *Allergy*. 2018;73(2):459-469.
55. Hong H, Chen F, Sun Y, et al. Nasal IL-25 predicts the response to oral corticosteroids in chronic rhinosinusitis with nasal polyps. *J Allergy Clin Immunol*. 2018;141(5):1890-1892.
56. Cheng D, Xue Z, Yi L, et al. Epithelial interleukin-25 is a key mediator in Th2-high, corticosteroid-responsive asthma. *Am J Respir Crit Care Med*. 2014;190(6):639-648.
57. Prefontaine D, Lajoie-Kadoch S, Foley S, et al. Increased expression of IL-33 in severe asthma: evidence of expression by airway smooth muscle cells. *J Immunol*. 2009;183(8):5094-5103.
58. Bartemes KR, Iijima K, Kobayashi T, Kephart GM, McKenzie AN, Kita H. IL-33-responsive lineage- CD25+ CD44(hi) lymphoid cells mediate innate type 2 immunity and allergic inflammation in the lungs. *J Immunol*. 2012;188(3):1503-1513.
59. Barlow JL, Peel S, Fox J, et al. IL-33 is more potent than IL-25 in provoking IL-13-producing nuocytes (type 2 innate lymphoid cells) and airway contraction. *J Allergy Clin Immunol*. 2013;132(4):933-941.
60. Imai Y, Yasuda K, Sakaguchi Y, et al. Skin-specific expression of IL-33 activates group 2 innate lymphoid cells and elicits atopic dermatitis-like inflammation in mice. *Proc Natl Acad Sci USA*. 2013;110(34):13921-13926.
61. Imai Y, Yasuda K, Nagai M, et al. IL-33-induced atopic dermatitis-like inflammation in mice is mediated by group 2 innate lymphoid cells in concert with basophils. *J Invest Dermatol*. 2019;139(10):2185-2194.
62. Buyschaert ID, Grulois V, Eloy P, et al. Genetic evidence for a role of IL33 in nasal polyposis. *Allergy*. 2010;65(5):616-622.
63. van Rijjt LS, Vos N, Willart M, et al. Essential role of dendritic cell CD80/CD86 costimulation in the induction, but not reactivation, of TH2 effector responses in a mouse model of asthma. *J Allergy Clin Immunol*. 2004;114(1):166-173.
64. Hammad H, Plantinga M, Deswarte K, et al. Inflammatory dendritic cells—not basophils—are necessary and sufficient for induction of Th2 immunity to inhaled house dust mite allergen. *J Exp Med*. 2010;207(10):2097-2111.
65. Yoshida K, Kubo A, Fujita H, et al. Distinct behavior of human Langerhans cells and inflammatory dendritic epidermal cells at tight junctions in patients with atopic dermatitis. *J Allergy Clin Immunol*. 2014;134(4):856-864.
66. Woodruff PG, Modrek B, Choy DF, et al. T-helper type 2-driven inflammation defines major subphenotypes of asthma. *Am J Respir Crit Care Med*. 2009;180(5):388-395.
67. Czarnowicki T, Gonzalez J, Shemer A, et al. Severe atopic dermatitis is characterized by selective expansion of circulating TH2/TC2 and TH22/TC22, but not TH17/TC17, cells within the skin-homing T-cell population. *J Allergy Clin Immunol*. 2015;136(1): 104-115.
68. Howell MD, Kim BE, Gao P, et al. Cytokine modulation of atopic dermatitis filaggrin skin expression. *J Allergy Clin Immunol*. 2007;120(1):150-155.
69. Hoyler T, Klose CS, Souabni A, et al. The transcription factor GATA-3 controls cell fate and maintenance of type 2 innate lymphoid cells. *Immunity*. 2012;37(4):634-648.
70. Neill DR, Wong SH, Bellosi A, et al. Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. *Nature*. 2010;464(7293):1367-1370.
71. Wong SH, Walker JA, Jolin HE, et al. Transcription factor RORalpha is critical for nuocyte development. *Nat Immunol*. 2012;13(3):229-236.
72. Leyva-Castillo JM, Galand C, Mashiko S, et al. ILC2 activation by keratinocyte-derived IL-25 drives IL-13 production at sites of allergic skin inflammation. *J Allergy Clin Immunol*. 2020;145(6):1606-1614.
73. Smith SG, Chen R, Kjarsgaard M, et al. Increased numbers of activated group 2 innate lymphoid cells in the airways of patients with severe asthma and persistent airway eosinophilia. *J Allergy Clin Immunol*. 2016;137(1):75-86 e8.
74. Chen R, Smith SG, Salter B, et al. Allergen-induced increases in sputum levels of group 2 innate lymphoid cells in subjects with asthma. *Am J Respir Crit Care Med*. 2017;196(6):700-712.
75. Winkler C, Hochdorfer T, Israelsson E, et al. Activation of group 2 innate lymphoid cells after allergen challenge in asthmatic patients. *J Allergy Clin Immunol*. 2019;144(1):61-69. e7.
76. Drake LY, Iijima K, Kita H. Group 2 innate lymphoid cells and CD4+ T cells cooperate to mediate type 2 immune response in mice. *Allergy*. 2014;69(10):1300-1307.
77. Liu S, Verma M, Michalec L, et al. Steroid resistance of airway type 2 innate lymphoid cells from patients with severe asthma: The role of thymic stromal lymphopoietin. *J Allergy Clin Immunol*. 2018;141(1):257-268.
78. Walford HH, Lund SJ, Baum RE, et al. Increased ILC2s in the eosinophilic nasal polyp endotype are associated with corticosteroid responsiveness. *Clin Immunol*. 2014;155(1):126-135.



79. Ho J, Bailey M, Zaunders J, et al. Group 2 innate lymphoid cells (ILC2s) are increased in chronic rhinosinusitis with nasal polyps or eosinophilia. *Clin Exp Allergy*. 2015;45(2):394-403.
80. Kim BS, Wang K, Siracusa MC, et al. Basophils promote innate lymphoid cell responses in inflamed skin. *J Immunol*. 2014;193(7):3717-3725.
81. Heyman B, Tianmin L, Gustavsson S. In vivo enhancement of the specific antibody response via the low-affinity receptor for IgE. *Eur J Immunol*. 1993;23(7):1739-1742.
82. Westman S, Gustavsson S, Heyman B. Early expansion of secondary B cells after primary immunization with antigen complexed with IgE. *Scand J Immunol*. 1997;46(1):10-15.
83. Perrigoue JG, Saenz SA, Siracusa MC, et al. MHC class II-dependent basophil-CD4+ T cell interactions promote T(H)2 cytokine-dependent immunity. *Nat Immunol*. 2009;10(7):697-705.
84. Maurer M, Altrichter S, Schmetzer O, Scheffel J, Church MK, Metz M. Immunoglobulin E-mediated autoimmunity. *Front Immunol*. 2018;9:689.
85. Shamji MH, Thomsen I, Layhadi JA, et al. Broad IgG repertoire in patients with chronic rhinosinusitis with nasal polyps regulates proinflammatory IgE responses. *J Allergy Clin Immunol*. 2019;143(6):2086-2094.
86. Ramirez GA, Yacoub MR, Ripa M, et al. Eosinophils from physiology to disease: A comprehensive review. *Biomed Res Int*. 2018;2018:9095275.
87. Mould AW, Ramsay AJ, Matthaei KI, Young IG, Rothenberg ME, Foster PS. The effect of IL-5 and eotaxin expression in the lung on eosinophil trafficking and degranulation and the induction of bronchial hyperreactivity. *J Immunol*. 2000;164(4):2142-2150.
88. Yang M, Hogan SP, Mahalingam S, et al. Eotaxin-2 and IL-5 cooperate in the lung to regulate IL-13 production and airway eosinophilia and hyperreactivity. *J Allergy Clin Immunol*. 2003;112(5):935-943.
89. Denlinger LC, Phillips BR, Ramratnam S, et al. Inflammatory and comorbid features of patients with severe asthma and frequent exacerbations. *Am J Respir Crit Care Med*. 2017;195(3):302-313.
90. Woodruff PG, Khashayar R, Lazarus SC, et al. Relationship between airway inflammation, hyperresponsiveness, and obstruction in asthma. *J Allergy Clin Immunol*. 2001;108(5):753-758.
91. Hulse KE, Stevens WW, Tan BK, Schleimer RP. Pathogenesis of nasal polyposis. *Clin Exp Allergy*. 2015;45(2):328-346.
92. Thompson CF, Price CP, Huang JH, et al. A pilot study of symptom profiles from a polyp vs an eosinophilic-based classification of chronic rhinosinusitis. *Int Forum Allergy Rhinol*. 2016;6(5):500-507.
93. Soy FK, Pinar E, Imre A, Calli C, Calli A, Oncel S. Histopathologic parameters in chronic rhinosinusitis with nasal polyposis: impact on quality of life outcomes. *Int Forum Allergy Rhinol*. 2013;3(10):828-833.
94. Matsuwaki Y, Ookushi T, Asaka D, et al. Chronic rhinosinusitis: risk factors for the recurrence of chronic rhinosinusitis based on 5-year follow-up after endoscopic sinus surgery. *Int Arch Allergy Immunol*. 2008;146(Suppl 1):77-81.
95. Liu FT, Goodarzi H, Chen HY. IgE, mast cells, and eosinophils in atopic dermatitis. *Clin Rev Allergy Immunol*. 2011;41(3):298-310.
96. Kiehl P, Falkenberg K, Vogelbruch M, Kapp A. Tissue eosinophilia in acute and chronic atopic dermatitis: a morphometric approach using quantitative image analysis of immunostaining. *Br J Dermatol*. 2001;145(5):720-729.
97. Holgate ST, Chuchalin AG, Hebert J, et al. Efficacy and safety of a recombinant anti-immunoglobulin E antibody (omalizumab) in severe allergic asthma. *Clin Exp Allergy*. 2004;34(4):632-638.
98. Humbert M, Beasley R, Ayres J, et al. Benefits of omalizumab as add-on therapy in patients with severe persistent asthma who are inadequately controlled despite best available therapy (GINA 2002 step 4 treatment): INNOVATE. *Allergy*. 2005;60(3):309-316.
99. Brusselle G, Michils A, Louis R, et al. "Real-life" effectiveness of omalizumab in patients with severe persistent allergic asthma: the PERSIST study. *Respir Med*. 2009;103(11):1633-1642.
100. Molimard M, Buhl R, Niven R, et al. Omalizumab reduces oral corticosteroid use in patients with severe allergic asthma: real-life data. *Respir Med*. 2010;104(9):1381-1385.
101. Gevaert P, Omachi TA, Corren J, et al. Efficacy and safety of omalizumab in nasal polyposis: 2 randomized phase 3 trials. *J Allergy Clin Immunol*. 2020;146(3):595-605.
102. Siegels D, Heratizadeh A, Abraham S, et al. Systemic treatments in the management of atopic dermatitis: a systematic review and meta-analysis. *Allergy*. 2021;76(4):1053-1076.
103. Ortega HG, Liu MC, Pavord ID, et al. Mepolizumab treatment in patients with severe eosinophilic asthma. *N Engl J Med*. 2014;371(13):1198-1207.
104. Bel EH, Wenzel SE, Thompson PJ, et al. Oral glucocorticoid-sparing effect of mepolizumab in eosinophilic asthma. *N Engl J Med*. 2014;371(13):1189-1197.
105. Castro M, Zangrilli J, Wechsler ME, et al. Reslizumab for inadequately controlled asthma with elevated blood eosinophil counts: results from two multicentre, parallel, double-blind, randomised, placebo-controlled, phase 3 trials. *Lancet Respir Med*. 2015;3(5):355-366.
106. Bleecker ER, FitzGerald JM, Chanez P, et al. Efficacy and safety of benralizumab for patients with severe asthma uncontrolled with high-dosage inhaled corticosteroids and long-acting beta2-agonists (SIROCCO): a randomised, multicentre, placebo-controlled phase 3 trial. *Lancet*. 2016;388(10056):2115-2127.
107. FitzGerald JM, Bleecker ER, Nair P, et al. Benralizumab, an anti-interleukin-5 receptor alpha monoclonal antibody, as add-on treatment for patients with severe, uncontrolled, eosinophilic asthma (CALIMA): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet*. 2016;388(10056):2128-2141.
108. Nair P, Wenzel S, Rabe KF, et al. Oral glucocorticoid-sparing effect of benralizumab in severe asthma. *N Engl J Med*. 2017;376(25):2448-2458.
109. Holguin F, Cardet JC, Chung KF, et al. Management of severe asthma: a European Respiratory Society/American Thoracic Society guideline. *Eur Respir J*. 2020;55(1):1900588.
110. Lugogo N, Domingo C, Chanez P, et al. Long-term efficacy and safety of mepolizumab in patients with severe eosinophilic asthma: a multi-center, open-label, phase IIIb study. *Clin Ther*. 2016;38(9):2058-2070.
111. Busse WW, Bleecker ER, FitzGerald JM, et al. Long-term safety and efficacy of benralizumab in patients with severe, uncontrolled asthma: 1-year results from the BORA phase 3 extension trial. *Lancet Respir Med*. 2019;7(1):46-59.
112. Murphy K, Jacobs J, Bjermer L, et al. Long-term safety and efficacy of reslizumab in patients with eosinophilic asthma. *J Allergy Clin Immunol Pract*. 2017;5(6):1572-1581.
113. Ortega H, Lemiere C, Llanos JP, et al. Outcomes following mepolizumab treatment discontinuation: real-world experience from an open-label trial. *Allergy Asthma Clin Immunol*. 2019;15:37.
114. Han JK, Bachert C, Fokkens W, et al. Mepolizumab for chronic rhinosinusitis with nasal polyps (SYNAPSE): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Respir Med*. 2021;9(10):1141-1153.
115. Oldhoff JM, Darsow U, Werfel T, et al. Anti-IL-5 recombinant humanized monoclonal antibody (mepolizumab) for the treatment of atopic dermatitis. *Allergy*. 2005;60(5):693-696.
116. Castro M, Corren J, Pavord ID, et al. Dupilumab efficacy and safety in moderate-to-severe uncontrolled asthma. *N Engl J Med*. 2018;378(26):2486-2496.
117. Rabe KF, Nair P, Brusselle G, et al. Efficacy and safety of dupilumab in glucocorticoid-dependent severe asthma. *N Engl J Med*. 2018;378(26):2475-2485.

