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Adaptive immunity to commensal skin fungi promotes inflammatory flares

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Charlotte Hurabielle-Claverie. Adaptive immunity to commensal skin fungi promotes inflammatory flares. Biochemistry, Molecular Biology. Université Paris Cité, 2019. English. NNT : 2019UNIP7059 . tel-03027465

HAL Id: tel-03027465

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Université de Paris

Ecole doctorale Hématologie, Oncogénèse, Biothérapies (HOB) 561
*Laboratoire: National Institutes of Health (NIH), NIAID, LPD then LISB, Metagenome
Immunology Section / INSERM U976*

Adaptive immunity to commensal skin fungi promotes inflammatory flares

Par Charlotte Hurabielle-Claverie

Thèse de doctorat en Immunologie

Dirigée par Yasmine Belkaid et
Et par Armand Bensussan

Présentée et soutenue publiquement le 27 septembre 2019

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Titre : L'immunité adaptative contre les champignons commensaux promeut les poussées de maladies inflammatoires cutanées

Résumé: Le microbiote cutané joue un rôle fondamental dans les réponses immunitaires physiologiques de l'hôte. Cependant, la façon dont les réponses immunitaires aux commensaux cutanés peuvent influencer la physiopathologie des maladies inflammatoires cutanées est encore mal comprise. Les champignons font partie du microbiote cutané normal et ils induisent de fortes signatures IL-17 (interleukine 17), une cytokine qui a également un rôle fondamental dans la promotion du psoriasis. Nous démontrons ici que les réponses cellulaires T aux champignons commensaux augmentent la dermatose psoriasiforme imiquimod-induite chez la souris. Lorsque la peau des souris était colonisée avec des champignons avant d'induire une inflammation cutanée avec de l'imiquimod, le phénotype de la maladie psoriasique humaine était reproduit, avec une augmentation des réponses T_H17 induites par les commensaux, ainsi que de *neutrophil extracellular traps* (NETs). Les réponses mémoires T_H17 aux champignons commensaux étaient suffisantes pour induire une nouvelle poussée de la maladie après la résolution de l'inflammation initiale. En conclusion, nos données démontrent qu'une réponse T_H17 aux champignons cutanés commensaux peut aggraver l'inflammation cutanée et que la reconnaissance des commensaux cutanés dans un contexte inflammatoire peut induire une cicatrice inflammatoire permanente chez les lymphocytes T conduisant à de nouvelles poussées inflammatoires lors de la réexposition aux commensaux initiaux.

Mots clefs : microbiote cutané, champignons, psoriasis, T_H17 , imiquimod

Title: Adaptive immunity to commensal skin fungi promotes inflammatory flares

Abstract: Skin microbiota plays a fundamental role in host physiological immune responses. However, how immune responses to skin commensals can alter the pathogenesis of inflammatory disorders remains unclear. Fungi are part of the normal microbiota of the skin and they induced strong interleukin-17 signatures, a cytokine that is also critical in psoriasis inflammation. Here we demonstrate that T cell responses to canonical fungal commensals increased the pathology of murine imiquimod-induced psoriasiform dermatitis. Skin colonization with fungi prior to inflammation triggering with imiquimod further resumed the human phenotype, with increased in the commensal-induced Th17 responses and neutrophil extracellular traps. Notably, memory Th17 responses to commensal fungi were sufficient to induce disease flare after resolution of the initial inflammation. Together, our data demonstrate that a Th17 response to commensal skin fungi can worsen skin inflammation and that sensing of the commensals in an inflammatory manner induce a permanent inflammatory scar in the T cells leading to a pro-inflammatory transcription signature.

Keywords: skin microbiota, fungi, psoriasis, T_H17 , imiquimod

Acknowledgements

A really big thanks to Yasmine. It has been awesome working under your supervision for the last few years. I have learnt a lot and really enjoyed my time at the NIH. I understand now better the science world! Your guidance and support have been precious to drive my project. I really enjoyed working around all the wonderful, smart and fun people you hired. I also really enjoyed to learn how to present data and create a 'story' out of it, it has been an incredibly helpful lesson for my future career.

I am infinitely grateful to have received your support in my career choices, your help navigating immigration issues and your advice going forward.

Thank you to Saeko who started this project and with whom I hope we can collaborate again in the future!

Thank you to Julie and Verena helping with the last experiments and analysis!

Thanks a lot, to Ollie, Samira, Seong-Ji, NickNeck, Michael George, Ai-Ing, Ivan, Juliana, Nico, Verena, Julie, Apollo, Michel, Taylor, Djalma, Pete, Sidd, Hugh, for all of your feedback and advice over the last few years. Being able to regularly present in front of amazing young scientists has been incredibly valuable to learn how to think of results and next steps. I also really enjoyed learning to know each of you, spending time with you, improving my English (or destroying it depending on the days) and receiving support whenever I needed some!

Thank you Jackie!! I don't know how I would have done anything without you, you have helped so much to solve any single problem I encountered with lab issues.

Thank you Teresa for the massive amount of sort you did for me! It was so helpful that you were able to accommodate any of the date I needed one, and it made those long days so pleasant because I knew I would laugh with you and share some passion about the science!

Thank you to Mihalis Lionakis and his postdocs, to allow me to get fundamental strains of *Candida albicans* and for the mice you kindly offered!

Thanks to my committee Julie Segre, Mihalis Lionakis, and David Sacks for listening to my presentation and providing guidance each year.

Thank you to Armand Bensussan, Martine Bagot, and Jean-David Bouaziz to make my stay in the US a possibility, and to have been so understanding of my decision to stay there for my personal life and career!

Importantly, thanks to my family! Maman, Papa, Adele et Marc, de m'avoir accompagnée, soutenue, d'être venus me voir et d'avoir accueilli Deanou pendant cette période!

Thank you ma choupinette chérie Marie, ma TouyTouy, Hélène Chérie, Marie J, and Chaton for the long hours of phone support while I was in the mouse house, allowing this to be a pleasant experience!

It has definitely been a challenge being away of all my family and friends but I'm infinitely grateful for your support and to have the feeling that you are always with me, to receive texts and calls almost every day, making the distance way more sustainable.

Thank you to my family in law, Nancy and Scott, Brit and Trevor, Libby and Ross, for welcoming me warmly in your family.

Thank you to the GIGOGY 3.4 team, Nick Neck, Ivan, SJ, Durant, M George, and Dean for helping balancing the hard work with balance, lift weight, squats, a lot of inappropriate comments, river dogs, sprints and going out. It was definitely a strong part of my daily wellbeing and I really appreciated all the laughs and snacks we shared together.

Thanks to the amazing women in science, Samira, Ai-Ing, SeongJi, Taylor (and Ollie obviously), for sharing inspiration together!

And ... Thank you to my love, Dean, my biggest discovery of this Ph.D. and probably of my lifetime, to agree to spend the rest of your life with me, to help making it possible, to help me every day, including during the difficult times, to (sometimes) help me with my English, to break my stereotypes on America, to have learnt French, to go on all this crazy French trips with me and my friends and family, to have welcomed them, to help me being able to develop my career here and lift me up every day. Merci pour les 'rigoles' et les bisous et tous les jours passés ensemble. I feel very honored to be your wife!

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Preface

The work presented in this thesis was conducted at the National Institutes of Health, National Institute of Allergy and Infectious Diseases (NIAID), Laboratory of Immune System Biology (LISB), Metagenome Immunology Section (MIS) of Doctor Yasmine Belkaid. The project was funded by NIH grants and the National Psoriasis Foundation.

Charlotte Hurabelle-Claverie was funded by Fondation pour la Recherche Médicale (FRM), Fondation La Roche-Posay, Collège des Enseignants en Dermatologie de France, Société Française de Dermatologie (SFD), Philippe Foundation, Fondation Groupe Pasteur Mutualité.