118. Bachert C, Han JK, Desrosiers M, et al. Efficacy and safety of dupilumab in patients with severe chronic rhinosinusitis with nasal polyps (LIBERTY NP SINUS-24 and LIBERTY NP SINUS-52): results from two multicentre, randomised, double-blind, placebo-controlled, parallel-group phase 3 trials. *Lancet*. 2019;394(10209):1638-1650.
119. Simpson EL, Bieber T, Guttman-Yassky E, et al. Two phase 3 trials of dupilumab versus placebo in atopic dermatitis. *N Engl J Med*. 2016;375(24):2335-2348.
120. Akinlade B, Guttman-Yassky E, de Bruin-Weller M, et al. Conjunctivitis in dupilumab clinical trials. *Br J Dermatol*. 2019;181(3):459-473.
121. Achten R, Bakker D, Ariens L, et al. Long-term follow-up and treatment outcomes of conjunctivitis during dupilumab treatment in patients with moderate-to-severe atopic dermatitis. *J Allergy Clin Immunol Pract*. 2021;9(3):1389-1392.
122. Popiela MZ, Barbara R, Turnbull AMJ, et al. Dupilumab-associated ocular surface disease: presentation, management and long-term sequelae. *Eye (Lond)*. 2021. <https://doi.org/10.1038/s41433-020-01379-9> [Epub ahead of print].
123. Hanania NA, Korenblat P, Chapman KR, et al. Efficacy and safety of lebrikizumab in patients with uncontrolled asthma (LAVOLTA I and LAVOLTA II): replicate, phase 3, randomised, double-blind, placebo-controlled trials. *Lancet Respir Med*. 2016;4(10):781-796.
124. Busse WW, Brusselle GG, Korn S, et al. Tralokinumab did not demonstrate oral corticosteroid-sparing effects in severe asthma. *Eur Respir J*. 2019;53(2):1800948. <https://doi.org/10.1183/13993003.00948-2018>
125. Panettieri RA Jr, Sjobring U, Peterffy A, et al. Tralokinumab for severe, uncontrolled asthma (STRATOS 1 and STRATOS 2): two randomised, double-blind, placebo-controlled, phase 3 clinical trials. *Lancet Respir Med*. 2018;6(7):511-525.
126. Gottlow M, Svensson DJ, Lipkovich I, et al. Application of structured statistical analyses to identify a biomarker predictive of enhanced tralokinumab efficacy in phase III clinical trials for severe, uncontrolled asthma. *BMC Pulm Med*. 2019;19(1):129.
127. Silverberg JI, Toth D, Bieber T, et al. Tralokinumab plus topical corticosteroids for the treatment of moderate-to-severe atopic dermatitis: results from the double-blind, randomized, multicentre, placebo-controlled phase III ECZTRA 3 trial. *Br J Dermatol*. 2020.
128. Wollenberg A, Blauvelt A, Guttman-Yassky E, et al. Tralokinumab for moderate-to-severe atopic dermatitis: results from two 52-week, randomized, double-blind, multicentre, placebo-controlled phase III trials (ECZTRA 1 and ECZTRA 2). *Br J Dermatol*. 2020.
129. Guttman-Yassky E, Blauvelt A, Eichenfield LF, et al. Efficacy and safety of lebrikizumab, a high-affinity interleukin 13 inhibitor, in adults with moderate to severe atopic dermatitis: a phase 2b randomized clinical trial. *JAMA Dermatol*. 2020;156(4):411-420.
130. Kuna P, Bjermer L, Tornling G. Two Phase II randomized trials on the CRTh2 antagonist AZD1981 in adults with asthma. *Drug Des Devel Ther*. 2016;10:2759-2770.
131. Bateman ED, O'Brien C, Rugman P, Luke S, Ivanov S, Uddin M. Efficacy and safety of the CRTh2 antagonist AZD1981 as add-on therapy to inhaled corticosteroids and long-acting  $\beta$ (2)-agonists in patients with atopic asthma. *Drug Des Devel Ther*. 2018;12:1093-1106.
132. Brightling CE, Gaga M, Inoue H, et al. Effectiveness of fevipiprant in reducing exacerbations in patients with severe asthma (LUSTER-1 and LUSTER-2): two phase 3 randomised controlled trials. *Lancet Respir Med*. 2021;9(1):43-56. [https://doi.org/10.1016/S2213-2600\(20\)30412-4](https://doi.org/10.1016/S2213-2600(20)30412-4)
133. Agnihotri G, Lio PA. Revisiting therapies for atopic dermatitis that failed clinical trials. *Clin Drug Investig*. 2020;40(5):421-431.
134. Kabashima K, Matsumura T, Komazaki H, Kawashima M, Nemolizumab JPSG. Trial of nemolizumab and topical agents for atopic dermatitis with pruritus. *N Engl J Med*. 2020;383(2):141-150.
135. Furue M, Yamamura K, Kido-Nakahara M, Nakahara T, Fukui Y. Emerging role of interleukin-31 and interleukin-31 receptor in pruritus in atopic dermatitis. *Allergy*. 2018;73(1):29-36.
136. Menzies-Gow A, Corren J, Bourdin A, et al. Tezepelumab in adults and adolescents with severe, uncontrolled asthma. *N Engl J Med*. 2021;384(19):1800-1809.
137. Kelsen SG, Agache IO, Soong W, et al. Astegolimab (anti-ST2) efficacy and safety in adults with severe asthma: a randomized clinical trial. *J Allergy Clin Immunol*. 2021;148(3):790-798.
138. Agache I, Beltran J, Akdis C, et al. Efficacy and safety of treatment with biologicals (benralizumab, dupilumab, mepolizumab, omalizumab and reslizumab) for severe eosinophilic asthma. A systematic review for the EAACI Guidelines - recommendations on the use of biologicals in severe asthma. *Allergy*. 2020;75(5):1023-1042.
139. Gevaert P, Van Bruaene N, Cattaert T, et al. Mepolizumab, a humanized anti-IL-5 mAb, as a treatment option for severe nasal polyposis. *J Allergy Clin Immunol*. 2011;128(5):989-995.
140. Straumann A, Conus S, Grzonka P, et al. Anti-interleukin-5 antibody treatment (mepolizumab) in active eosinophilic oesophagitis: a randomised, placebo-controlled, double-blind trial. *Gut*. 2010;59(1):21-30.
141. Agache I, Akdis CA, Akdis M, et al. EAACI Biologicals Guidelines-dupilumab for children and adults with moderate-to-severe atopic dermatitis. *Allergy*. 2021;76(4):988-1009.
142. Agache I, Song Y, Alonso-Coello P, et al. Efficacy and safety of treatment with biologicals for severe chronic rhinosinusitis with nasal polyps: A systematic review for the EAACI guidelines. *Allergy*. 2021;76(8):2337-2353.
143. Chung KF, Wenzel SE, Brozek JL, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J*. 2014;43(2):343-373.
144. Hekking PP, Wener RR, Amelink M, Zwinderman AH, Bouvy ML, Bel EH. The prevalence of severe refractory asthma. *J Allergy Clin Immunol*. 2015;135(4):896-902.
145. Buhl R, Humbert M, Bjermer L, et al. Severe eosinophilic asthma: a roadmap to consensus. *Eur Respir J*. 2017;49(5):1700634.
146. Horvath I, Barnes PJ, Loukides S, et al. A European Respiratory Society technical standard: exhaled biomarkers in lung disease. *Eur Respir J*. 2017;49(4):1600965.
147. Lim S, Jatakanon A, Meah S, Oates T, Chung KF, Barnes PJ. Relationship between exhaled nitric oxide and mucosal eosinophilic inflammation in mild to moderately severe asthma. *Thorax*. 2000;55(3):184-188.
148. Jatakanon A, Lim S, Kharitonov SA, Chung KF, Barnes PJ. Correlation between exhaled nitric oxide, sputum eosinophils, and methacholine responsiveness in patients with mild asthma. *Thorax*. 1998;53(2):91-95.
149. Harvey ES, Langton D, Katelaris C, et al. Mepolizumab effectiveness and identification of super-responders in severe asthma. *Eur Respir J*. 2020;55(5):1902420.
150. Goldman M, Hirsch I, Zangrilli JG, Newbold P, Xu X. The association between blood eosinophil count and benralizumab efficacy for patients with severe, uncontrolled asthma: subanalyses of the Phase III SIROCCO and CALIMA studies. *Curr Med Res Opin*. 2017;33(9):1605-1613.
151. Corren J, Weinstein S, Janka L, Zangrilli J, Garin M. Phase 3 study of reslizumab in patients with poorly controlled asthma: effects across a broad range of eosinophil counts. *Chest*. 2016;150(4):799-810.
152. Fokkens WJ, Lund VJ, Hopkins C, Hellings PW, Kern R, Reitsma S, et al. European position paper on rhinosinusitis and nasal polyps 2020. *Rhinology*. 2020;58(Suppl S29):1-464.

153. Bakker DS, Nierkens S, Knol EF, et al. Confirmation of multiple endotypes in atopic dermatitis based on serum biomarkers. *J Allergy Clin Immunol*. 2021;147(1):189-198.
154. He H, Olesen CM, Pavel AB, et al. Tape-strip proteomic profiling of atopic dermatitis on dupilumab identifies minimally invasive biomarkers. *Front Immunol*. 2020;11:1768.
155. Renert-Yuval Y, Thyssen JP, Bissonnette R, et al. Biomarkers in atopic dermatitis—a review on behalf of the International Eczema Council. *J Allergy Clin Immunol*. 2021;147(4):1174-1190.
156. Brunner PM, Pavel AB, Khattri S, et al. Baseline IL-22 expression in patients with atopic dermatitis stratifies tissue responses to fezakinumab. *J Allergy Clin Immunol*. 2019;143(1):142-154.
157. Damsky W, Peterson D, Ramseier J, et al. The emerging role of Janus kinase inhibitors in the treatment of autoimmune and inflammatory diseases. *J Allergy Clin Immunol*. 2021;147(3):814-826.
158. Schlosser RJ, Smith TL, Mace J, Soler ZM. Asthma quality of life and control after sinus surgery in patients with chronic rhinosinusitis. *Allergy*. 2017;72(3):483-491.
159. Banoub RG, Phillips KM, Hoehle LP, Caradonna DS, Gray ST, Sedaghat AR. Relationship between chronic rhinosinusitis exacerbation frequency and asthma control. *Laryngoscope*. 2018;128(5):1033-1038.
160. Sella GCP, Tamashiro E, Sella JA, et al. Asthma is the dominant factor for recurrence in chronic rhinosinusitis. *J Allergy Clin Immunol Pract*. 2020;8(1):302-309.
161. Alvaro-Lozano M, Akdis CA, Akdis M, et al. EAACI allergen immunotherapy user's guide. *Pediatr Allergy Immunol*. 2020;31(Suppl 25):1-101.
162. Kristiansen M, Dhami S, Netuveli G, et al. Allergen immunotherapy for the prevention of allergy: a systematic review and meta-analysis. *Pediatr Allergy Immunol*. 2017;28(1):18-29.

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# Bronchial smooth muscle cell in asthma: where does it fit?

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## ABSTRACT

Asthma is a frequent respiratory condition whose pathophysiology relies on altered interactions between bronchial epithelium, smooth muscle cells (SMC) and immune responses. Those leads to classical hallmarks of asthma: airway hyper-responsiveness, bronchial remodelling and chronic inflammation. Airway smooth muscle biology and pathophysiological implication in asthma are now better understood. Precise deciphering of intracellular signalling pathways regulating smooth muscle contraction highlighted the critical roles played by small GTPases of Rho superfamily. Beyond contractile considerations, active involvement of airway smooth muscle in bronchial remodelling mechanisms is now established. Not only cytokines and growth factors, such as fibroblasts growth factor or transforming growth factor- $\beta$ , but also extracellular matrix composition have been demonstrated as potent phenotype modifiers for airway SMC. Although basic science knowledge has grown significantly, little of it has translated into improvement in asthma clinical practice. Evaluation of airway smooth muscle function is still limited to its contractile activity. Moreover, it relies on tools, such as spirometry, that give only an overall assessment and not a specific one. Interesting technics such as forced oscillometry or specific imagery (CT and MRI) give new perspectives to evaluate other aspects of airway muscle such as bronchial remodelling. Finally, except for the refinement of conventional bronchodilators, no new drug therapy directly targeting airway smooth muscle proved its efficacy. Bronchial thermoplasty is an innovative and efficient therapeutic strategy but is only restricted to a small proportion of severe asthmatic patients. New diagnostic and therapeutic strategies specifically oriented toward airway smooth muscle are needed to improve global asthma care.