Abbreviations

A

AF	Alexa-fluor
APC	Antigen Presenting Cell

B

BCR	B cell receptor
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C

<i>C. albicans, C. alb</i>	<i>Candida albicans</i>
CD	Cluster of differentiation
CDR	Complementarity Determining Regions
CFU	Colony forming unit
CLA	Cutaneous Lymphocyte Antigen
CLEC7A	C-type lectin domain family 7 member A
CLP	Common Lymphoid Progenitor

D

DC	Dendritic cell
DETCs	Dendritic epidermal T cells

G

GWAS	Genetic-wide association studies
------	----------------------------------

H

HSC	Hematopoietic Stem Cell
-----	-------------------------

I

IFN	Interferon
IL	Interleukin
ILCs	Innate lymphoid cells

Introduction

1. The immune system

1.1. Introduction

While all the interfaces with the environment represent both a physical and chemical barrier against the invasion of the host by pathogens, they are also the home for a myriad of micro-organisms such as bacteria, viruses, fungi and archea, commonly referred to as the microbiota. Each of the body barriers co-evolved with the microbes they are surrounded with and developed a relationship that is mutually beneficial for the microbes and their host. These microbes exist on a spectrum ranging from symbiotic to pathogenic and form complex relationships with the host immune system. The skin, oral and intestinal mucosa are examples of the anatomic barriers that prevent intrusion of the host by microbes. In case of a breach or an invasion of these barriers, the host developed an efficient immune system to defend against these micro-organisms, broadly divided into two branches: the innate and the adaptive immune system. While those two branches are traditionally separated from each other, there is a constant crosstalk between innate and adaptive immunity, allowing to provide the best protection against infections.

1.2. Innate immunity

The innate immune system is evolutionarily conserved and allows rapid sensing and responses to infections.

There are three types of phagocytes in the immune system, represented by macrophages, granulocytes (such as neutrophils, eosinophils, basophils and mast cells), and dendritic cells (DCs). They share the ability to engulf and kill pathogens.

Among granulocytes, neutrophils represent the majority of the circulating white blood cells (50 to 75% of the blood WBC). They are one of the first responders to infection and injury

sites, and they play a key role in first line defense against pathogens, using different methods to clear pathogens: phagocytosis, degranulation of antimicrobial agents (such as myeloperoxidase, reactive oxygen species, cathepsin, cathelicidin etc.) and neutrophil extracellular traps (NETs). The neutrophil extracellular traps are a recently described mechanism of neutrophil death where they extrude webs of fibers composed of chromatin and serin proteases (Brinkmann et al., 2004a). Mastocytes, eosinophils and basophils are also involved in defense against pathogens, especially parasites, but have also a prominent role in allergy.

Macrophages and DCs are also professional antigen presenting cells (APCs), that are able to uptake antigens, process them, and present them to adaptive immune cells, as well as producing inflammatory mediators such as cytokines and chemokines. As such, they can be considered as bridges between innate and adaptive immunity. Antigen presentation is done through the major histocompatibility complex (MHC). MHC-I molecules are expressed by almost all the body cells with the exception of non-nucleated cells (such as mature red blood cells). MHC-I molecules mainly bind peptides derived from the cytosolic compartment, such as host-derived peptides or peptides generated from an intracellular pathogen. More recently, it has been discovered that MHC-I molecules can present peptides generated from exogenous proteins, a process called cross-presentation. In contrast, MHC-II molecules expression is largely confined to APCs. MHC-II molecules present extracellular proteins that have been internalized in the APCs in phagolysosomes.

In addition to the myeloid cells described above, innate lymphoid cells (ILCs) and natural killer cells are cells that do not recognize a specific antigen and are therefore not considered part of the adaptive immune system but share some similarities with lymphoid lineages. Innate lymphoid cells (ILCs) are a recently discovered class of immune cells that are defined by a classic lymphoid cell morphology, but lack lineage markers and rearranged antigen receptors. The ILC family comprises classic cytotoxic natural killer cells but also three distinct groups of non-cytotoxic ILCs based on their transcription factor expression profile and expression of effector cytokines: ILC1s, ILC2s, and ILC3s, the latter including lymphoid tissue inducer cells (Vivier et al., 2018). ILCs are enriched at barrier surfaces. Natural killer cells (NK cells) are effector cells that are able to recognize and kill tumor cells and virus infected cells. This can happen because of lack of expression of MHC-I-peptide complex – a process known as ‘missing

self'. In this case, they release the contents of their granules (perforin, granzymes), which leads to induction of apoptosis in the target cell or to cell lysis. NK cells also play a role in antibody-dependent cell-mediated toxicity (ADCC), which is the release of cytolytic granules upon recognition of infected cells opsonized with antibodies.

All these cells provide rapid response to pathogens, taking only a few minutes to hours to be recruited on the site of injury, and are therefore indispensable to provide an immediate response. However, their response may not be powerful enough to clear the pathogen, they can create a lot of collateral damages because of their untargeted mode of action, and they mostly lack memory (with the exception of NK cells that display a type of memory). In contrast, the adaptive immune cells respond slower, but they are maintaining long term memory.

1.3. Receptors of innate immunity

When a pathogen is able to cross the physical and chemical barriers of the host, they are immediately detected by the immune system. Indeed, during an infection, conserved pathogen-associated molecular patterns (PAMPs) are recognized by pattern recognition receptors (PRR) (Takeuchi and Akira, 2010). PRRs include toll-like receptors (TLRs), nod-like receptors (NLRs), Retinoic acid-inducible gene (RIG)-I-like receptors (RLRs) and C-type lectin receptors (CLR). TLR and CLR are transmembrane proteins while RIG-I-like receptors and NLR are cytoplasmic. PRRs are expressed mainly in innate immune cells, but also in various cells not traditionally thought of as immune cells such as keratinocytes. Activation of the PRRs lead to intracellular signaling cascades such as nuclear factor- κ B (NF- κ B)-dependent and interferon (IFN)-regulatory factor (IRF)-dependent signaling pathways, leading to expression of inflammatory mediators (Trinchieri and Sher, 2007).

TLRs collectively recognize lipids, carbohydrates, peptides and nucleic-acid structures that are broadly expressed and conserved by the different categories of microbes. Some TLRs are expressed at the cell surface, whereas others are expressed on the membrane of intracellular endosomes and lysosomes, allowing responses to both extra- and intra-cellular pathogens. At least 10 TLRs exist in humans and 12 in mice. The table below shows the ligand and specificity of each TLR.

TLR	PAMPs recognized	Origin of the PAMP	Localization of the TLR
1	Triacyl lipoprotein	Bacteria	Plasma membrane
2	Lipoprotein	Bacteria, virus, parasite, self, fungi	Plasma membrane
3	Double-stranded RNA (dsRNA)	Virus	Endolysosome
4	Lipopolysaccharides (LPS)	Bacteria, virus, self	Plasma membrane
5	Flagellin	Bacteria	Plasma membrane
6	Diacyl lipoprotein	Bacteria, virus	Plasma membrane
7	Single-stranded RNA (ssRNA), imidazoquinoline compound	Bacteria, virus, self	Endolysosome
8	Single-stranded RNA (ssRNA), imidazoquinoline compound	Bacteria, virus, self	Endolysosome (human)
9	CpG-DNA	Bacteria, virus, parasite, self	Endolysosome
10	Unknown	Unknown	Endolysosome
11	Profilin-like molecule	Protozoa	Plasma membrane

C-type lectin receptors such as Dectin-1 and Dectin-2 have an important role in the recognition of β -glucan from fungi.

NLRs encompass NOD receptors and NALPs (NACHT-, LRR- and pyrin-domain-containing proteins) and are important for the recognition of intracellular PAMPs.

RLRs comprise RIG-I, melanoma differentiation-associated gene 5 (MDA5), and LGP2 and play a role in the recognition of viruses.

1.4. Adaptive immunity

The B and T cells are the two mainstays of the adaptive immune system. B cells represent the humoral component of adaptive immunity, while T cells represent the cellular component.

B cells

B cells develop in the bone marrow in mammals and migrate to the secondary lymphoid organs (SLO) such as the spleen and lymph nodes when they are mature. They are able to bind

directly to a specific antigen through the B cell receptor (BCR) at their surface. The BCR is composed of a membrane-bound immunoglobulin that has a unique specificity for an antigen, and of CD79, a transmembrane molecule allowing for signal transduction after antigen recognition. B cells can be activated directly after the interaction with their specific antigen through the BCR, which is called T-cell independent activation. In this case, the antigen is usually a polysaccharide or an unmethylated CpG DNA. B cells can also be activated with the help from T helper cells, typically follicular helper T cells (T_{FH}). These responses take a longer time to develop compared to the T-independent activation but they allow responses to a broader spectrum of antigens with more precise responses and longer memory. In T-dependent B cell activation, the antigen is internalized and processed in small peptides and then presented to the T cells on the MHC II of the B cell. T helper cells that are able to recognize the MHC II – antigen complex through their T cell receptor (TCR) will then express co-stimulatory molecules such as CD40L, as well as cytokines such as IL-4 and IL-21, leading to B cell activation. Activated B cells undergo clonal expansion and some differentiate into plasma cells while other become memory B cells for long-lived protection. Plasma cells are able to secrete immunoglobulins in high quantities. B cells activated through T cells help also undergo immunoglobulin class switching (a mechanism allowing to change from one immunoglobulin type to another such as from IgM to IgG or IgA), and somatic hypermutation which is a mechanism allowing for the development of the highest affinity of the immunoglobulin for its antigen.

Antibodies are Y-shaped proteins that can bind to antigens through their 2 variable regions (Fab) and can communicate with other components of the immune system through their constant region (Fc region). Immunoglobulins work by

- neutralizing pathogens, preventing their interaction and entry with host cells
- antibody-dependent cell-mediated cytotoxicity which is the process of opsonization of a pathogen with antibodies, resulting in activation of phagocytosis by scavenger cells that will destroy the pathogens
- complement activation, which leads to lysis of the target cell and chemoattraction of other components of the immune system
- immunoprecipitation allowing elimination of serum-soluble antigens.

Naïve T cells and antigen recognition

All T cells originate from a common lymphoid progenitor (CLP) itself developing from hematopoietic stem cells (HSC) from the bone marrow. CLP migrate and engraft to the thymus, where they start the maturation process as early thymic progenitors until they become naïve T cells. The maturation process starts with division of the early thymic progenitor and downregulation of c-kit. These cells are double negative for CD4 and CD8 co-receptor and are called double negative (DN) 1. Several steps allow the maturation into a naïve T cell:

- **T cell receptor (TCR)- β rearrangement and selection:** the TCR is a heterodimeric surface protein consisting of an α and a β chain. Each of these chains is made up of a constant and a highly variable region, the latter allowing the recognition of a different epitope for each of the TCR chains (Bentley and Mariuzza, 1996). Most of the variation in the variable region is due to differences in the three complementarity determining regions (CDRs). Diversity in the TCR repertoire is created through the random rearrangement of the DNA encoded segment of the V-J chains and V-D-J chains respectively in α and a β chain. RAG1 and RAG2 recombinases are the enzymes responsible of these rearrangements (Davis and Bjorkman, 1988). After both chains rearrange and successfully pair, the TCR is expressed at the cell surface, alongside with both CD4 and CD8 receptors and the cell is then referred to as double positive (DP).
- **Positive selection:** this step allows the survival of the thymocytes with a TCR recognizing with a strong enough affinity a self-antigen presented by the thymus cortical epithelial cells. Thymocytes selected on their ability to recognize an antigen presented on an MHC-I molecule will become CD8⁺ T cells and downregulate their expression of CD4, while thymocytes recognizing MHC-II-antigen complex will become CD4⁺ T cells.
- **Negative selection:** this step removes the majority of the thymocytes with a too strong affinity with a self-peptide. The rest of the ones with high affinity then become regulatory T cells (T_{reg}). This process is called central tolerance.

Naïve T cells then leave the thymus. They express the chemokine receptor CCR7 and L-selectin (CD62L), two molecules allowing second lymphoid organs homing. Priming of naïve T cells occur when APCs in peripheral tissues process an antigen and migrate to the draining lymph node and present the antigen molecule on their MHC.

CD8⁺ T cells recognize peptides presented on MHC-I molecules of APCs through their TCR, while the TCR of CD4⁺ T cells recognizes peptides presented on MHC-II molecules of APCs.

CD8⁺ T cells are also called cytotoxic T cells as they are able to recognize and kill cells that are infected, cancerous, or damaged in another way. They can do so through the secretion of perforin, a molecule that forms pores in the membrane of the target cell, and granzymes, a cytotoxic molecule. Cytotoxic T cells can also produce pro-inflammatory cytokines such as IFN γ and TNF α . The role of CD4⁺ T cells is described further below. Briefly, their main role is to product cytokines and enhance other cell types responses to infections, such as B cells and innate cells.

In addition to the classic $\alpha\beta$ T cells, other subpopulations of T cells exist:

- Mucosal-associated invariant T (MAIT) cells are a population characterized by a semi-invariant TCR α and restricted by the MHC class I-related protein 1 (MR1) (Adams and Luoma, 2013). They represent around 5% of the human T cells
- $\gamma\delta$ T cells have a TCR made of a γ chain and a δ chain instead of the $\alpha\beta$ TCR. They are also way less diverse than the $\alpha\beta$ T cells. They recognize non-classical antigens such as lipid antigens presented by CD1 molecules (in particular CD1d). They also represent only 5% of the T cells in the blood but are more abundant in the gut.
- Natural killer T cells (NKT) share properties of both NK and T cells. They represent around 0.1% of the circulating T cells and are also restricted by CD1d molecules.

Priming of naïve CD4⁺ T cells into effector T cells

When naïve CD4⁺ T cells encounter an antigen, TCR stimulation via MHC class II on APCs and cytokine signaling induce expansion and lineage differentiation into at least seven functionally

distinct T_H effector subsets: T_H1 , T_H2 , T_H9 , T_H17 , T_H22 , T_{FH} and inducible regulatory T cells. The lineage commitment depends upon the nature of the enviroing cytokines signals, activating various signal transducers and activator of transcription (STAT) proteins, ultimately leading to the expression of master transcription factors (Christie and Zhu, 2014; Loo et al., 2018). The master transcription factors then lead to the downstream expression of a differentiation program and the repression of other T_H effector subsets developmental programs. After stimulation of the TCR in presence of $IFN\gamma$ and IL-12, $CD4^+$ T cells differentiate in T_H1 cells through the activation of STAT1, leading to an upregulation of T-bet which is the master transcription for T_H1 cells (Szabo et al., 2000). This increases downstream expression of the $IFN-\gamma$ gene and upregulation of the inducible chain of the IL-12 receptor (IL-12Rb2), while suppressing T_H2 -associated factors. T_H1 cells have a central role in immune responses against intracellular pathogens (intracellular bacteria, viruses, and some protozoa) (Hsieh et al., 1993; Mosmann et al., 1986). In the presence of IL-4, T cells differentiate into T_H2 $CD4^+$ T cells: T_H2 cells produce IL-4, IL-5 and IL-13, which are potent activators of B cell IgE production and eosinophil recruitment, and target helminths. T_H9 cells develop in the presence of $TGF\beta$ and IL-4, which induces the expression of the transcription factors PU.1, IRF4, BATF, Foxo1. They have a role in anti-helminths and anti-tumor immunity (Kaplan et al., 2015). T_H17 cells produce pro-inflammatory cytokines such as IL-17A, IL-17F, IL-21 and IL-22 and have a role in protection against extracellular bacteria and fungi. As explained in the B cells chapter, T_{FH} cells are important in the formation and maintenance of B cell germinal centers by providing CD40-CD40L interactions and producing IL-4 and IL-21. T_H22 cells produce IL-22, IL-13 and $TNF-\alpha$ and are involved in epidermal immunity and tissue remodeling (Eyerich et al., 2009).