## INTRODUCTION

Airway smooth muscle cells (aSMC) derive mainly during embryogenesis from mesenchymal precursors, in parallel with epithelial buds, and are associated with the correct development of the airways tree.<sup>1</sup> Present all along the respiratory tree from the trachea to the bronchioles, it is conserved in vertebrae through evolution.<sup>2</sup> aSMCs are thought to maintain basal tone in bronchi and homogeneous lung ventilation through modulation of local airflow resistance.<sup>3</sup> Though, its real

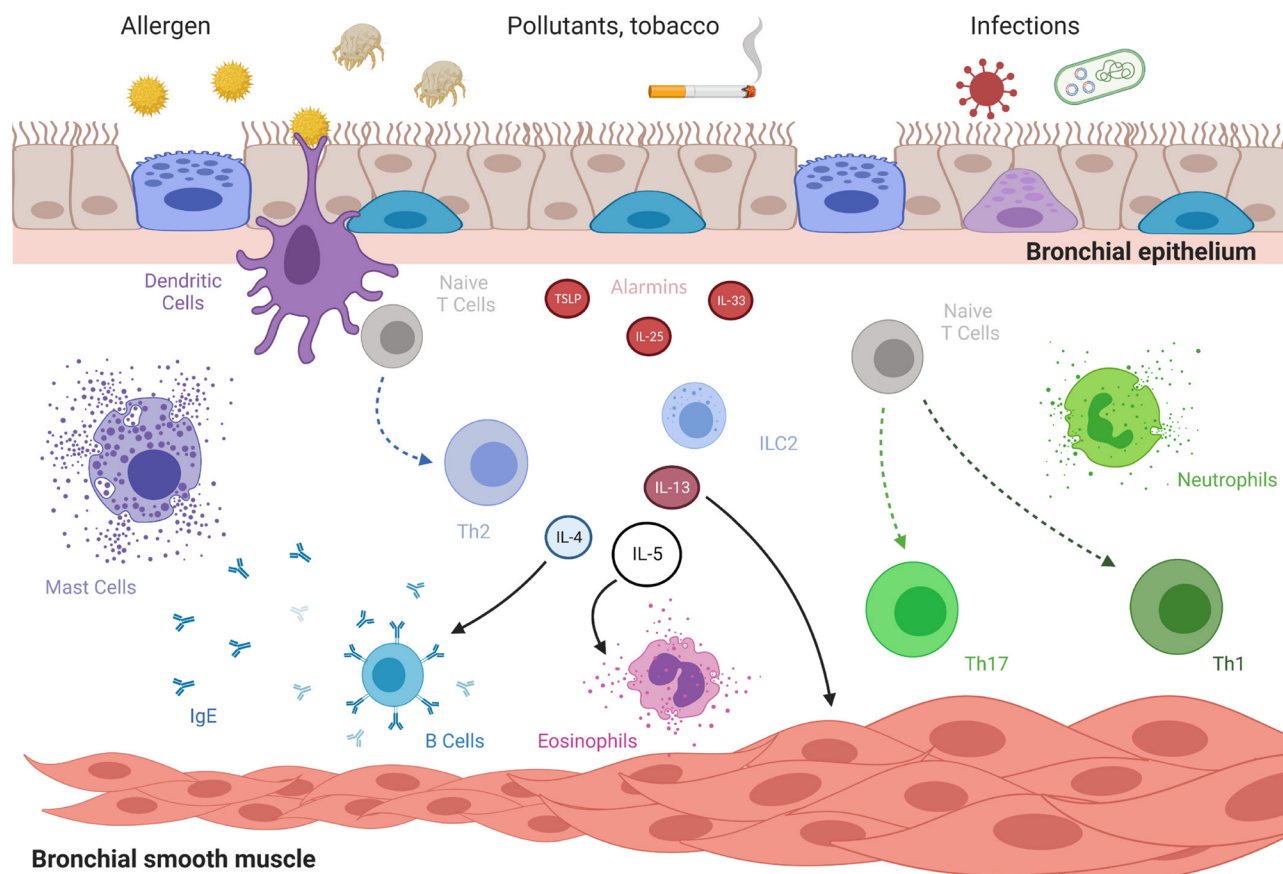
physiological role after development remains controversial in part due to the difficulties to design experimental procedure to test specific hypothesis (impact on mucus expulsion and cough, ventilation to perfusion matching etc).<sup>4</sup> However, while the physiological role of aSMC is controversial, their involvement in airways diseases, especially in asthma, is now better understood.

Asthma is a respiratory condition defined by the association of variable respiratory symptoms, such as acute dyspnoea, chest tightness, wheezing and chronic cough, associated with impaired airflow and chronic bronchial inflammation.<sup>5</sup> It is a frequent disease affecting around 250 million patients worldwide with an increased incidence for the last decade.<sup>6</sup> Asthma treatment is mainly based on inhaled corticosteroids associated with long-acting and short-acting bronchodilators. The main objective of those treatments is to achieve a complete control of the disease.<sup>7</sup>

Asthma pathophysiological hallmark can principally be divided into three inter-related components: airway inflammation, airway hyper-responsiveness and airway remodelling (figure 1).

Inflammatory pathways involved in asthma course have been extensively studied. Two main pathophysiological pathways are now commonly accepted: eosinophilic (or type-2-driven asthma) and non-eosinophilic.<sup>8</sup> Considering type-2-driven asthma, key inflammatory cytokines can be highlighted: interleukine (IL)-4 implicated in T-helper 2 polarisation and IgE switching, IL-5 associated with eosinophils production and trafficking and IL-13 that plays a central role in airway remodelling. Some biotherapies targeting those cytokines pathways had proven their efficiency in selected severe asthmatics and are now available in clinical practice.<sup>9</sup> On the contrary, non-eosinophilic asthma, including paucigranulocytic and neutrophilic asthma, remains poorly understood.

Airway remodelling is defined by an association of structural modifications of bronchial



**Figure 1** Pathophysiology of asthma. Under stimulation by noxious and/or harmless environment, bronchial epithelium secretes alarmins. Such molecules stimulate innate and adaptive immunity giving rise to infiltration of bronchi by eosinophils or neutrophils or both. Conversely, under sustained inflammation, bronchial remodelling developed with increased basal membrane thickness and hypertrophy and hyperplasia of bronchial smooth muscle. Along with specific bronchial smooth muscle cells acquired hypercontractility, all those mechanisms participate to airway hyper-responsiveness. IgE, immunoglobulin E; IL, interleukine; Th, T-helper cell, TSLP, thymic stromal lymphopoietin. Created with BioRender.com.

wall including aSMC alterations, epithelial dysfunction, reticular membrane thickening and oedema.<sup>10 11</sup> Such lesions can be irreversible and lead to the progressive loss of respiratory function through time.<sup>12</sup> Airway remodelling unlinked with airway smooth muscle biology is presented elsewhere.<sup>13</sup>

Airways hyper-responsiveness (AHR) is defined by an exaggerated response of airways to harmless or harmful stimuli. This altered response of bronchi to environment depends on bronchial smooth muscle's activity, principal actor of bronchoconstriction. Noteworthy, inflammation, by acting on bronchial smooth muscle, is also closely linked to this phenomenon.

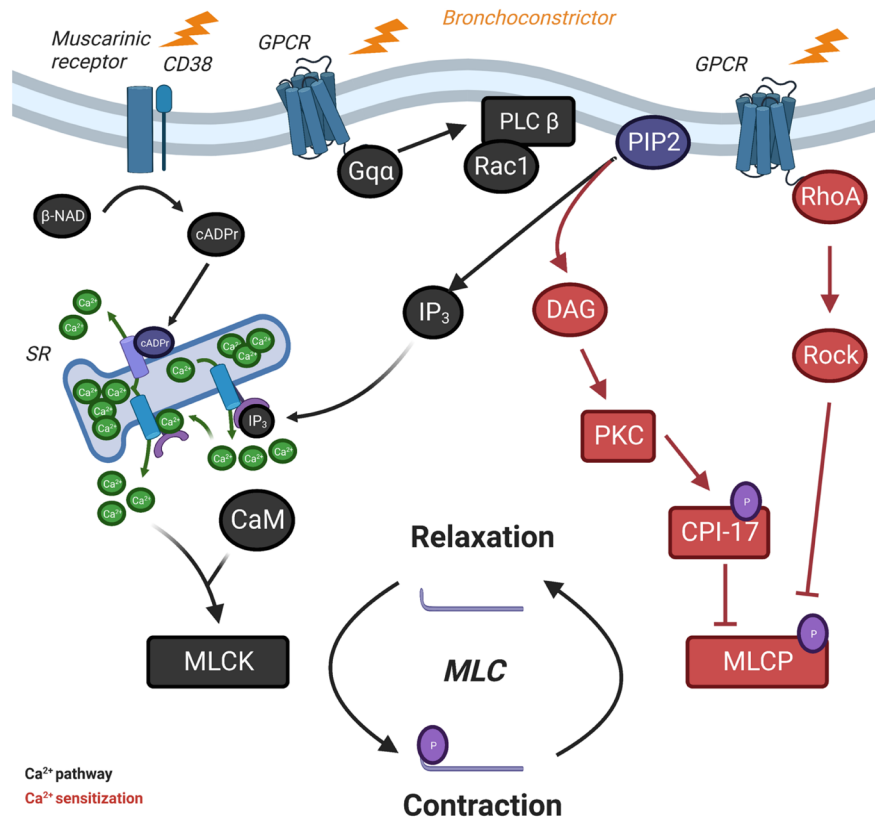
Although recent biotherapies critically improved asthma outcomes in severe asthmatic patients, some inflammatory endotypes, noteworthy non-eosinophilic asthma, remain orphan of efficient treatment.<sup>14–17</sup> New strategies are evaluated in preclinical setup on non-inflammatory components, notably in aSMC. In this review, we will discuss the biological dysfunctions of the bronchial smooth muscle during asthma, the different techniques of evaluation of these dysfunctions in the

clinic as well as the existing or developing therapeutic strategies to manage them.

### Biology of aSMC and role in asthma pathophysiology

#### aSMC contraction dysfunction in asthma

aSMC is essential in the development and sustainment of AHR. Ex vivo studies demonstrated that aSMC harvested from asthmatic subjects displayed increased maximum capacity and shortening velocity compared with controls.<sup>18</sup> Moreover, aSMCs isolated from asthmatic patients presented a stronger contraction in response to histamine compared with controls.<sup>19</sup> Contractile capacity of bronchial SMC principally depends on the phosphorylation of the 20 kDa myosin light chain (MLC<sub>20</sub>), which leads to the activation of the contractile apparatus (figure 2). The phosphorylation level of MLC<sub>20</sub> is regulated by two enzymes: the myosin light chain kinase (MLCK) and the myosin light chain phosphatase (MLCP). Two distinct signalling pathways regulate the activity of these enzymes: the Ca<sup>2+</sup> and the Ca<sup>2+</sup> sensitisation pathway.



**Figure 2** Intracellular regulation of airway smooth muscle cells contraction. Smooth muscle cells contraction and relaxation cycle depends on phosphorylation and dephosphorylation of MLC, respectively. Increase of intracellular  $\text{Ca}^{2+}$  concentration in response to bronchoconstrictor stimuli leads to the CaM-dependent activation of MLCK, which phosphorylates MLC: it is the  $\text{Ca}^{2+}$  pathway. In parallel, activation of RhoA–Rock pathways and CPI-17 deactivates MLCP, which prevents MLC from dephosphorylation: it is the  $\text{Ca}^{2+}$  sensitisation.  $\beta$ -NAD, beta-nicotinamide adenine dinucleotide; cADPr, cyclic adenosine diphosphate ribose; CaM, calmodulin; CD, cluster of differentiation; DAG, diacylglycerol; GPCR, G protein-coupled receptors; IP<sub>3</sub>, inositol triphosphate; MLC, myosin light chain; MLCK, myosin light chain kinase; MLCP, myosin light chain phosphatase; PKC, protein kinase C; PLC, phospholipase C; PIP<sub>2</sub>, phosphatidylinositol-4,5-bisphosphate; Rac1, Rac family small GTPase 1; ROCK, Rho kinase; RhoA, Rho family small GTPase A; SR, sarcoplasmic reticulum.

Considering the  $\text{Ca}^{2+}$ -pathway, the phosphorylation of MLC<sub>20</sub> by MLCK leads to cross bridges with actin that conducts the aSMC contraction cycle. Interestingly, over-expression of MLCK in aSMC has been observed during asthma associated with its overcontractility.<sup>18–20</sup> MLCK activity is principally controlled by the rise in cytosolic  $\text{Ca}^{2+}$  concentration coming from extracellular calcium influx through ion channels and sarcoplasmic reticulum (SR) calcium stores.<sup>21</sup> SR stores release is principally triggered by the inositol 1,4,5-trisphosphate (IP<sub>3</sub>) produced by activated phospholipase C (PLC) after the binding of contractile agonists to G protein-coupled receptors or to M<sub>3</sub> muscarinic receptors.<sup>22</sup>

Recently, this signalling pathway has been complemented by studies demonstrating the involvement of the monomeric GTPase Rac1 in aSMC contraction. Rac1 protein is activated in murine and human aSMC by bronchoconstrictors such as methacholine leading to its association with the pleckstrin homology domain of PLC  $\beta$ 2 to potentiate the production of IP<sub>3</sub> required for aSMC contraction.<sup>23</sup> The relevance of this signalling pathway in

aSMC was highlighted by demonstrating that Rac1 was overactivated in aSMC from asthmatic patients as well as in aSMC from mice developing allergic asthma. In this experimental model, deletion of the Rac1 gene specifically in SMCs or pharmacological inhibition of Rac1 activity prevents AHR. These results identify the Rac1 protein as a new therapeutic target in respiratory pathologies associated with AHR.<sup>23</sup>

Independently of IP<sub>3</sub> production, cyclic adenosine diphosphate ribose (cADPr) could activate the ryanodine receptors channel (RyR) on the SR leading to the liberation of  $\text{Ca}^{2+}$  from the internal SR stores.<sup>24–25</sup> Such metabolite is produced from beta-nicotinamide adenine dinucleotide next to the stimulation of the muscarinic receptor M<sub>3</sub>. Moreover,  $\text{Ca}^{2+}$  liberation is increased by  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release through RyRs, resulting in  $\text{Ca}^{2+}$  wave propagation and the simultaneous aSMC twitching.<sup>26</sup> In asthma, overexpression of CD38 induced by proinflammatory cytokines such as IL-1 $\beta$ , IL-13 and tumor necrosis factor- $\alpha$  leads to increased RyR activation by cADPr.<sup>24–27</sup>

Calcium uptake and sustainment of ATP synthesis by mitochondria are essential to smooth muscle contraction.<sup>28</sup> Calcium uptake is mediated by the mitochondrial  $\text{Ca}^{2+}$  uniporter while its release mainly depends on  $\text{Na}^{2+}/\text{Ca}^{2+}$  or  $\text{H}^{+}/\text{Ca}^{2+}$  exchanger.<sup>29</sup> In aSMC from asthmatic patients, mitochondrial dysfunction can be observed. Downregulation of the expression of sarcoendoplasmic  $\text{Ca}^{2+}$  ATPases 2 is associated with the dysregulation of  $\text{Ca}^{2+}$  homeostasis in asthma.<sup>30</sup>