The table below describes the lineage commitment of each of the known T_H subsets after receiving their respective cytokine stimuli, and the master transcription factor and cytokines expressed after initiation of the commitment process:

T helper cell subset	Cytokines in the microenvironment triggering the lineage commitment	Signal transducers and activators of transcription (STAT)	Master transcription factor	Cytokine(s) produced	Protective function
T _{H1}	IFN- γ , IL-12	STAT1, STAT4	T-bet	IFN- γ	Intracellular pathogens
T _{H2}	IL-2, IL-4	STAT5, STAT6	Gata3	IL-4, IL-5, IL-13	Helminths
T _{H9}	TGF β , IL-2, IL-4	SMADs, STAT5, STAT6	PU.1, IRF4, BATF, Foxo1	IL-9	Helminths
T _{H17}	TGF β , IL-6, IL-21, IL-23	SMADs, STAT3	ROR γ t	IL-17, IL-21, IL-22	Extracellular pathogens
T _{H22}	IL-6, TNF- α , IL-23, IL-1 β		Aryl hydrocarbon receptor (AhR), T-bet	IL-22, IL-13, TNF- α	Tissue repair, epidermal immunity
T _{FH}	IL-6	STAT3	Bcl6	IL-21	Humoral immunity
T _{reg}	TGF β , IL-2	SMADs, STAT5	Foxp3	IL-10, TGF β	Immuno-regulation

Tolerance mechanisms and regulatory T cells (T_{reg})

To be efficient but not harmful, the immune system must avoid to mount deleterious responses against environmental, food, and commensal antigens. One of the important way the immune system achieves tolerance is to negatively select self-reactive T cells in the thymus, a process leading to elimination or inactivation of self-reactive T cell clones and known as central tolerance (Starr et al., 2003). In the periphery, other mechanisms of tolerance avoid autoimmunity. Anergy is a state of inactivation of the T cells that happen when there is chronic engagement of TCRs by self-antigens, without simultaneous engagement of T cell co-stimulatory receptor CD28 by CD80 and CD86 on APCs.

However, circulation of reactive T cells escaping those mechanisms of regulation are a threat to the host as they can result in autoimmunity. Therefore, another important mechanism

allowing immunological homeostasis is the development of regulatory T cells as they help to suppress excessive and destructive immune responses against self and foreign antigens. Importantly, in absence of T_{reg} , severe inflammation and autoimmunity develop, with failure to thrive, lymphadenopathy, splenomegaly, immune dysregulation, polyendocrinopathy, enteropathy etc., demonstrating the importance of T_{reg} in providing proper immune homeostasis (Bennett et al., 2001; Kim et al., 2006).

Natural T_{reg} (nT_{reg}) develop in the thymus through selection of naïve T cells, while induced T_{reg} (iT_{reg}) differentiate in the peripheral tissues. Thymic selection of T_{reg} starts with recognition of a self-peptide-MHC2 complex by the TCR, with co-stimulation and IL-2-STAT5 signaling. iT_{reg} develops in the periphery in a mechanism similar to the T helper cells: TGF β and IL-2 cytokines signal through SMADs, STAT5 (Josefowicz et al., 2012). The master transcription factor for all T_{reg} is Foxp3, leading to the downstream expression of immunosuppressive cytokines such as IL-10 and TGF- β . All T_{reg} lack expression of the IL-7 receptor and express high levels of the IL-2 receptor alpha chain (CD25).

Memory T cells

After clearance of an infection, the majority of effector T cells die via apoptosis, but a small percentage of the T cells persist and differentiate into long-lived memory cells (Badovinac et al., 2002; Pepper and Jenkins, 2011). Memory T cells respond more rapidly and more efficiently to secondary infections than naïve T cells. Memory cells express markers of antigen-experience such as CD44. They lack expression of CD45RA but express CD45RO. In addition, two main subsets of memory T cells were initially described depending on their expression of CD62L and CCR7 (Sallusto et al., 1999). Central memory T cells (T_{CM}) express these lymph node homing receptors and are therefore mostly found in the SLO and in the circulation. On the other hand, effector memory T cells T_{EM} don't express these receptors, and are mostly found in the peripheral tissues and in the circulation. T_{EM} therefore migrate rapidly to secondary infection sites where they have immediate and potent effector functions. T_{CM} lack this immediate effector function in the periphery but have a high proliferative capacity and efficiently stimulate dendritic cells.

Other memory T cells subsets have been more recently discovered, such as resident memory T cells (T_{RM}), which remain poised in the previously infected tissue and don't re-enter in the circulation (Schenkel and Masopust, 2014).

T_H1 and T_H2 cells in inflammatory diseases

Adaptive immune responses are essential for fighting against invasion of the host with pathogens. Indeed, in absence of the main transcription factors for the different effector T cell subsets, or if there are mutations of STATs or cytokines genes, various primary immunodeficiency diseases can occur. Many functions of different T cell effector subsets have been learnt through the discovery of these primary human immunodeficiencies and by creating mouse models specifically deleting or overexpressing T_H subsets associated factors such as transcription factors, STATs, or cytokines. For example, T-bet transcription factor deletion are more susceptible to several intracellular pathogens, such as *Salmonella typhimurium* or *Mycobacterium tuberculosis* (Ravindran et al., 2005; Sullivan et al., 2005).

On the other hand, dysregulated immune responses may happen and lead to autoimmune and chronic inflammatory disorders. These responses can be targeted against self or commensal floral antigens.

Figure: transcriptional regulation of CD4+ T helper (T_H) cells that mediate tissue inflammation.

From Loo TT et al, *J Leuk Biol.*

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Dysregulated T_H1 responses can promote tissue destruction and chronic inflammation (Lazarevic and Glimcher, 2011). In human, genetic studies have shown associations between single nucleotide polymorphisms (SNPs) in T-bet binding sites with inflammatory bowel diseases (Soderquest et al., 2017), or with asthma (Munthe-Kaas et al., 2008). In mice, lack of T-bet prevents the development of inflammatory bowel disease (Neurath et al., 2002), experimental autoimmune encephalomyelitis (Bettelli et al., 2004), diabetes (Juedes et al., 2004), arthritis (Wang et al., 2006), and systemic lupus erythematosus (Peng et al., 2002). On the other hand, T-bet regulated the balance between T_H1 and T_H2 cells, and in absence of T-

bet, mice can develop more severe asthma-like disease. This can happen both independently of allergic responses, due to lack of repression of pro-fibrotic cytokines, and due to unregulated T_H2 responses (Finotto et al., 2002).

While T_H2 responses are primarily aiming at expelling parasitic infections, dysregulated T_H2 responses in human can lead to atopy and allergic reactions (Walker and McKenzie, 2017). Indeed, damaged tissue induces the release of alarmins, such as TSLP and IL-33, which triggers the T_H2 cytokine production, itself leading to the stimulation of IgE production by B cells, recruitment of eosinophils and mast cells, and increased mucus production, smooth muscle contractility, and ultimately leading to the expulsion of the parasites (Anthony et al., 2007). Helminths invasion also triggers tissue repair by increasing angiogenesis, myofibroblast activation and extracellular matrix deposition, as well as promoting the differentiation of regulatory M2 macrophages (Gause et al., 2013). However, these responses are also amplified in response to allergens in atopic patients, and lead to the development of pathologies such as asthma, atopic dermatitis, or chronic allergic sinusitis. Genome-wide associated studies have identified polymorphisms in genes encoding alarmins, cytokines and epithelial barrier proteins in asthma or atopic dermatitis (Moffatt et al., 2010). Sputum and blood cytology in asthma patients demonstrate evidence of T_H2 involvement, with eosinophils and IL-5 producing $CD4^+$ T cells, hyper-IgE production, while mouse models lacking expression of T_H2 cytokines often have improved asthma-associated features (Lambrecht et al., 2019; Papi et al., 2018). Asthma patients also exhibit airway remodeling, with epithelial damage, goblet cell hyperplasia, and increased myofibroblast and fibrocyte proliferation, leading to smooth muscle hyperplasia. Monoclonal antibodies targeting IL-5, or IL-4 and IL-13 are used in clinic to reduce exacerbation frequency in patients with asthma (Rabe et al., 2018; Yancey et al., 2017). (Of note, only half of the patients with asthma have responses strongly skewed towards T_H2 , while the rest of the patients have asthma that are described as ‘non-eosinophilic’, with involvement of T_H1 , T_H17 responses and neutrophils). Atopic dermatitis is also considered a T_H2 disease, and monoclonal antibody blocking IL-4 and IL-13 signaling are also improving this disease (Beck et al., 2014; Gittler et al., 2012).