The  $\text{Ca}^{2+}$  sensitisation pathway leads to a maximal contraction independently of the intracellular  $\text{Ca}^{2+}$  concentration by the regulation of the  $\text{MLC}_{20}$  phosphorylation state by MLCP. On the one hand, activation of protein kinase C by diacylglycerol leads to the phosphorylation of protein kinase C-potentiated phosphatase inhibitor protein of 17 kDa (CPI-17), which binds to the catalytic subunit of MLCP, inhibiting its phosphatase activity.<sup>31</sup> On the other hand, activated RhoA interacts with its downstream effector Rho kinase (ROCK), which inactivates MLCP by phosphorylating its myosin-binding subunit.<sup>32</sup> Moreover, ROCK can also regulate the activity of MLCP by the phosphorylation of CPI-17.<sup>31 33</sup> Interestingly, expression and activity of CPI-17 are increased in aSMC from rats model of allergic asthma.<sup>34</sup> In parallel, several studies have shown an increase of RhoA expression in aSMC in animal models.<sup>35</sup> Inhibition of RhoA activity prevents and reverses AHR induced in allergic asthma models of guinea pigs.<sup>36</sup> Conversely, inflammatory cytokines can significantly influence  $\text{Ca}^{2+}$  sensitisation pathway. IL17A, a cytokine secreted by Th17 cells, is able to induce an upregulation of RhoA protein in human aSMC.<sup>37</sup> Conversely, it can also increase aSMC contraction by the activation of RhoA–ROCK2 through NF- $\kappa$ B pathway.<sup>38</sup> Targeting IL-17 pathway with neutralising antibody decreased the expression of NF- $\kappa$ B, ROCK-I and ROCK-II in lung parenchyma in a mouse model of asthma compared with controls.<sup>39</sup> Interestingly, it has been recently proven in mouse that combination of anti-IL17 antibodies along with ROCK inhibitor (Y-27632) significantly improved respiratory resistance, bronchial remodelling and inflammation.<sup>40</sup> Similar findings were observed with IL-13, which induces an increase in RhoA expression by aSMC.<sup>41</sup> Those results suggest a potential role of  $\text{Ca}^{2+}$  sensitisation pathway dysfunction associated with asthma AHR.

Aside inflammatory cytokines, small molecules can also interact with the contractile apparatus such as nitric oxide (NO). In physiological conditions, NO is a potent bronchodilator through the production of cGMP by activated cytosolic guanylate cyclase leading to decreased intracellular  $\text{Ca}^{2+}$ .<sup>42</sup> Bronchial NO mainly derived from epithelial cells on the one hand and inhibitory non-adrenergic non-cholinergic nerve terminals.<sup>43 44</sup> Inhibition of NO synthesis by L-NG-Nitro arginine methyl ester in vitro and in vivo in guinea pigs enhances the airway hyperresponsiveness in response to histamine.<sup>45</sup> Though, its precise role in the context of asthma remains controversial.<sup>46</sup> Indeed, inflammatory environment, especially type

2 driven, is a potent activator of inducible NO synthase of type 2 in epithelial cells.<sup>47 48</sup> Exhaled NO measured in asthmatic patients is correlated with AHR.<sup>49 50</sup> Association of NO with worsened AHR, despite its physiological bronchodilator effect, can be partly explained by collateral damages linked with peroxynitrite production and side effects on vessels with increased permeability and bronchial oedema.

Autonomous innervation of aSMCs is another modulator of AHR. TRPA1 channels are located at sensory nerves, predominantly on C fibres and is also expressed in non-neuronal cells including airway inflammatory cells, SMC, epithelial cells and fibroblasts.<sup>51</sup> Activation of TRPA1 by environmental irritants such as cigarette smoke or air pollution leads to the activation of bronchopulmonary C fibres in an experimental model and is implicated in cough.<sup>52</sup> Inhibition or knockout of TRPA1 channels leads to an inhibition of neuropeptide release and airway hyperreactivity in an ovalbumin-challenged mice model of asthma.<sup>53</sup> Whether such pathway is implicated in human asthma pathophysiology remains to be demonstrated.

#### aSMC beyond contraction: bronchial remodeling

Airway remodelling presentation may differ between groups of moderate-to-severe asthmatic patients in terms of aSMC mass and basement membrane thickening.<sup>54</sup> Interestingly, airway remodelling can appear early in asthma, even in children.<sup>55 56</sup> Significant bronchial remodelling in childhood, characterised by reticular basement thickening and increased aSMC mass, is associated with severe disease.<sup>57</sup> Moreover, airway remodelling is also associated with persistent obstruction in severe asthmatic children.<sup>58</sup> Those elements are in favour of an early role played by airway remodelling in asthma natural course.

Physiologically, aSMC presents low proliferation capacities and a contractile phenotype characterised by the expression of sm- $\alpha$ -actin, smooth muscle  $\gamma$ -actin, smooth muscle myosin heavy chain, calponin, h-caldesmon, SM22, smoothelin and metavinculin.<sup>59</sup> Though, in inflammatory environment, aSMC have the ability to switch their phenotype with an increase of their proliferation and migration capacity leading to hyperplasia.<sup>60</sup> Mitochondria biogenesis is also affected. In proliferative aSMC from asthmatic patients, increased mitochondrial mass and activity can be observed associated with altered calcium homeostasis.<sup>61</sup>

Phenotype switch can be induced in vitro by several growth factors and cytokines presented in [table 1](#). Analysis of induced sputum in asthmatics showed that many of these molecules are indeed oversecreted.<sup>62</sup> Bronchial inflammation associated with remodelling can be driven by the environment through the ability of bronchial epithelium to secrete alarmins, namely thymic stromal lymphopoietin (TSLP), IL-25 and IL-33, in response to harm.<sup>11</sup> It has been demonstrated that human aSMC expressed receptor to TSLP and IL-25 and that their

**Table 1** Mitogenic factors of aSMC

Factors	Cellular source	Effects	Ref
Growth factors			
PDGF	Platelets, monocytes/macrophages, ASMC, epithelium	Pro-proliferative	123–126
FGF	Extracellular matrix, monocytes/macrophages, ASMC	Pro-proliferative	126–129
EGF	Epithelium, platelets	Pro-proliferative	130
Cytokines			
TGF- $\beta$	ASMC, T-lymphocytes, epithelium	Pro-proliferative	128 131–133
TNF- $\alpha$	ASMC, epithelium, T cells, monocytes/macrophages	Pro-proliferative, antiproliferative	126 134–136
IL-1 $\beta$	T cells, monocytes/macrophages, ASMC, epithelium	Pro-proliferative	137 138
IL-6	T cells, monocytes/macrophages, ASMC, epithelium	Pro-proliferative	135 138
IFN- $\gamma$	T cells, NK cells	Antiproliferative	139
IL-4	T-cell, mast cells	Antiproliferative	140 141
Inflammatory mediators			
Histamine	Mast cells, basophils	Pro-proliferative	142 143
Thromboxane A2	Mast cells, monocytes/macrophages	Pro-proliferative	144 145
Sphingosine 1-phosphate	Plasma, platelets	Pro-proliferative	146
Enzymes and diverse			
Tryptase	Mast cells	Pro-proliferative	143 147
Thrombin	Plasma	Pro-proliferative	148
Elastase	Neutrophils	Pro-proliferative	149
Reactive oxygen species	Monocytes–macrophages, neutrophils, eosinophils, mast cells	Pro-proliferative	150

aSMC, airway smooth muscle cells; EGF, epidermal growth factor; FGF, fibroblast growth factor; IFN, interferon; IL, interleukine; PDGF, platelet-derived growth factor; TGF- $\beta$ , transforming growth factor- $\beta$ ; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ .

stimulation lead to proinflammatory and synthetic phenotypes.<sup>63 64</sup> Conversely, it has been shown by air–liquid interface coculture that injured epithelial cells stimulate aSMC proliferation through the production of proinflammatory molecules (IL-6, IL-8, monocyte chemoattractant protein-1) and matrix metalloproteinase-9.<sup>65</sup> Mechanical stimulation of epithelial cells is another path leading to phenotype switch of aSMC. A significant proliferation of aSMC could be induced in vitro by compression of epithelial cells.<sup>66</sup> Combination of inflammatory cytokines, such as type 2 cytokines, leads to complex modification of aSMC phenotype. For example, whereas IL-13 alone have an antiproliferative effect on cultured aSMC, it also increases the expression of CysLT1 receptor enhancing leukotriene induced proliferation.<sup>67 68</sup> Interestingly, stimulated aSMC are also able to synthesise proinflammatory factors such as the platelet-derived growth factor (PDGF), fibroblast growth factor, IL-1 $\beta$ , transforming growth factor- $\beta$ , IL-5, IL-6, IL-8, IL-17, which further amplifies phenotype switch.<sup>3</sup>

Composition of the extracellular matrix (ECM) itself also influences the phenotype and functions of aSMC.<sup>69</sup> Laminin reduces the aSMC proliferation and increases the expression of contractile proteins such as sm- $\alpha$ -actin and smooth muscle myosin heavy chain.<sup>70</sup> On the contrary, fibronectin promotes the aSMC proliferation

but decreases the expression of contractile proteins.<sup>70</sup> During asthma, the synthesis of laminin is reduced while the fibronectin synthesis is increased promoting the switch of aSMC to a proliferative phenotype.<sup>71</sup> In response, aSMC participate to the deposition of the ECM through increased MMP-9 and MMP-12 expressions as it has been shown in bronchial biopsies from severe asthmatic patients.<sup>72</sup>

At a cell signalling level, aSMC proliferation is principally under the control of extracellular signal-regulated kinase (ERK) and PI3K pathways by increased expression of cyclin D1. ERK protein, a member of the MAPK family, is a central regulator of cell cycle entry and G1 progression essential to aSMC proliferation.<sup>73</sup> In parallel, Akt1, an effector of PI3K, inhibits the constitutively active glycogen synthase kinase 3 and an activator mTOR and p70 S6 kinase which are important for transcriptional activation and protein translation leading to aSMC proliferation and hypertrophy.<sup>74</sup> PI3K can also activate Rac1 and Cdc42 in order to promote the cell proliferation thanks to cyclin D1.<sup>75</sup> In addition, Rac1 forms part of the Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase complex and also participates in the reactive oxygen species production which is involved in the aSMC mitogenesis.<sup>76</sup> We recently demonstrated that Rac1 was essential to the increased proliferation capacities of aSMC



from asthmatic patients in comparison to controls in basal condition and after mitogenic stimulation.<sup>77</sup> We identified the signal transducer and activator of transcription 3 as the main effectors involved in such Rac1-dependent mechanism. Interestingly, inhibition of Rac1 activity in a mouse model of asthma prevented aSMC hyperplasia. aSMC hyperplasia also results from the migration of aSMC or progenitors in response to mitogenic factors such as the PDGF, the vascular endothelial growth factor, the transforming growth factor  $\beta$  (TGF- $\beta$ ) or IL-1 $\beta$ . The P38 MAPK, PI3K and ERK pathways are involved in migration as their inhibition leads to decreased aSMC migration due to reduced phosphorylation of heat shock protein 27 implicated in the F-actin polymerization necessary for cell motility.<sup>78</sup> Moreover, the migration of aSMC in response to PDGF is significantly impacted by the inhibition of PI3K, ERK or ROCK pathway.<sup>79 80</sup>

Aside its proliferative capacity, activated aSMC also demonstrate improved capacity to interact with immune cells through increased expression of their surface molecules such as VCAM-1, ICAM-1, CD44 and LFA-1.<sup>81</sup> Noteworthy, aSMC display closed interaction with mast cells and are able to synthesize powerful mastocyte chemoattractants such as the stem cell factor but also TGF- $\beta$ 1 and tumour suppressor in lung cancer-1.<sup>82</sup> In parallel, aSMC express both CD44 and CD51 which are involved in the mast cell adhesion.<sup>83</sup> Interaction of mast cells with aSMC promotes the degranulation and cytokines production by mast cells ultimately leading to aSMC contraction and proliferation.<sup>84</sup> aSMC can also interact with other immune cell types such as T cells via CD44, which induces DNA synthesis of aSMC and promotes its proliferation.<sup>85</sup>

As presented, aSMC has important impacts on asthma pathophysiology not solely due to their contractile activity but also to their ability to interact with other cell types leading to complex remodelling activity. Such a broad involvement makes the clinical evaluation of its action at a patient level complex, as it is discussed in the next section.

### Bronchial smooth muscle in asthma clinical practice

In routine practice, evaluation of aSMC's function cannot be fulfilled directly. Indeed, conventional pulmonary function tests, such as spirometry and plethysmography only give access to an overall sight of their implication in airway obstruction. New tools currently developed to further assess new facets of its function are described below.

### Airway obstruction in asthma

Airway obstruction is necessary to asthma diagnosis along with relevant and consistent respiratory symptoms. Pulmonary function tests in asthma, particularly forced spirometry, are standardised and aim to prove such obstruction.<sup>86</sup> Evolution of obstruction through time is critical considering asthma care. Data from birth cohorts showed that children with low forced expiratory volume

in one second (FEV1)/forced vital capacity (FVC) ratio had a steeper slope of evolution of FEV1/FVC ratio through time until adulthood that increases the risk of developing asthma.<sup>87</sup> In parallel, patients self-reporting asthma experienced a faster decline of FEV1 through time than healthy volunteers in a 15-year prospective study performed in Denmark.<sup>88</sup>

Bronchodilators response tests are available to assess the role of bronchial smooth muscle contraction in obstruction for an individual patient.<sup>86</sup> However, negative bronchodilator test does not imply that aSMC are not relevant in asthma symptoms. Indeed, a fixed obstruction (FEV1/FVC ratio under lower limit of normal values and/or FEV1 under 80% of predicted value after bronchodilators) can appear in about 20% of never ever-smoking adult asthmatic patients after 10 years of follow-up.<sup>89</sup> Such persistent airway obstruction had been linked with increased airway smooth muscle area in a population of severe asthmatics under standardised high dose anti-inflammatory treatment.<sup>90</sup> Though, airway remodelling is not only dependent on aSMC. It has been shown recently by cluster analysis of pathological examination of bronchial biopsies from asthmatic patients and healthy individuals that bronchial remodelling could be classified into several groups depending on the component involved (bronchial smooth muscle, basal membrane).<sup>54</sup>

Considering small airways impairment in asthma, available explorations are currently imperfect. A study highlighted that mid-expiratory and instantaneous flows (FEF25-75 and FEF75) did not significantly add informations to FEV1 and FEV1 to FVC ratio.<sup>91</sup> Use of impulse oscillometry and nitrogen breath washout techniques identified around 1/3 of asthmatic patients displaying markers of small airways dysfunction.<sup>92</sup> Though, data clearly linking pathology with oscillometric data are still lacking. Conversely, specific imagery approaches are currently assessed. Non-invasive evaluation of obstruction by hyperpolarized <sup>3</sup>HE MRI showed that ventilation defects due to obstruction often persisted in time and location under stable or provoked (methacholine) conditions in a small series of patients.<sup>93</sup> Interestingly, markers of ventilation heterogeneity linked to small airways involvement inversely correlated with variation of asthma control score under inhaled corticosteroid treatment of asthmatic patients.<sup>94</sup> Nevertheless, MRI lacks availability needed for clinical practice.

### Airway hyper-responsiveness in asthma

AHR is an important argument in favour of asthma diagnosis that can be sought by direct and indirect provocation bronchial tests.<sup>95 96</sup> Methacholine and histamine tests are the principal direct provocation tests available. They aim at triggering direct bronchial smooth muscle contraction through inhalation of determined cumulative doses of stimulant. However, this test explores only selected pathways of bronchial smooth muscle contraction to the exclusion of the others described in the previous sections. In addition, its overall sensitivity is around 60%–90% and

**Table 2** Effect of approved biotherapies on pulmonary function tests in allergic and eosinophilic severe asthma (phase III trials)

Phenotype Endotype	Molecule Type	Target	Main inclusion criteria	Effect on respiratory function	Ref
Allergic	Omalizumab Humanised monoclonal antibody	IgE	Severe asthma with high dose ICS Positive SPT to aeroallergen Serum total IgE 30–700 IU/mL	Improved morning PEF +2.8% predicted FEV1 in comparison with placebo	15 106
Eosinophilic	Mepolizumab Humanised monoclonal antibody	IL-5	Severe asthma with high dosage ICS. Peripheral blood eosinophils count $\geq 150/\text{mm}^3$ at screening or $\geq 300/\text{mm}^3$ during the previous year. + Maintenance treatment with systemic corticosteroids (5–35 mg of prednisone or equivalent) <sup>4</sup>	Slight improvement of pre-BD and post-BD FEV1 (+98 mL, +138 mL, respectively) in comparison with placebo Statistically non-significant improvement of pre-BD and post-BD FEV1 in the corticosteroid weaning trial	14 151
Eosinophilic	Reslizumab Humanised monoclonal antibody	IL-5	Inadequately controlled asthma despite at least medium dosage ICS. Peripheral blood eosinophils count $\geq 400/\text{mm}^3$	Statistically significant improvement of pre-BD FEV1 in comparison with placebo (+0.11 L LS mean)	152
Eosinophilic	Benralizumab Humanised monoclonal antibody	IL-5 receptor	Severe asthma with high dosage ICS. Baseline peripheral blood eosinophils count $\geq 300/\text{mm}^3$	Statistically significant improvement of pre-BD FEV1 in comparison with placebo in SIROCCO and CALIMA studies (+159 mL and +116 mL, respectively, LS mean)	16 153 154
Type-2 inflammation	Dupilumab Fully human monoclonal antibody	IL-4 receptor $\alpha$	Uncontrolled asthma despite medium to high dosage ICS and up to two controller	Statistically significant improvement of pre-BD FEV1 in comparison with placebo at 12 wks (+130 mL LS mean) and 24 wks (+ 220 mL LS mean).	17 108
Eosinophilic and non-eosinophilic	Tezepelumab Fully human monoclonal antibody	TSLP	Uncontrolled asthma despite medium to high dosage ICS	Statistically significant improvement of pre-BD FEV1 in comparison with placebo at 52 wks (+130 mL LS mean)	110

BD, bronchodilators; FEV1, forced expiratory volume in one second; ICS, inhaled corticosteroid; IgE, immunoglobuline E; IL-5, interleukine 5; LS, least squares; PEF, peak expiratory flow; SPT, skin prick test; TSLP, thymic stromal lymphopoietin; wks, weeks.

specificity around 90%.<sup>97</sup> Its use in clinical practice is then principally reserved to intermediate probability of asthma at diagnosis and is not recommended for follow-up.

Indirect tests mainly include exercise-induced and eucapnic hyperventilation tests. The objective of such tests is to provoke a deshydration of respiratory airways that stimulates the secretion of various cytokines and inflammatory mediators by bronchial epithelium and sub-mucosa triggering hypersensitive aSMC contraction.<sup>96</sup> Exercise-induced bronchoconstriction is associated with asthma with a good specificity but low sensitivity. In an overall population, exercise-induced bronchoconstriction under standardised exercise correlated with airflow limitation (FEV1) but also with age and sex.<sup>98</sup> Noteworthy, some patients displaying significant positive indirect AHR tests would not react to direct stimulation of airway smooth muscle contraction.<sup>99</sup>

#### Unmet needs in aSMC functional evaluation in asthma

Diagnostic tools determining the exact role played by bronchial smooth muscle in asthma at an individual level

need to be developed. Indeed, spirometry isn't sufficient to precisely incriminate the responsible agent (inflammation, bronchial smooth muscle, infection triggers, combination of those). Tests specifically exploring bronchial contractility and its determinants could potentially be of interest in order to guide therapy, particularly concerning the use of long-acting bronchodilators. Conversely, it would be interesting to evaluate the proliferative activity of bronchial smooth muscle in order to early detect and prevent bronchial remodelling. Finally, the improvement of physiopathological knowledge could lead to the development of new targeted therapeutic strategies alongside their specific biomarkers.

#### aSMC as a target in asthma

##### Conventional therapies: inhaled and oral treatments

Conventional therapies in asthma principally target 2 pathophysiological mechanisms: inflammation and bronchoconstriction. Inhaled bronchodilators (beta-2 agonist and anticholinergic) directly target aSMC by decreasing

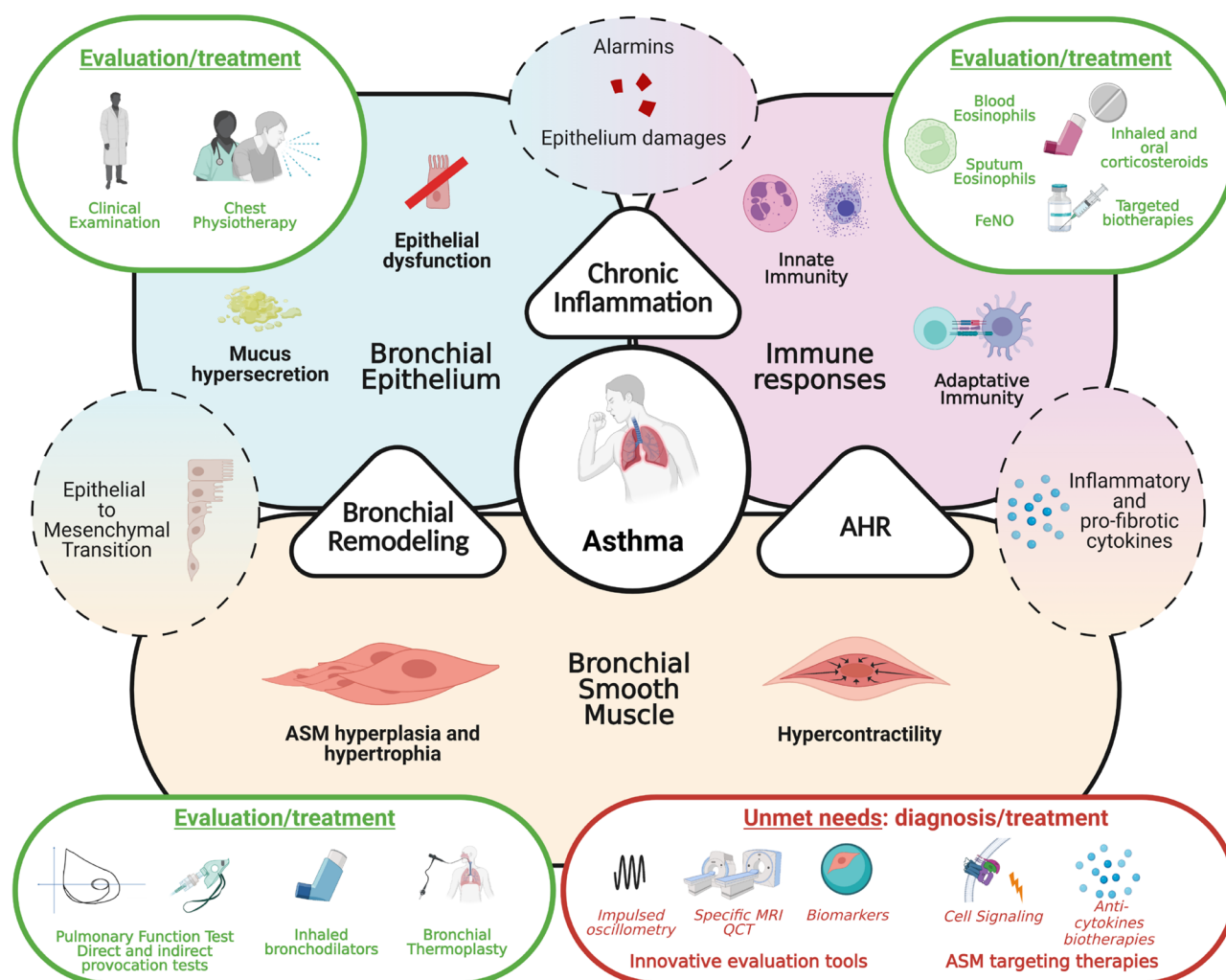
its contractility in order to improve airflow and limit chronic and acute symptoms. Though, pharmacological researches mainly focused on the improvement of the length and/or delay of action. Interestingly, a proof-of-concept clinical trial showed in a small cohort of severe asthmatics (31 patients) that gallopamil, a calcium ion channel inhibitor, could reduce the aSMC bronchial area and thickness after 1 year of treatment in comparison with baseline associated with reduced exacerbation after the end of treatment.<sup>100</sup> However, further clinical trials were withdrawn by the pharmaceutical companies.

Even if inhaled corticosteroids target principally inflammatory effectors, it also affects aSMC contractility and proliferation. Glucocorticoids reduce the expression of  $\alpha$ -smooth actin and the short isoform of MLCK by aSMC in response to TGF- $\beta$  which dampens its contractility.<sup>101</sup>

It also decreases the expression and phosphorylation of CPI-17 by aSMC in a rat model leading to lower MLC phosphorylation and improved AHR.<sup>102</sup> Furthermore, ciclesonide effectively reduced key bronchial remodeling features, such as goblet cell hyperplasia or immune reactive aSMC, in a rat model of asthma.<sup>103</sup> Considering bronchial remodelling, high doses of inhaled steroids also improved the submucosal hypervascularity but also the basement membrane thickness in small clinical studies.<sup>104 105</sup>

### Biotherapies and aSMC

No biotherapy directly targeting aSMC is currently under clinical development to our knowledge. However, available biotherapies that target inflammation can have implication in aSMC (table 2).



**Figure 3** Bronchial smooth muscle cells in asthma from pathophysiological consideration to evaluation and treatments. Asthma development relies on three main pathophysiological processes, bronchial remodelling, chronic inflammation and airway hyper-responsiveness, consequences of alteration of bronchial epithelium and ASM and development of inadequate immune responses. Whereas anti-inflammatory therapeutic strategies got significantly improved in the last years, little progress has been made considering ASM. Innovative precise evaluation tools of ASM function along with specific targeting strategies need to be developed. AHR, airway hyper-responsiveness; ASM, airway smooth muscle; FeNO, exhaled fraction of azote monoxide.

Omalizumab is a humanised monoclonal antibody targeting immunoglobulin (Ig) E to treat severe allergic asthmatic patients. Phase III study demonstrated that omalizumab significantly improves asthma exacerbation rate compared with placebo.<sup>15 106</sup> It also showed that omalizumab could slightly improve morning peak expiratory flow and FEV1. Monoclonal antibodies targeting IL-5 pathway namely mepolizumab, reslizumab and benralizumab are now approved to be used to treat severe eosinophilic asthma.<sup>107</sup> Considering improvement of prebronchodilator and postbronchodilator FEV1, biotherapies targeting IL-5 reached statistical significance overall, though clinical significance remains questionable (about +100 mL vs placebo). Conversely, dupilumab, a fully human monoclonal antibody targeting IL-4 receptor  $\alpha$  also demonstrated a significant reduction of exacerbation rate and efficient oral corticosteroid tapering in patients suffering of uncontrolled moderate to severe asthma.<sup>17 108</sup> Interestingly, a significant improvement of FEV1 under treatment with dupilumab was observed at 24 weeks of treatment with mean difference above 200 mL.<sup>108</sup> In a real-life asthma cohort, dupilumab also improved FEV1 by 10% (predicted values) after 1 year of treatment.<sup>109</sup> Recently, a treatment with tezepelumab, a human monoclonal antibody targeting the TSLP, lead to a 130 mL increase of pre bronchodilator FEV1 in comparison with the control group.<sup>110</sup> Such improvements, to be confirmed in the long term, tends to underline the importance of the interactions between inflammation and bronchial smooth muscle and the interest in simultaneously targeting multiple actors.