Th17 cells and diseases

At steady state, T_H17 cells are mostly found at barrier sites such as the gastrointestinal tract, the skin and the lungs, where they provide protection against extracellular bacteria and fungi as well as homeostatic functions. These functions are achieved thanks to the production of the signature cytokines IL-17A, IL-17F, IL21 and IL-22. They have an essential role in preventing invasion of the host by extracellular microbes. Indeed, T_H17 signature cytokines induce the expression of antimicrobial peptides (lipocalin-2, Reg3 β , Reg3 γ , S100A8, S100A9), chemokines (CXCL1, CXCL2, CXCL8, CXCL10) and metalloproteases helping wound healing and tissue remodeling, and pro-inflammatory cytokines (IL-1 β , IL-6, TNF- α , GM-CSF) (Stockinger and Omenetti, 2017). IL-17A also promotes the expression of tight junction proteins (Lee et al., 2015). All these responses help to contain potential pathogens outside of the host. Interestingly, commensal microbes themselves promote the differentiation of T_H17 cells, which in turn control the burden of these bacteria and prevent infiltration of the host : in the gut, one single commensal microbe, segmented filamentous bacterium, is able to induce T_H17 cells (Ivanov et al., 2009).

Importantly, T_H17 cells have a fundamental role in the defense against fungi, especially *Candida albicans*, and extracellular bacteria such as *Staphylococcus aureus*, especially at mucocutaneous barrier sites. This is highlighted by the development of chronic and / or severe infections with those microbes in patients lacking the cytokines IL-17A, IL-17F, their receptor IL-17 receptor A (IL-17RA) or with mutations in the gene coding for the T_H17 master transcription factor, *RORc* (Okada et al., 2015; Puel et al., 2011). Loss of function mutations in *STAT3* also result in recurrent *Candida albicans* and *Staphylococcus aureus* infections and in increased colonization with *Candida* species (Abusleme et al., 2018; Holland et al., 2007). Several murine models recapitulate the importance of T_H17 cells and signature cytokines for mucocutaneous protection against *Candida albicans* infection (Khader et al., 2009). For example, mice deficient in IL-17 receptor A (IL-17RA) have significantly less survival compared to control mice in a model of systemic candidiasis and have significantly more severe infection in models of oral candidiasis, with increased fungal burden and decreased mobilization of neutrophils at the sites of infection (Conti et al., 2009; Huang et al., 2004). To be able to clear potential pathogens, T_H17 induce rapid recruitment of granulocytes and macrophages through the production of IL-17. IL-17 cytokines are potent inducer of neutrophils expansion and survival, as well as chemoattraction (Onishi and Gaffen, 2010). T_H17 cytokines also induce the up-

regulation of antimicrobial peptides, which directly contribute to host defense by direct killing of invading organisms.

On the other hand, several human diseases have been associated with dysregulated T_H17 cells. T_H17 cells master transcription factor is ROR γ t, which is induced in response to cytokines such as TGF- β and IL-6, with or without additional IL-23 and IL-1 β (Ivanov et al., 2006). However other transcription factors are necessary to specify the full differentiation of T_H17 cells (Ciofani et al., 2012). The basic leucine zipper transcriptional factor ATF-like (BATF) and interferon-regulatory factor 4 (IRF4) complexes are essential to remodel chromatin accessibility, allowing the ROR γ t-dependent upregulation of T_H17 signature genes. Other transcription factors regulate the fate of T_H17, leading to maintenance or repression of T_H17 signature genes, such as AP-1, Fos12, c-Maf, or Ahr.

Interestingly, the cytokine TGF- β is important both for the development of T_{reg} and T_H17 (Bettelli et al., 2006; Weaver and Hatton, 2009). Depending on the relative abundance of TGF- β and IL-6 in the environment, a CD4⁺ T cell can develop either toward a T_{reg} or a T_H17 cell, with an initial and transient co-expression of Foxp3 and ROR γ t. If there is a high TGF- β , Foxp3 inhibits ROR γ t activity, and the cell will develop as a T_{reg}, while at low TGF- β concentrations, TGF- β and IL-6 act synergistically to induce ROR γ t transcription of T_H17 program (Veldhoen et al., 2006). IL-23 acts synergistically with IL-6, IL-21, and TGF- β and also induces differentiation of T_H17 cells (Zhou et al., 2007).

The mechanisms described above are important because they show that T_H17 lineage is plastic and therefore susceptible to environmental modulations. This is useful when apprehending the concept of beneficial versus pathogenic T_H17 cells. This concept was developed following the *in vitro* differentiation of T_H17 cells with different antibody cocktails, their transfer *in vivo* in mice and the observation of their abilities to induce experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis in mice with inflammation of the central nervous system (Lee et al., 2012). When culturing CD4⁺ T cells with IL-6 and TGF- β , T_H17 cells producing IL-10 developed and they did not induce EAE (McGeachy et al., 2007). Alternatively, when cultures contained IL-23 in combination with IL-6 and IL-1 β , pathogenic T cells were able to induce EAE were generated (McGeachy et al., 2009; Meyer zu Horste et al., 2016).

In addition to EAE, a number of autoimmune diseases are mediated through IL-17 and it is yet unclear if all the T_H17 in this context are functionally different compared to physiologic T_H17 or if it is due to dysregulated responses in a predisposed genetic environment.

Rheumatoid arthritis is an autoimmune disease affecting the joints, leading to inflamed joints, cartilage and bone erosions, ultimately leading to irreversible deformities in joints. Long thought to be due to dysregulated T_H1 responses, it was later demonstrated that IL-17 induces the production of other pro-inflammatory cytokines in the joints such as TNF- α , IL-1 β and IL-6 from chondrocytes, synoviocytes, macrophages and osteocytes. These cytokines in turn leads to the recruitment of neutrophils and macrophages to the synovium (Shen et al., 2005). IL-17 cytokines also contribute to the destruction of the cartilage and bone through the induction of the expression of matrix metalloproteinases (MMP) 1, 2, 3, 9 and 13, which drive degradation of extracellular matrix within the joint (Chabaud et al., 2000). IL-17 induces the expression of RANKL (receptor activator of nuclear factor- κ B ligand), a membrane-bound receptor of the TNF superfamily that promotes differentiation of osteoclasts, leading to bone destruction (Sato et al., 2006).

Inflammatory bowel disease (IBD) encompasses both Crohn's disease and ulcerative colitis, which are inflammatory disorders affecting the whole GI tract and the colon and rectum respectively. Patients with IBD experience chronic abdominal pain, diarrhea, rectal bleeding, as well as complications outside of the GI tract such as arthritis, pyoderma gangrenosum, or primary sclerosing cholangitis. Increased amounts of IL-17 and IL-23 have also been found in the intestinal mucosa in humans as well as in experimental models of IBD (Fujino et al., 2003). GWAS studies have found that polymorphisms in IL23R or STAT3, two T_H17 -related genes, as well as in NOD2 (nucleotide-binding oligomerization domain-containing protein 2) which encodes a sensor of bacterial cell wall components (Duerr et al., 2006; Lesage et al., 2002). It has therefore been hypothesized that IBD is caused by dysregulated inflammatory T_H17 responses against commensal gastrointestinal microbes. However, monoclonal antibodies targeting IL-17 pathway have failed to treat Crohn's disease (Hueber et al., 2012).

Psoriasis is another IL-17 mediated disease and will be developed further in a specific chapter of this thesis (see chapter 4 of the introduction).

2. The skin

2.1 Skin structure

The skin represents a physical, chemical and immune barrier to the environment. With the gut and the lungs, it represents one the largest interface with the environment. It has a surface area of 25 m² when including the skin appendage openings into the calculation (Gallo, 2017). It is also densely innervated and is an important sensory organ, relaying information to the central nervous system. Given its large surface area and proximity to the external environment, the skin is an important place of interaction with the microbiota.

The skin consists of two major components: the epithelial compartment, consisting of the hair follicle and the epidermis, and the underlying connective tissue, the dermis, which is vascularized and therefore provides nutritional and structural support (Pasparakis et al., 2014).