Few trials specifically studied the effect of biotherapies on pulmonary functions. Benralizumab failed to significantly improve prebronchodilator FEV1 and hyperinflation in SOLANA trial.<sup>111</sup> To note, this trial aimed at assessing benralizumab efficiency at short-term (84 days) and potentially lack interesting effects on obstruction through prolonged treatment. Small-sized clinical studies have shown that mepolizumab could possibly improve small airway function evaluated by multiple-breath nitrogen washout test and forced oscillometry after a few months of treatments.<sup>112</sup>

### Bronchial thermoplasty

In order to specifically target bronchial wall including aSMC, an interventional endoscopic technic was developed: bronchial thermoplasty. Its objective is to lower the airway wall thickness by direct thermic energy application. In AIR-2 clinical trial, bronchial thermoplasty significantly improved quality of life with a trend in favour of better asthma control through lower exacerbation rate in comparison with the sham group.<sup>113</sup> Good long-term tolerance has been shown after 5 years of follow-up.<sup>114</sup> Considering bronchial remodelling, in a small prospective case series (n=11), bronchial thermoplasty slightly decreased the airway wall thickness and air-trapping 2 years after the procedure, even if no significant improvement of airway lumen could be observed.<sup>115</sup> However,

no significant effect had been observed on small airways evaluated by oscillometry 6 months after the procedure, despite significantly improved clinical markers.<sup>116</sup> Interestingly, a proof-of-concept pilot study found that bronchial thermoplasty could nevertheless improve dynamic hyperinflation in selected patients.<sup>117</sup> Though, such results need to be confirmed in larger and control clinical studies.

Data about bronchial thermoplasty effects on airways physiology and asthma pathophysiology are now available. aSMC area ( $\alpha$ -SMA staining) significantly decreased in short term (6 weeks) after bronchial thermoplasty consistent with lower smooth muscle mass.<sup>118</sup> The TASMA randomised clinical study confirmed such data by including a parallel delayed bronchial thermoplasty group of severe asthmatic patients as controls.<sup>119</sup> Nevertheless, modification of aSMC mass did not correlate with improvement of asthma control and related quality of life scores (Asthma Control Questionnaire, Asthma Quality of Life Questionnaire). In parallel, it was recently reported that bronchial thermoplasty induced a decrease of mucus production assessed by MUC5AC epithelial expression at 12 months post procedure.<sup>120</sup> Bronchial thermoplasty can also modify cell cross-talk between the different components of airway wall. It has been reported that it blocked the production and secretion of heat-shock protein-60 by the epithelium that triggered in part remodelling in asthma by fibroblasts.<sup>121</sup>

### Unmet needs in targeted treatment of aSMC in asthma

Few innovative strategies targeting specifically aSMC are available. Although bronchial thermoplasty has shown interesting results, this technique remains complex and reserved to a limited number of patients. Besides, in an era of precision medicine, tools that predict response to a treatment strategy in an individual setting are essential. Few studies are available concerning such biomarkers to predict efficacy of bronchial thermoplasty. Fixed or reversible obstruction status was not significantly associated with clinical response to bronchial thermoplasty.<sup>122</sup> Small molecules that directly target key signalling pathways implicated in aSMC contraction and proliferation could be an interesting therapeutic opportunity. For example, inhibition of Rho and Rac activation could potentially reduce airway hyper-responsiveness and remodelling. Nevertheless, those have long been considered as undruggable due to their ubiquitous expression and critical biological roles that could lead to serious side effects. The identification of tissue and condition specific regulators of Rho superfamily activation, such as guanine exchange factors, could be a promising way to overcome such limit.

### CONCLUSION

Asthma represents a broad-spectrum disease involving at different levels epithelial dysfunction, sustained bronchial inflammation and bronchial smooth muscle

dysfunction. Knowledge about bronchial smooth muscle role in asthma pathophysiology had been considerably improved (figure 3). Mechanisms of bronchial contraction are better understood and new intracellular pathways had been discovered. However, aSMC should not be only considered as simple contraction actors. Indeed, implication of aSMC airway remodelling and their secretion capacities are far more important than previously expected.

In routine practice, aSMC functions are mainly assessed through pulmonary function tests and provocation tests. Innovative tools such as forced oscillometry and dedicated imaging are used in clinical research but did not reach clinical practice yet. In parallel, improvement of basic sciences comprehension of airway smooth muscle biology doesn't lead to real targeted strategies for now. On the one hand, bronchodilators developed for the last decades mainly targeted the same pathways (adrenergic and muscarinic receptors) and on the other hand, bronchial thermoplasty still lacks predictors of success even though promising results have been described. Development of tools to better assess aSMC activity in clinical practice along with new targeted treatment are mandatory to identify patients for whom SMC dysfunction is preponderant and need to be specifically treated.

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#### REFERENCES

- 1 Badri KR, Zhou Y, Schuger L. Embryological origin of airway smooth muscle. *Proc Am Thorac Soc* 2008;5:4–10.
- 2 Cieri RL. Pulmonary smooth muscle in vertebrates: a comparative review of structure and function. *Integr Comp Biol* 2019;59:10–28.
- 3 Berger P, Marthan R, Tunon de Lara J-M. [The pathophysiological role of smooth muscle cells in bronchial inflammation]. *Rev Mal Respir* 2002;19:778–94.
- 4 Mitzner W. Airway smooth muscle: the appendix of the lung. *Am J Respir Crit Care Med* 2004;169:787–90.
- 5 Reddel HK, Bateman ED, Becker A, et al. A summary of the new GINA strategy: a roadmap to asthma control. *Eur Respir J* 2015;46:622–39.
- 6 D'Amato G, Vitale C, Molino A, et al. Asthma-related deaths. *Multidiscip Respir Med* 2016;11:37.
- 7 Chung KF, Wenzel SE, Brozek JL, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J* 2014;43:343–73.
- 8 Carr TF, Zeki AA, Kraft M. Eosinophilic and Noneosinophilic asthma. *Am J Respir Crit Care Med* 2018;197:22–37.
- 9 Agache I, Beltran J, Akdis C, et al. Efficacy and safety of treatment with biologicals (benralizumab, dupilumab, mepolizumab, omalizumab and reslizumab) for severe eosinophilic asthma. A systematic review for the EAAACI Guidelines - recommendations on the use of biologicals in severe asthma. *Allergy* 2020;75:1023–42.
- 10 Pascual RM, Peters SP. Airway remodeling contributes to the progressive loss of lung function in asthma: an overview. *J Allergy Clin Immunol* 2005;116:quiz 487:477–86.
- 11 Lambrecht BN, Hammad H. The airway epithelium in asthma. *Nat Med* 2012;18:684–92.
- 12 Bumbacea D, Campbell D, Nguyen L, et al. Parameters associated with persistent airflow obstruction in chronic severe asthma. *Eur Respir J* 2004;24:122–8.
- 13 Hough KP, Curtiss ML, Blain TJ, et al. Airway remodeling in asthma. *Front Med* 2020;7:191.
- 14 Ortega HG, Liu MC, Pavord ID, et al. Mepolizumab treatment in patients with severe eosinophilic asthma. *N Engl J Med* 2014;371:1198–207.
- 15 Holgate ST, Chuchalin AG, Hebert J. Omalizumab 011 International study G. efficacy and safety of a recombinant anti-immunoglobulin E antibody (omalizumab) in severe allergic asthma. *Clin Exp Allergy* 2004;34:632–8.
- 16 FitzGerald JM, Bleecker ER, Nair P, et al. Benralizumab, an anti-interleukin-5 receptor  $\alpha$  monoclonal antibody, as add-on treatment for patients with severe, uncontrolled, eosinophilic asthma (CALIMA): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet* 2016;388:2128–41.
- 17 Castro M, Corren J, Pavord ID, et al. Dupilumab efficacy and safety in moderate-to-severe uncontrolled asthma. *N Engl J Med* 2018;378:2486–96.
- 18 Ma X, Cheng Z, Kong H, et al. Changes in biophysical and biochemical properties of single bronchial smooth muscle cells from asthmatic subjects. *Am J Physiol Lung Cell Mol Physiol* 2002;283:L1181–9.
- 19 Matsumoto H, Moir LM, Oliver BGG, et al. Comparison of gel contraction mediated by airway smooth muscle cells from patients with and without asthma. *Thorax* 2007;62:848–54.
- 20 Benayoun L, Druilhe A, Dombret M-C, et al. Airway structural alterations selectively associated with severe asthma. *Am J Respir Crit Care Med* 2003;167:1360–8.
- 21 Murray RK, Fleischmann BK, Kotlikoff MI. Receptor-activated Ca influx in human airway smooth muscle: use of Ca imaging and perforated patch-clamp techniques. *Am J Physiol* 1993;264:C485–90.
- 22 Sanderson MJ, Delmotte P, Bai Y, et al. Regulation of airway smooth muscle cell contractility by Ca<sup>2+</sup> signaling and sensitivity. *Proc Am Thorac Soc* 2008;5:23–31.
- 23 André-Grégoire G, Dilasser F, Chesné J, et al. Targeting of Rac1 prevents bronchoconstriction and airway hyperresponsiveness. *J Allergy Clin Immunol* 2018;142:e823:824–33.
- 24 Deshpande DA, Walseth TF, Panettieri RA, et al. CD38/Cyclic ADP-ribose-mediated Ca<sup>2+</sup> signaling contributes to airway smooth muscle hyper-responsiveness. *FASEB J* 2003;17:452–4.
- 25 Deshpande DA, White TA, Guedes AGP, et al. Altered airway responsiveness in CD38-deficient mice. *Am J Respir Cell Mol Biol* 2005;32:149–56.
- 26 Perez JF, Sanderson MJ. The frequency of calcium oscillations induced by 5-HT, ACh, and KCl determine the contraction of smooth muscle cells of intrapulmonary bronchioles. *J Gen Physiol* 2005;125:535–53.
- 27 Deshpande DA, Dogan S, Walseth TF, et al. Modulation of calcium signaling by interleukin-13 in human airway smooth muscle: role of CD38/cyclic adenosine diphosphate ribose pathway. *Am J Respir Cell Mol Biol* 2004;31:36–42.
- 28 Tarasov AI, Griffiths EJ, Rutter GA. Regulation of ATP production by mitochondrial Ca(2+). *Cell Calcium* 2012;52:28–35.
- 29 Delmotte P, Sieck GC. Interaction between endoplasmic/sarcoplasmic reticulum stress (ER/SR stress), mitochondrial signaling and Ca(2+) regulation in airway smooth muscle (ASM). *Can J Physiol Pharmacol* 2015;93:97–110.
- 30 Mahn K, Hirst SJ, Ying S, et al. Diminished sarco/endoplasmic reticulum Ca<sup>2+</sup> ATPase (SERCA) expression contributes to airway remodelling in bronchial asthma. *Proc Natl Acad Sci U S A* 2009;106:10775–80.
- 31 Sakai H, Hirano T, Takeyama H, et al. Acetylcholine-induced phosphorylation of CPI-17 in rat bronchial smooth muscle: the roles of Rho-kinase and protein kinase C. *Can J Physiol Pharmacol* 2005;83:375–81.