The epidermis is the outermost layer of the skin and is in direct contact with the environment. It is primarily a mechanical barrier, preventing external insults such as invasion of pathogens and chemical penetration, as well as loss from within, such as water and solute loss. The most numerous cell type in the epidermis is the keratinocyte, originating from stem cells located in the basal cell layer. Keratinocytes migrate upward to the surface as they undergo maturation, ultimately forming the different layers of the epidermis, namely from the innermost to the outermost the basal layer, the stratum spinosum, the stratum granulosum, and the stratum corneum.

The human epidermis also contains melanocytes, Langerhans cells, CD8⁺ T cells and Merkel cells in humans. In mice, in addition to the former, there is a prominent population of dendritic epidermal T cells (DETCs), a cell type that is absent in humans. In addition, in comparison to humans, mice have a way higher density of hair follicles and a thinner epidermis (Pasparakis et al., 2014).

There are anchoring fibers of the basement membrane which allow adhesion of the epidermis to the dermis at the rete ridges.

Figure: The mouse and skin structure.

From Pasparakis M, Haase I and Nestle FO, *Nature review immunology* 2014.

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The dermis is divided in the more superficial papillary dermis and the deeper reticular dermis. It mainly comprises extracellular matrix and stromal cells (the fibroblasts) that produce the collagen and elastic fibers. They provide most of the mechanical strength to the skin as well as a structural framework for blood and lymphatic vessels, nerves, glands and hair follicles. Fluid regulation happens in the dermis through the blood and lymphatic vessels. The neuronal dendrites are located in the skin and allow for sensory feedback.

This layer contains many types of immune cells, such as APCs (DCs and macrophages), $\alpha\beta$ - and $\gamma\delta$ -T cells, ILCs, mast cells, and basophils (Di Meglio et al., 2011).

2.2 Skin immune system

Antigen presenting cells in the skin

Multiple APCs exist in the skin, with at least four population of dendritic cells under steady state conditions (Malissen et al., 2014). The professional antigen-presenting cells in the epidermis are represented by Langerhans cells (LCs). They are found both in the interfollicular epidermis and the epithelium of the hair follicles. Langerhans cells develop from yolk sac-derived myeloid precursors and from fetal liver-derived monocytes and are recruited to the epidermis during early embryogenesis. The LCs pool renews direct in the tissue, through a slow proliferation rate. In addition to their APCs function, LCs have some regulatory functions and are able to induce regulatory T cells (Bobr et al., 2010; Igyarto et al., 2009). In the dermis, the three major subsets of conventional DCs are represented by XCR1+ DC (that can be CD103+ or CD103-), CD11b+ DCs, and double negative DCs. Dermal CD11b+ DCs are the most abundant DCs in normal dermis. The dermal conventional DCs come from a bone marrow derived precursor that continually renews the pool of tissue conventional DCs. All the aforementioned antigen presenting cells are able to migrate to the lymph nodes upon activation by bacterial, fungal, viral or protozoal stimuli and present antigens to naïve antigen-

specific T cells, which then will undergo clonal expansion and acquisition of the T cell effector functions.

Macrophages are also present in the dermis. They have primarily phagocytic and anti-inflammatory properties but are not able to migrate to the lymph nodes.

Finally, plasmacytoid DCs (pDCs) can be found in inflamed skin and mediate systemic pro-inflammatory responses.

Figure: APCs in the skin.

From Belkaid Y & Tamoutounour S, *Nature Rev Immunol* 2016.

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Adaptive immune cells in the skin

The skin is an amazing reservoir of T cells, with $\alpha\beta$ T cells representing the majority of the T cells in the skin of humans, while $\gamma\delta$ T cells represent 1-10% of the T cells in normal human skin. It has been estimated that around 20 billions of $\alpha\beta$ T cells are present in the total skin of a normal adult (Clark et al., 2006), around twice as much as in the circulation, and these cells are phenotypically, functionally and clonally diverse. In normal mouse skin, the majority of $\alpha\beta$ T cells are dermal CD4⁺ T cells, while CD8⁺ T cells are mostly localized in the epidermis (Belkaid et al., 2002; Gebhardt et al., 2011). In human skin, the majority of T cells reside in the dermal-epidermal junction, around blood vessels in the dermis, and in appendages such as the hair follicle (Clark et al., 2006), while the human epidermis is populated with unique phenotypes of resident T cells such as CD103⁺ CD8⁺ and CD4⁺ T cells (Watanabe et al., 2015). Contrarily to humans, mouse epidermis also contains a high number of $\gamma\delta$ T cells, especially dendritic epidermal T cells (DETCs) that are localized in the epidermis at steady state. These cells express a conserved V γ 5V δ 1 TCR, are relatively immobile, divide slowly, and have a key role in promoting tissue repair (Chodaczek et al., 2012; Havran and Jameson, 2010).

Other than DETCs, which reside solely in the epidermis of murine skin, the other subtypes of $\gamma\delta$ T cells are predominantly located in the dermis. These dermal $\gamma\delta$ T cells, whose TCR receptor includes the V γ 4 or V γ 6 TCR chains are predominant producers of IL-17 within mice at steady

state (Nakamizo et al., 2015; Ramírez-Valle et al., 2015). Both dermal subsets appear to be radioresistant and are largely resident in the dermis (Cai et al., 2014). Dermal $\gamma\delta$ T cells have been implicated in host immunity to commensals and pathogens where they proliferate in response to multiple cutaneous bacteria including *Staphylococcus aureus* (Dillen et al., 2018), *Corynebacterium accolans* (Ridaura et al., 2018), *Mycobacterium bovis* (Sumaria et al., 2011). Additionally, $\gamma\delta$ T cells have been found to be increased in lesions of human psoriasis and in murine models of psoriasis, where depletion of $\gamma\delta$ T cells leads to amelioration of disease (Sandrock et al., 2018).

Skin-homing $\alpha\beta$ T cells are generated from naïve T cells activated by APCs travelling in skin draining lymph nodes after they encounter an antigen in the skin. These APCs then present the antigen to the TCR through their MHC, stimulate the T cells via the co-stimulatory molecules such as CD80 and CD86 (and their ligand CD28 on T cells) and lead the T cells to their effector function depending on the cytokine environment. Recruitment to the skin of the effector T cells is facilitated by the expression of adhesion molecules on the T cells to ligands in the skin. T cells expressing the cutaneous lymphocyte antigen-1 (CLA) bind to E-selectin expressed by endothelial cells during inflammation (Kupper and Fuhlbrigge, 2004). Other factors able to attract T cells in the skin are the chemokines, which signal through G-protein coupled receptors in T cells (Griffith et al., 2014). For example, CCR10 and CCR8 are utilized for T cell homing to inflamed skin via CCL27 and CCL8 respectively, which are produced by keratinocytes (Homey et al., 2002). In addition, blood vessel endothelium-derived CCL1 also attracts T cells expressing CCR8 (Gombert et al., 2005). During skin infections, T cells infiltrate the skin and memory T cells can remain in the skin to provide rapid protection in case of a reinfection: up to 95% of the $\alpha\beta$ T cells in the skin are CD45RO memory T cells (Bos et al., 1987). Contrarily to CD8⁺ T cells which contract and become true resident in the skin after clearance of an infection, the majority of CD4⁺ T cells recirculate, and are sometimes called migratory memory T cells (T_{MM}) (Debes et al., 2005).

Role of epithelial cells in skin immunity

Keratinocytes, which represent the outer barrier of the skin, are not only a physical barrier but also have an important role to direct the skin immunity. They produce a number of chemokines allowing the recruitment of T cells to the skin. They also detect pathogen-

associated molecular patterns (PAMPs) because they express pattern recognition receptor (PRR) (Takeuchi and Akira, 2010), including toll-like receptors (TLRs) Keratinocytes express TLR 1 to 6 and TLR 9, allowing them to act as first-responders when encountering pathogens (Miller, 2008). Keratinocytes also produce antimicrobial peptides (AMPs) that directly kill pathogens (Harder and Schroder, 2005). Finally, keratinocytes also produce cytokines, such as IL-1 and TNF α . All these factors can initiate anti-microbial responses in the infected tissue, and further recruit innate and adaptive professional immune cells such as neutrophils, monocytes and T cells.

3. The microbiome

3.1 Introduction

While all the interfaces with the environment represent both a physical and chemical barrier against the invasion of the host by pathogens, they are also the home for a myriad of micro-organisms such as bacteria, viruses, fungi and archea, commonly referred to as the microbiota. The whole microbiota represents a higher number of cells and genes than their host's genome! Each of the body barriers co-evolved with the microbes they are surrounded with and developed a relationship that is mutually beneficial for the microbes and their host. The microbiota coevolved with humans, establishing this interdependent relationship where the microbiota cannot survive without the host, but it also has an important role in calibrating the immune system, contribute to local regulation of tissues as well as participate in metabolic pathways. While the host-microbiota relationship is mainly symbiotic, it has been shown that this interaction can become dysregulated and participate in the pathogenesis of auto-immune or allergic diseases. Indeed, the microbiota has shaped the immune system for millennia, and rapid changes in the composition of the microbiota, because of antibiotic treatment, changes in diet or in hygiene rules over a few decades may have transformed the mutually benefic relationship between the microbiota and the host.

3.2 The gut microbiota

The gut microbiota is probably the richest in terms of number and diversity. The intestinal tract is home to up to 10^{14} microbes, the majority of them living in the colon (Ley et al., 2006). While the gut microbes can be useful for the host, the host also tends to establish a clear separation between the inside and the outside and tries to compartmentalize microbes on the outer part. This is helped by the production of mucus by goblet cells, but also through the production of multiple antimicrobial peptides, that can have direct microbicidal functions, with enzymatic attack of the bacterial wall or disruption of the bacterial inner membranes (Cash et al., 2006; Hooper and Macpherson, 2010). The gut microbiota also has a role in directly shaping the development of hematopoietic and innate cells. It has indeed been shown that germ free animals have a global defect in innate immune cells and in the number of hematopoietic stem cells and precursors (Belkaid and Harrison, 2017). Microbiota-derived metabolites, such as short-chain fatty acids, represent an energy source for enterocytes, but are also able to increase the generation of macrophages and dendritic cell precursors (Trompette et al., 2014). Microbe-derived riboflavin is indispensable for the development of MAIT cells, and the MAIT cells are absent in germ-free animals (Treiner et al., 2003). Importantly, the gut microbiota has a major role in shaping the adaptive immune system. Microbiota-derived antigens induce the production of a mucosal compartmentalized IgA response, which prevent adhesion of the commensal bacteria to the epithelial cells, but also reduces intestinal proinflammatory signaling (Peterson et al., 2007). The role of the gut microbiota has been extensively proven in the induction of the different T effector cell subsets. T_H17 cells frequencies are severely reduced in germ-free mice or mice treated with broad spectrum antibiotics (Ivanov et al., 2008). Interestingly, a single bacterial species, segmented filamentous bacteria (SFB), is able to induce the differentiation of intestinal T_H17 cells. These antigen-specific T_H17 cells are in turn able to control the SFB burden through the production of IL-17 signaling in intestinal epithelial cells (Yang et al., 2014). The T_H17 signature cytokines have an important role in the development of epithelial cells tight junction and reinforce the epithelial barriers (Weaver et al., 2013).

3.3 The skin microbiota

The skin is constantly exposed to the environment and is also a site of encounter with skin microbes. Unlike the gastrointestinal tract, the skin is relatively weakly colonized by microbes: it is estimated that there is about 1 million bacteria per cm², with around 10¹⁰ bacteria over a human skin surface (Belkaid and Segre, 2014; Grice et al., 2008). This can be explained by the relative inhospitality of the skin compared to the GI tract. The surface of the skin is dry with a low pH and produces many proteins that deprive microbes of essential nutrients. Microbiome composition of the skin varies depending on the clinical site, in relation with factors such as pH, temperature, degree of sebum and moisture of the site. For example, sebaceous sites are mainly represented by lipophilic bacterial species such as *Cutibacterium acnes* (formerly known as *Propionibacterium acnes*), while *Proteobacteria* are more frequent in dry sites. *Staphylococcus epidermidis* can tolerate the high salt concentrations present in sweat and may use urea and aminoacids from the eccrine sweat as a nutrient source (Scharschmidt and Fischbach, 2013).

The skin microbiota changes throughout the course of life. Initial seeding of the skin with microbes begins when the fetus exits out of the womb, and the type of delivery leads to different types of microbes seeding the skin: the neonate skin microbiota resembles most the maternal vaginal flora if the baby is delivered vaginally and the maternal skin microbiota if the baby is delivered through cesarean section (Dominguez-Bello et al., 2010). During adolescence, hormonal changes create a sebum-rich environment that allows for lipophilic bacteria to flourish (Oh et al., 2012). Microbial communities stabilize during adulthood, but continue to evolve depending on environmental factors (such as climate, antibiotics exposure, hygiene, etc.) and host factors.

Figure: the skin microbiota.

From Belkaid Y & Segre J, *Science* 2014.

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The microbes isolated from the skin depend on the depth and location in the skin itself: different microbes can be found in the outer epidermis and in the depth of the hair follicle, as demonstrated by the different results of microbiota mapping generated by swabbing versus biopsies (Prast-Nielsen et al., 2019). This can be explained by the different environment at different sites of the skin: hair follicles are anaerobic when going deeper in the follicle, while the top of the skin represents an aerobic environment (Chen et al., 2018).

Interestingly, the composition of the skin microbiota dramatically changes during episodes of skin inflammation, and it is not completely understood if these changes are secondary to the skin inflammation or if they drive the skin inflammation.

It is interesting to speculate on the role of the microbiota at each unique stage, and emerging evidence ties the date of encounter with the microbes with the response they induce in the host.

Recognition of the skin microbes by the host

Increasing evidence suggest that the manner in which antigens and microbial products are recognized determines the type of immune response. Using two different models of infections, it has been shown that CD11b⁺ DCs elicit a Th1 response when confronted to a pseudo-hyphae *Candida albicans* invasive (dermal) infection, while Langerhans cells were capable of eliciting a Th17 response to *Candida albicans* yeast in an epidermal infection (Kashem et al., 2015). In another model, Langerhans cells were required to sense *Staphylococcus aureus* and promote inflammatory responses with development of IL-17 $\gamma\delta$ T cells and IL-17 CD4 T cells (Kobayashi et al., 2015). These observations were made in the context of an altered skin barrier, but even with an intact skin barrier, the immune system is able to sense skin commensals, inducing cognate, long-lasting immune responses.

For commensal encounter, while one would expect that the surface commensals would be sensed by the most superficial APCs (the Langerhans cells), there are actually increasing body of evidence demonstrating that both Langerhans cells and dermal dendritic cell populations can sense the skin microbes and that the different cell types involved in microbe sensing are responsible for different response types. *Staphylococcus epidermidis* induces IL-17A producing CD8⁺ T cells, which is dependent on dermal resident CD103⁺ DCs and independent of Langerhans cells, and IL-17 production by these cells is licensed through the IL-1-producing

CD11b DCs (Naik et al., 2015a). This demonstrates that different DC subsets within the skin cooperate to respond to defined skin commensals.

The way skin-resident DCs sense microbiota-derived antigens remains unclear. Dermal DCs surrounding skin appendages such as hair follicle may extend dendrites within those appendages and capture microbes or their products. Microbial products could also passively diffuse through the epithelial barrier, especially in the hair follicles (Gallo and Nakatsuji, 2011).

Role of the microbiome in cutaneous immunity

The immune system has evolved closely with the skin commensal microbes, allowing colonization with those microbes while limiting pathogenic microbial invasion.

Several studies have shown how the skin microbiota can enhance the barrier function of the skin through the immune system, to prevent invasion with microbes.

Though much focus has been placed on the IL-1, IL-23 and IL-17 axis in disease settings, the IL-23 – IL-17 axis also provides barrier defense as demonstrated by patients who have defects in the axis are more susceptible to infections with extracellular pathogens (Lehman, 2014). Evidence continues to mount that the microbiota can modulate this axis, which is important for both immune homeostasis as well as disease. After production of IL-1 and IL-23 by APCs, multiple subsets of both innate-like and adaptive T-cells are capable of producing IL-17 family cytokines such as IL17, IL22, GM-CSF, which in turn regulate the inflammatory response and bolster barrier defense through increased expression of AMPs and chemokines (Gaffen et al., 2014).