- 32 Feng J, Ito M, Ichikawa K, *et al.* Inhibitory phosphorylation site for Rho-associated kinase on smooth muscle myosin phosphatase. *J Biol Chem* 1999;274:37385–90.
- 33 Koyama M, Ito M, Feng J, *et al.* Phosphorylation of CPI-17, an inhibitory phosphoprotein of smooth muscle myosin phosphatase, by Rho-kinase. *FEBS Lett* 2000;475:197–200.
- 34 Sakai H, Chiba Y, Hirano T, *et al.* Possible involvement of CPI-17 in augmented bronchial smooth muscle contraction in antigen-induced airway hyper-responsive rats. *Mol Pharmacol* 2005;68:145–51.
- 35 Chiba Y, Ueno A, Shinozaki K, *et al.* Involvement of RhoA-mediated Ca<sup>2+</sup> sensitization in antigen-induced bronchial smooth muscle hyperresponsiveness in mice. *Respir Res* 2005;6:4.
- 36 Schaafsma D, Bos IST, Zuidhof AB, *et al.* The inhaled Rho kinase inhibitor Y-27632 protects against allergen-induced acute bronchoconstriction, airway hyperresponsiveness, and inflammation. *Am J Physiol Lung Cell Mol Physiol* 2008;295:L214–9.
- 37 Chiba Y, Tanoue G, Suto R, *et al.* Interleukin-17A directly acts on bronchial smooth muscle cells and augments the contractility. *Pharmacol Rep* 2017;69:377–85.
- 38 Kudo M, Melton AC, Chen C, *et al.* IL-17A produced by  $\alpha\beta$  T cells drives airway hyper-responsiveness in mice and enhances mouse and human airway smooth muscle contraction. *Nat Med* 2012;18:547–54.
- 39 Camargo LdoN, Righetti RF, Aristóteles LRdeCRB, *et al.* Effects of Anti-IL-17 on inflammation, remodeling, and oxidative stress in an experimental model of asthma exacerbated by LPS. *Front Immunol* 2017;8:1835.
- 40 Dos Santos TM, Righetti RF, Camargo LdoN, *et al.* Effect of Anti-IL17 antibody treatment alone and in combination with Rho-kinase inhibitor in a murine model of asthma. *Front Physiol* 2018;9:1183.
- 41 Chiba Y, Nakazawa S, Todoroki M, *et al.* Interleukin-13 augments bronchial smooth muscle contractility with an up-regulation of RhoA protein. *Am J Respir Cell Mol Biol* 2009;40:159–67.
- 42 Carvajal JA, Germain AM, Huidobro-Toro JP, *et al.* Molecular mechanism of cGMP-mediated smooth muscle relaxation. *J Cell Physiol* 2000;184:409–20.
- 43 Ward JK, Barnes PJ, Springall DR, *et al.* Distribution of human i-NANC bronchodilator and nitric oxide-immunoreactive nerves. *Am J Respir Cell Mol Biol* 1995;13:175–84.
- 44 Asano K, Chee CB, Gaston B, *et al.* Constitutive and inducible nitric oxide synthase gene expression, regulation, and activity in human lung epithelial cells. *Proc Natl Acad Sci U S A* 1994;91:10089–93.
- 45 Nijkamp FP, van der Linde HJ, Folkerts G. Nitric oxide synthesis inhibitors induce airway hyperresponsiveness in the guinea pig in vivo and in vitro. Role of the epithelium. *Am Rev Respir Dis* 1993;148:727–34.
- 46 Prado CM, Martins MA, Tibério IFLC. Nitric oxide in asthma pathophysiology. *ISRN Allergy* 2011;2011:832560.
- 47 Hamid Q, Springall DR, Riveros-Moreno V, *et al.* Induction of nitric oxide synthase in asthma. *Lancet* 1993;342:1510–3.
- 48 Dweik RA, Sorkness RL, Wenzel S, *et al.* Use of exhaled nitric oxide measurement to identify a reactive, at-risk phenotype among patients with asthma. *Am J Respir Crit Care Med* 2010;181:1033–41.
- 49 Jatakanon A, Lim S, Kharitonov SA, *et al.* Correlation between exhaled nitric oxide, sputum eosinophils, and methacholine responsiveness in patients with mild asthma. *Thorax* 1998;53:91–5.
- 50 Dupont LJ, Rochette F, Demedts MG, *et al.* Exhaled nitric oxide correlates with airway hyperresponsiveness in steroid-naïve patients with mild asthma. *Am J Respir Crit Care Med* 1998;157:894–8.
- 51 Grace MS, Baxter M, Dubuis E, *et al.* Transient receptor potential (TRP) channels in the airway: role in airway disease. *Br J Pharmacol* 2014;171:2593–607.
- 52 Birrell MA, Belvisi MG, Grace M, *et al.* TRPA1 agonists evoke coughing in guinea pig and human volunteers. *Am J Respir Crit Care Med* 2009;180:1042–7.
- 53 Caceres AI, Brackmann M, Elia MD, *et al.* A sensory neuronal ion channel essential for airway inflammation and hyperreactivity in asthma. *Proc Natl Acad Sci U S A* 2009;106:9099–104.
- 54 Siddiqui S, Shikotra A, Richardson M, *et al.* Airway pathological heterogeneity in asthma: visualization of disease microclusters using topological data analysis. *J Allergy Clin Immunol* 2018;142:1457–68.
- 55 Saglani S, Payne DN, Zhu J, *et al.* Early detection of airway wall remodeling and eosinophilic inflammation in preschool wheezers. *Am J Respir Crit Care Med* 2007;176:858–64.
- 56 Regamey N, Ochs M, Hilliard TN, *et al.* Increased airway smooth muscle mass in children with asthma, cystic fibrosis, and non-cystic fibrosis bronchiectasis. *Am J Respir Crit Care Med* 2008;177:837–43.
- 57 Bossley CJ, Fleming L, Gupta A, *et al.* Pediatric severe asthma is characterized by eosinophilia and remodeling without T(H)2 cytokines. *J Allergy Clin Immunol* 2012;129:e913:974–82.
- 58 Tillie-Leblond I, de Blic J, Jaubert F, *et al.* Airway remodeling is correlated with obstruction in children with severe asthma. *Allergy* 2008;63:533–41.
- 59 Halayko AJ, Salari H, Ma X, *et al.* Markers of airway smooth muscle cell phenotype. *Am J Physiol* 1996;270:L1040–51.
- 60 Berair R, Saunders R, Brightling CE. Origins of increased airway smooth muscle mass in asthma. *BMC Med* 2013;11:145.
- 61 Trian T, Benard G, Begueret H, *et al.* Bronchial smooth muscle remodeling involves calcium-dependent enhanced mitochondrial biogenesis in asthma. *J Exp Med* 2007;204:3173–81.
- 62 Zou H, Fang Q-H, Ma Y-M, *et al.* Analysis of growth factors in serum and induced sputum from patients with asthma. *Exp Ther Med* 2014;8:573–8.
- 63 Shan L, Redhu NS, Saleh A, *et al.* Thymic stromal lymphopoietin receptor-mediated IL-6 and CC/CXC chemokines expression in human airway smooth muscle cells: role of MAPKs (ERK1/2, p38, and JNK) and STAT3 pathways. *J Immunol* 2010;184:7134–43.
- 64 Lajoie-Kadoch S, Joubert P, Létuvé S, *et al.* Tnf-Alpha and IFN-gamma inversely modulate expression of the IL-17E receptor in airway smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 2006;290:L1238–46.
- 65 Malavia NK, Raub CB, Mahon SB, *et al.* Airway epithelium stimulates smooth muscle proliferation. *Am J Respir Cell Mol Biol* 2009;41:297–304.
- 66 Lan B, Mitchel JA, O'Sullivan MJ, *et al.* Airway epithelial compression promotes airway smooth muscle proliferation and contraction. *Am J Physiol Lung Cell Mol Physiol* 2018;315:L645–52.
- 67 Risse P-A, Jo T, Suarez F, *et al.* Interleukin-13 inhibits proliferation and enhances contractility of human airway smooth muscle cells without change in contractile phenotype. *Am J Physiol Lung Cell Mol Physiol* 2011;300:L958–66.
- 68 Espinosa K, Bossé Y, Stankova J, *et al.* CysLT1 receptor upregulation by TGF-beta and IL-13 is associated with bronchial smooth muscle cell proliferation in response to LTD4. *J Allergy Clin Immunol* 2003;111:1032–40.
- 69 Keglłowicz LF, Borger P. The three A's in asthma - airway smooth muscle, airway remodeling & angiogenesis. *Open Respir Med J* 2015;9:70–80.
- 70 Hirst SJ, Twort CH, Lee TH. Differential effects of extracellular matrix proteins on human airway smooth muscle cell proliferation and phenotype. *Am J Respir Cell Mol Biol* 2000;23:335–44.
- 71 Tran T, McNeill KD, Gerthoffer WT, *et al.* Endogenous laminin is required for human airway smooth muscle cell maturation. *Respir Res* 2006;7:117.
- 72 Araujo BB, Dolhnikoff M, Silva LFF, *et al.* Extracellular matrix components and regulators in the airway smooth muscle in asthma. *Eur Respir J* 2008;32:61–9.
- 73 Orsini MJ, Krymskaya VP, Eszterhas AJ, *et al.* Mapk superfamily activation in human airway smooth muscle: mitogenesis requires prolonged p42/p44 activation. *Am J Physiol* 1999;277:L479–88.
- 74 Krymskaya VP, Penn RB, Orsini MJ, *et al.* Phosphatidylinositol 3-kinase mediates mitogen-induced human airway smooth muscle cell proliferation. *Am J Physiol* 1999;277:L65–78.
- 75 Schaafsma D, Roscioni SS, Meurs H, *et al.* Monomeric G-proteins as signal transducers in airway physiology and pathophysiology. *Cell Signal* 2008;20:1705–14.
- 76 Page K, Li J, Hodge JA, *et al.* Characterization of a Rac1 signaling pathway to cyclin D(1) expression in airway smooth muscle cells. *J Biol Chem* 1999;274:22065–71.
- 77 Dilasser F, Rose L, Hassoun D, *et al.* Essential role of smooth muscle Rac1 in severe asthma-associated airway remodelling. *Thorax* 2021;76:326–334.
- 78 Hedges JC, Dechert MA, Yamboliev IA, *et al.* A role for p38(MAPK)/HSP27 pathway in smooth muscle cell migration. *J Biol Chem* 1999;274:24211–9.
- 79 Carlin SM, Roth M, Black JL. Urokinase potentiates PDGF-induced chemotaxis of human airway smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 2003;284:L1020–6.
- 80 Parameswaran K, Cox G, Radford K, *et al.* Cysteinyl leukotrienes promote human airway smooth muscle migration. *Am J Respir Crit Care Med* 2002;166:738–42.
- 81 Tiliba O, Panettieri RA. Noncontractile functions of airway smooth muscle cells in asthma. *Annu Rev Physiol* 2009;71:509–35.
- 82 Yang W, Kaur D, Okayama Y, *et al.* Human lung mast cells adhere to human airway smooth muscle, in part, via tumor suppressor in lung cancer-1. *J Immunol* 2006;176:1238–43.

- 83 Girodet P-O, Ozier A, Trian T, *et al.* Mast cell adhesion to bronchial smooth muscle in asthma specifically depends on CD51 and CD44 variant 6. *Allergy* 2010;65:1004–12.
- 84 Hamawy MM, Mergenhagen SE, Siraganian RP. Adhesion molecules as regulators of mast-cell and basophil function. *Immunol Today* 1994;15:62–6.
- 85 Lazaar AL, Albelda SM, Pilewski JM, *et al.* T lymphocytes adhere to airway smooth muscle cells via integrins and CD44 and induce smooth muscle cell DNA synthesis. *J Exp Med* 1994;180:807–16.
- 86 Graham BL, Steenbruggen I, Miller MR, *et al.* Standardization of spirometry 2019 update. an official American thoracic Society and European respiratory Society technical statement. *Am J Respir Crit Care Med* 2019;200:e70–88.
- 87 Karmaus W, Mukherjee N, Janjanam VD, *et al.* Distinctive lung function trajectories from age 10 to 26 years in men and women and associated early life risk factors - a birth cohort study. *Respir Res* 2019;20:98.
- 88 Lange P, Parner J, Vestbo J, *et al.* A 15-year follow-up study of ventilatory function in adults with asthma. *N Engl J Med* 1998;339:1194–200.
- 89 Ulrik CS, Backer V. Nonreversible airflow obstruction in life-long nonsmokers with moderate to severe asthma. *Eur Respir J* 1999;14:892–6.
- 90 Ferreira DS, Carvalho-Pinto RM, Gregório MG, *et al.* Airway pathology in severe asthma is related to airflow obstruction but not symptom control. *Allergy* 2018;73:635–43.
- 91 Quanjer PH, Weiner DJ, Pretto JJ, *et al.* Measurement of FEF25–75% and FEF75% does not contribute to clinical decision making. *Eur Respir J* 2014;43:1051–8.
- 92 Kjellberg S, Houlitz BK, Zetterström O, *et al.* Clinical characteristics of adult asthma associated with small airway dysfunction. *Respir Med* 2016;117:92–102.
- 93 de Lange EE, Altes TA, Patrie JT, *et al.* The variability of regional airflow obstruction within the lungs of patients with asthma: assessment with hyperpolarized helium-3 magnetic resonance imaging. *J Allergy Clin Immunol* 2007;119:1072–8.
- 94 Farah CS, King GG, Brown NJ, *et al.* The role of the small airways in the clinical expression of asthma in adults. *J Allergy Clin Immunol* 2012;129:e381:381–7.
- 95 Coates AL, Wanger J, Cockcroft DW, *et al.* ERS technical standard on bronchial challenge testing: general considerations and performance of methacholine challenge tests. *Eur Respir J* 2017;49. doi:10.1183/13993003.01526-2016. [Epub ahead of print: 01 05 2017].
- 96 Hallstrand TS, Leuppi JD, Joos G, *et al.* ERS technical standard on bronchial challenge testing: pathophysiology and methodology of indirect airway challenge testing. *Eur Respir J* 2018;52. doi:10.1183/13993003.01033-2018. [Epub ahead of print: 15 11 2018].
- 97 Sumino K, Sugar EA, Irvin CG, *et al.* Methacholine challenge test: diagnostic characteristics in asthmatic patients receiving controller medications. *J Allergy Clin Immunol* 2012;130:e66:69–75.
- 98 Satia I, Priel E, Al-Khazraji BK, *et al.* Exercise-induced bronchoconstriction and bronchodilation: investigating the effects of age, sex, airflow limitation and FEV<sub>1</sub>. *Eur Respir J* 2021;58:2004026.
- 99 Anderson SD, Charlton B, Weiler JM, *et al.* Comparison of mannitol and methacholine to predict exercise-induced bronchoconstriction and a clinical diagnosis of asthma. *Respir Res* 2009;10:4.
- 100 Girodet P-O, Dournes G, Thumerel M, *et al.* Calcium channel blocker reduces airway remodeling in severe asthma. A proof-of-concept study. *Am J Respir Crit Care Med* 2015;191:876–83.
- 101 Goldsmith AM, Hershenson MB, Wolbert MP, *et al.* Regulation of airway smooth muscle alpha-actin expression by glucocorticoids. *Am J Physiol Lung Cell Mol Physiol* 2007;292:L99–106.
- 102 Goto K, Chiba Y, Sakai H, *et al.* Glucocorticoids inhibited airway hyperresponsiveness through downregulation of CPI-17 in bronchial smooth muscle. *Eur J Pharmacol* 2008;591:231–6.
- 103 Leung SY, Eynott P, Nath P, *et al.* Effects of ciclesonide and fluticasone propionate on allergen-induced airway inflammation and remodeling features. *J Allergy Clin Immunol* 2005;115:989–96.
- 104 Chetta A, Zanini A, Foresi A, *et al.* Vascular component of airway remodeling in asthma is reduced by high dose of fluticasone. *Am J Respir Crit Care Med* 2003;167:751–7.
- 105 Ward C, Pais M, Bish R, *et al.* Airway inflammation, basement membrane thickening and bronchial hyperresponsiveness in asthma. *Thorax* 2002;57:309–16.
- 106 Humbert M, Beasley R, Ayres J, *et al.* Benefits of omalizumab as add-on therapy in patients with severe persistent asthma who are inadequately controlled despite best available therapy (GINA 2002 step 4 treatment): INNOVATE. *Allergy* 2005;60:309–16.
- 107 Holguin F, Cardet JC, Chung KF, *et al.* Management of severe asthma: a European respiratory Society/American thoracic Society guideline. *Eur Respir J* 2020;55. doi:10.1183/13993003.00588-2019. [Epub ahead of print: 02 01 2020].
- 108 Rabe KF, Nair P, Brusselle G, *et al.* Efficacy and safety of Dupilumab in glucocorticoid-dependent severe asthma. *N Engl J Med* 2018;378:2475–85.
- 109 Dupin C, Belhadi D, Guilleminault L, *et al.* Effectiveness and safety of dupilumab for the treatment of severe asthma in a real-life French multi-centre adult cohort. *Clin Exp Allergy* 2020;50:789–98.
- 110 Menzies-Gow A, Corren J, Bourdin A, *et al.* Tezepelumab in adults and adolescents with severe, uncontrolled asthma. *N Engl J Med* 2021;384:1800–9.
- 111 Panettieri RA, Welte T, Shenoy KV, *et al.* Onset of effect, changes in airflow obstruction and lung volume, and health-related quality of life improvements with Benralizumab for patients with severe eosinophilic asthma: phase IIIB randomized, controlled trial (SOLANA). *J Asthma Allergy* 2020;13:115–26.
- 112 Farah CS, Badal T, Reed N, *et al.* Mepolizumab improves small airway function in severe eosinophilic asthma. *Respir Med* 2019;148:49–53.
- 113 Castro M, Rubin AS, Laviolette M, *et al.* Effectiveness and safety of bronchial thermoplasty in the treatment of severe asthma: a multicenter, randomized, double-blind, sham-controlled clinical trial. *Am J Respir Crit Care Med* 2010;181:116–24.
- 114 Thomson NC, Rubin AS, Niven RM, *et al.* Long-term (5 year) safety of bronchial thermoplasty: asthma intervention research (air) trial. *BMC Pulm Med* 2011;11:8.
- 115 Konietzke P, Weinheimer O, Welpütz MO, *et al.* Quantitative CT detects changes in airway dimensions and air-trapping after bronchial thermoplasty for severe asthma. *Eur J Radiol* 2018;107:33–8.
- 116 Langton D, Ing A, Sha J, *et al.* Measuring the effects of bronchial thermoplasty using oscillometry. *Respirology* 2019;24:431–6.
- 117 Guibert N, Guilleminault L, Lepage B. Bronchial thermoplasty in patients with dynamic hyperinflation: results from the proof-of-concept heat trial. *Eur Respir J* 2020.
- 118 Ichikawa T, Panariti A, Audusseau S, *et al.* Effect of bronchial thermoplasty on structural changes and inflammatory mediators in the airways of subjects with severe asthma. *Respir Med* 2019;150:165–72.
- 119 Goorsenberg AWM, d'Hooghe JNS, Srikanthan K. Bronchial thermoplasty induced airway smooth muscle reduction and clinical response in severe asthma: the TASMA randomized trial. *Am J Respir Crit Care Med* 2020.
- 120 Haj Salem I, Gras D, Joubert P, *et al.* Persistent reduction of mucin production after bronchial thermoplasty in severe asthma. *Am J Respir Crit Care Med* 2019;199:536–8.
- 121 Sun Q, Fang L, Roth M, *et al.* Bronchial thermoplasty decreases airway remodelling by blocking epithelium-derived heat shock protein-60 secretion and protein arginine methyltransferase-1 in fibroblasts. *Eur Respir J* 2019;54:1900300.
- 122 Langton D, Ing A, Fielding D, *et al.* Bronchodilator responsiveness as a predictor of success for bronchial thermoplasty. *Respirology* 2019;24:63–7.
- 123 Hirst SJ, Barnes PJ, Twort CH. Quantifying proliferation of cultured human and rabbit airway smooth muscle cells in response to serum and platelet-derived growth factor. *Am J Respir Cell Mol Biol* 1992;7:574–81.
- 124 Hirst SJ, Barnes PJ, Twort CH. PDGF isoform-induced proliferation and receptor expression in human cultured airway smooth muscle cells. *Am J Physiol* 1996;270:L415–28.
- 125 Simeone-Penney MC, Severgnini M, Roza L, *et al.* PDGF-Induced human airway smooth muscle cell proliferation requires STAT3 and the small GTPase Rac1. *Am J Physiol Lung Cell Mol Physiol* 2008;294:L698–704.
- 126 Stamatou R, Paraskeva E, Gourgoulianis K, *et al.* Cytokines and growth factors promote airway smooth muscle cell proliferation. *ISRN Inflamm* 2012;2012:731472.
- 127 Ediger TL, Toews ML. Synergistic stimulation of airway smooth muscle cell mitogenesis. *J Pharmacol Exp Ther* 2000;294:1076–82.
- 128 Bossé Y, Thompson C, Stankova J, *et al.* Fibroblast growth factor 2 and transforming growth factor beta1 synergism in human bronchial smooth muscle cell proliferation. *Am J Respir Cell Mol Biol* 2006;34:746–53.
- 129 Zou H, Nie X-hong, Zhang Y, *et al.* Effect of basic fibroblast growth factor on the proliferation, migration and phenotypic modulation of airway smooth muscle cells. *Chin Med J* 2008;121:424–9.
- 130 Krymskaya VP, Hoffman R, Eszterhas A, *et al.* EGF activates ErbB-2 and stimulates phosphatidylinositol 3-kinase in human airway smooth muscle cells. *Am J Physiol* 1999;276:L246–55.

- 131 Chen G, Khalil N. Tgf-Beta1 increases proliferation of airway smooth muscle cells by phosphorylation of MAP kinases. *Respir Res* 2006;7:2.
- 132 Cohen MD, Ciocca V, Panettieri RA. TGF-Beta 1 modulates human airway smooth-muscle cell proliferation induced by mitogens. *Am J Respir Cell Mol Biol* 1997;16:85–90.
- 133 Xie S, Sukkar MB, Issa R, et al. Mechanisms of induction of airway smooth muscle hyperplasia by transforming growth factor-beta. *Am J Physiol Lung Cell Mol Physiol* 2007;293:L245–53.
- 134 Stewart AG, Tomlinson PR, Fernandes DJ, et al. Tumor necrosis factor alpha modulates mitogenic responses of human cultured airway smooth muscle. *Am J Respir Cell Mol Biol* 1995;12:110–9.
- 135 Knobloch J, Yanik SD, Körber S, et al. TNFalpha-Induced airway smooth muscle cell proliferation depends on endothelin receptor signaling, GM-CSF and IL-6. *Biochem Pharmacol* 2016;116:188–99.
- 136 Li X, Zou F, Lu Y, et al. Notch1 contributes to TNF- $\alpha$ -induced proliferation and migration of airway smooth muscle cells through regulation of the Hes1/PTEN axis. *Int Immunopharmacol* 2020;88:106911.
- 137 De S, Zelazny ET, Souhrada JF, et al. Interleukin-1 beta stimulates the proliferation of cultured airway smooth muscle cells via platelet-derived growth factor. *Am J Respir Cell Mol Biol* 1993;9:645–51.
- 138 De S, Zelazny ET, Souhrada JF, et al. Il-1 beta and IL-6 induce hyperplasia and hypertrophy of cultured guinea pig airway smooth muscle cells. *J Appl Physiol* 1995;78:1555–63.
- 139 Amrani Y, Tliba O, Choubey D, et al. IFN-Gamma inhibits human airway smooth muscle cell proliferation by modulating the E2F-1/Rb pathway. *Am J Physiol Lung Cell Mol Physiol* 2003;284:L1063–71.
- 140 Hawker KM, Johnson PR, Hughes JM, et al. Interleukin-4 inhibits mitogen-induced proliferation of human airway smooth muscle cells in culture. *Am J Physiol* 1998;275:L469–77.
- 141 Shim JY, Park SW, Kim DS, et al. The effect of interleukin-4 and amphiregulin on the proliferation of human airway smooth muscle cells and cytokine release. *J Korean Med Sci* 2008;23:857–63.
- 142 Panettieri RA, Yadavish PA, Kelly AM, et al. Histamine stimulates proliferation of airway smooth muscle and induces c-fos expression. *Am J Physiol* 1990;259:L365–71.
- 143 Chhabra J, Li Y-Z, Alkhoury H, et al. Histamine and tryptase modulate asthmatic airway smooth muscle GM-CSF and RANTES release. *Eur Respir J* 2007;29:861–70.
- 144 Noveral JP, Grunstein MM. Role and mechanism of thromboxane-induced proliferation of cultured airway smooth muscle cells. *Am J Physiol* 1992;263:L555–61.
- 145 Capra V, Habib A, Accomazzo MR, et al. Thromboxane prostanoid receptor in human airway smooth muscle cells: a relevant role in proliferation. *Eur J Pharmacol* 2003;474:149–59.
- 146 Ammit AJ, Hastie AT, Edsall LC, et al. Sphingosine 1-phosphate modulates human airway smooth muscle cell functions that promote inflammation and airway remodeling in asthma. *FASEB J* 2001;15:1212–4.
- 147 Berger P, Perng DW, Thabrew H, et al. Tryptase and agonists of PAR-2 induce the proliferation of human airway smooth muscle cells. *J Appl Physiol* 2001;91:1372–9.
- 148 Panettieri RA, Hall IP, Maki CS, et al. Alpha-thrombin increases cytosolic calcium and induces human airway smooth muscle cell proliferation. *Am J Respir Cell Mol Biol* 1995;13:205–16.
- 149 Huang C-D, Chen H-H, Wang C-H, et al. Human neutrophil-derived elastase induces airway smooth muscle cell proliferation. *Life Sci* 2004;74:2479–92.
- 150 Brar SS, Kennedy TP, Sturrock AB, et al. NADPH oxidase promotes NF-kappaB activation and proliferation in human airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 2002;282:L782–95.
- 151 Bel EH, Wenzel SE, Thompson PJ, et al. Oral glucocorticoid-sparing effect of mepolizumab in eosinophilic asthma. *N Engl J Med* 2014;371:1189–97.
- 152 Castro M, Zangrilli J, Wechsler ME, et al. Reslizumab for inadequately controlled asthma with elevated blood eosinophil counts: results from two multicentre, parallel, double-blind, randomised, placebo-controlled, phase 3 trials. *Lancet Respir Med* 2015;3:355–66.
- 153 Bleecker ER, FitzGerald JM, Chanez P, et al. Efficacy and safety of benralizumab for patients with severe asthma uncontrolled with high-dosage inhaled corticosteroids and long-acting  $\beta_2$ -agonists (SIROCCO): a randomised, multicentre, placebo-controlled phase 3 trial. *Lancet* 2016;388:2115–27.
- 154 Nair P, Wenzel S, Rabe KF, et al. Oral glucocorticoid-sparing effect of Benralizumab in severe asthma. *N Engl J Med* 2017;376:2448–58.



**Titre : Nouvelles stratégies thérapeutiques dans l'asthme sévère**

Inhibition de la voie de signalisation dépendante de la GTPase Rac

**Mots clés :** Asthme sévère, Rac GTPase, Eosinophiles, Muscle lisse bronchique

**Résumé :** L'asthme est une pathologie chronique des voies aériennes caractérisée par une hyperréactivité bronchique, un remodelage tissulaire et une inflammation chronique. Les formes sévères résistantes aux traitements conventionnels requièrent de nouvelles stratégies thérapeutiques.

Mon équipe d'accueil a démontré dans un modèle murin d'asthme sévère une augmentation d'activité de la GTPase Rac1 dans les cellules musculaires lisses (CML) bronchiques qui participe à leur hypercontractilité et leur prolifération. Cette suractivation de Rac1 est aussi observée dans d'autres types cellulaires, dont les cellules inflammatoires.

L'objectif principal de ma thèse a été (i) de confirmer chez les asthmatiques sévères la suractivation de Rac1, (ii) d'identifier les populations inflammatoires concernées et (iii) les fonctions effectrices dépendantes de Rac dans ces différents types cellulaires.

Dans le cadre du protocole de recherche clinique NARACAS (NCT NCT03325088), j'ai confirmé sur des biopsies bronchiques de patients asthmatiques sévères la suractivation de Rac dans les CML et dans les cellules infiltrantes. Les polynucléaires éosinophiles ont été identifiés comme la principale population inflammatoire suractivant Rac dans le cadre d'un modèle murin d'asthme allergique sévère aux acariens. Par approches biochimiques et cytométriques, mes résultats démontrent une activation de Rac durant la maturation de l'éosinophile. Par ailleurs, l'inhibition de l'activité de Rac réduit la dégranulation des éosinophiles.

L'ensemble de ces résultats démontrent que les niveaux d'activité de Rac régulent à la fois la maturation et la dégranulation des éosinophiles. Le développement d'inhibiteurs des voies de signalisation dépendante de Rac pourrait constituer une stratégie innovante de lutte contre le remodelage bronchique et ses déterminants inflammatoires.

**Title: New therapeutic strategies in severe asthma**

Inhibition of the Rac GTPase-dependent signaling pathway

**Keywords:** Severe asthma, Rac GTPase, Eosinophils, Bronchial Smooth Muscle

**Abstract:** Asthma is an airway disease characterized by bronchial hyper-responsiveness, tissue remodeling and chronic inflammation. Severe asthma which resists conventional treatments requires new therapeutic strategies.

My host team demonstrated in a mouse model of severe asthma an increased activity of the Rac1 GTPase in bronchial smooth muscle cells (SMCs) involved in their hypercontractility and proliferation. This overactivation of Rac1 also existed in other cells, including inflammatory ones.

The main objective of my thesis was (i) to confirm the over-activation of Rac1 in severe asthmatics, (ii) to identify the involved inflammatory populations and (iii) the Rac-dependent effector functions in these different cell types.

In the NARACAS clinical research protocol (NCT NCT03325088), I confirm in bronchial biopsies from severe asthma patients that Rac is overactivated in SMCs and in infiltrating cells. Eosinophils are identified as the main inflammatory population overactivating Rac in a mouse model of severe house dust mite allergic asthma. By biochemical and cytometric approaches, my results demonstrate Rac activation during eosinophil maturation. Furthermore, inhibition of Rac activity reduces eosinophil degranulation.

Together, these results demonstrate that Rac activity levels regulate both eosinophil maturation and degranulation. The development of inhibitors of Rac-dependent signaling pathways could be an innovative strategy to combat bronchial remodeling and its inflammatory determinants.