Commensals also have the ability to control other arms of the immune system such as IL-1 signaling, which is an evolutionarily conserved arm of the innate immune system that may have evolved as a mediator of host-commensal cross-talk. Germ free mice have reduced IL-1 α from the skin compared to SPF mice. Additionally, keratinocytes of germ free mice have increased levels of IL-1ra compared to SPF mice. Mono-association with the commensal *S. epidermidis* increases IL-1 α and decreases IL-1ra to levels similar to SPF mice (Naik et al., 2012).

IL-1 and IL-23 are also capable of polarizing $\alpha\beta$ T-cells to produce IL-17 in response to microbiota in the skin (Naik et al., 2015a). Additionally, IL-17 induced by the microbiota in an IL-1 dependent manner plays a role in immune homeostasis and can also provide protective

immunity to the pathogens *Leishmania major* and *Candida albicans* (Naik et al., 2015a; Naik et al., 2012). A diverse microbiota is essential to ensure protection against infections. Mice living in SPF conditions have a lack of tissue resident and effector differentiated memory T-cells compared to wild mice and the latter have better response and control of infection with *L. monocytogenes* or LCMV than the former (Beura et al., 2016; Rosshart et al., 2017)

Importantly, the time of encounter with skin commensals is fundamental in the type of immune responses that they induce. In contrast to adults, microbial sensing in the neonatal period leads to a tolerogenic response to the microbes encountered and the development of antigen-specific regulatory T cells seeding the skin (Scharschmidt et al., 2015). However, skin microbes are not essential for the development of T_{reg}, as there are an increased number and proportion of T_{reg}, while there is a decrease in effector IFN- γ or IL-17A producing T cells in the skin of germ free mice (Naik et al., 2012).

On the other hand, disturbance of the skin microbiome can have long term impact on the immune system and on the risk of inflammatory skin diseases. Treatment of neonate mice with antibiotics durably alters the gut microbiota and worsens imiquimod-induced skin inflammation (Zanvit et al., 2015).

Immune responses to the microbiota can also promote wound healing and limit skin inflammation after an injury, by modulating both the innate and adaptive immune responses: *S. epidermidis* TLR2 signaling inhibits responses to dsRNA coming from damaged host cells (Gallo and Nakatsuji, 2011). Commensal-specific T cells can also accelerate wound healing through expression of tissue repair gene (Linehan et al., 2018a).

While immune responses to the skin microbiota has multiple beneficial roles for the host, barrier defects or genetic pro-inflammatory conditions can challenge these physiological responses.

Microbiome and skin inflammatory diseases

Atopic dermatitis

Atopic dermatitis is a chronic inflammatory skin disease that typically develops in individual with epidermal barrier dysfunction, such as filaggrin deficiency and impaired lipids in the stratum corneum. It is characterized clinically by flares of erythematous papules and plaques, associated with variable vesiculation, oozing, scaling, and crusting depending on the age of the lesions, and associated with severe pruritus. Decades ago, the association between *Staphylococcus aureus* and atopic dermatitis was first made (LEYDEN et al., 1974) and it has long been recognized that patients with atopic dermatitis are more susceptible to various infections, including with *S. aureus* (Brüssow, 2016). While the association has stood the test of time, whether *S. aureus* is the inciting event, involved in disease proliferation and progression, or merely an opportunist replicating in the setting of underlying host inflammation has been and is currently an active area of research.

Microbiome analysis have shown that atopic dermatitis flares are associated with dysbiosis dominated by blooms of *Staphylococcus aureus*, but also by overall decrease in skin microbial diversity (Kong et al., 2012). Though compelling, such associations between prevalence and disease severity cannot differentiate if *S. aureus* is a cause or a consequence of the skin inflammation. Interventional studies have largely shown that decreasing *S. aureus* during disease flares tends to reduce the severity of the disease, suggesting that *S. aureus* plays a role in disease progression (Brüssow, 2016). Even more, *S. aureus* secretes various toxins and superantigens, and atopic dermatitis patients tend to have a higher degree of toxin secreting strains of *S. aureus* than controls (Hoeger et al., 1992; Zollner et al., 2000). In particular, *S. aureus* δ -toxin is capable of inducing mast cell degranulation, which can in turn promote innate and adaptive Type 2 immunity (Nakamura et al., 2013). Taken together, *S. aureus* appears to play a role in disease progression, which can at least partially be explained by production of various toxins.

Though *S. aureus* may be involved in disease progression, the inciting event remains unknown. However, genomic analysis has pointed to epidermal structural proteins. The findings that mutations in FLG, the gene encoding the skin barrier protein filaggrin (Barker et al., 2007; Irvine et al., 2011; Palmer et al., 2006), and SPINK5, which encodes lymphoepithelial Kazal-type 5 serine protease inhibitor (LEKTI) and is involved in cleavage of pro-filaggrin to filaggrin

(Walley et al., 2001), both can lead to atopic dermatitis suggests that barrier defects may be an inciting event in disease pathogenesis. Even more, filaggrin deficiency associated atopic dermatitis often precedes allergic rhinitis (Barker et al., 2007), indicating epicutaneous sensitization may be the inciting in the atopic march. Another study looking at ADAM17 deficient mice, which have barrier defects in an epidermal growth factor receptor EGFP dependent mechanism, display an atopic dermatitis-like phenotype, which is dependent on *S. aureus* and *Corynebacterium bovis* (Kobayashi et al., 2015). It seems reasonable to propose a model where a barrier defect leads to a leaky barrier, which is exposed to more microbes and microbial products such as *S. aureus* δ -toxin, which leads to proliferation of T_H2 cytokines, which may further favor *S. aureus* growth on toxin production.

4. Psoriasis

Psoriasis is a chronic inflammatory skin disease of unknown etiology, affecting approximately 2-3% of the population. It is believed to be driven by aberrant activation of the innate and adaptive immune system in genetically predisposed individuals, with flares triggered by environmental factors.

Clinical and histological features

Psoriasis vulgaris accounts for 90% of all skin presentations of psoriasis. It is characterized by raised, sharply demarcated, scaly, erythematous plaques (Nestle et al., 2009). The sites commonly involved in classic plaque psoriasis are the elbows, the knees, the scalp, the umbilicus and the lumbosacral area; less frequently, there may be an 'inverse' pattern of the lesions, localized in the folds. The nails are also a classic site of involvement. Other variants exist, such as guttate psoriasis or pustular variants (either generalized or localized).

Psoriasis may involve the joints, most often causing destructive asymmetric oligoarthritis. In addition, patients with psoriasis have considerable psychosocial disability and a major impairment in patients' quality of life but also have increased risks of metabolic syndrome, atherosclerotic cardiovascular diseases and depression (Gelfand et al., 2006; Nestle et al., 2009; Parisi et al., 2015).

Histologically, psoriasis is characterized by acanthosis, hyperkeratosis and parakeratosis of the epidermis, with an infiltrate of T cells and neutrophils. The epidermis may be spongiotic. Granular layer in the psoriatic skin is absent. In the dermis, the capillaries are elongated and tortuous, and there is an infiltrate of dendritic cells, macrophages and T cells.

Genetic factors

Psoriasis incidence is higher when there is a family history of psoriasis in first- and second-degree relatives. In addition, analysis of concordance rates among twins shows a higher concordance rate of psoriasis in monozygotic twins as compared to dizygotic twins, supporting a role for a genetic component of the disease.

Recent genome-wide association studies (GWAS) have identified ~ 50 genetic loci at genome-wide significance ($p < 5e-8$), together explaining ~ 22% of the genetic heritability. Major genes in which polymorphisms are associated with increased risks of psoriasis include genes involved in:

- Skin barrier formation, such as LCE3B/C/D or KLF4
- Innate immunity, such as IL-28RA, ELMO1, NOS2, CARD14, NFKBIA
- Interface innate/adaptive immunity, such as TRAF31P3, IL-12B, IL-23A, TYK2
- Adaptive immunity, such as IL-23R, STAT3, IRF4, RUNX3
- Metabolism, such as PRDX5 or B3GNT2 (Di Meglio et al., 2011).

Immunopathogenesis

Even though the main histologic feature in psoriasis is an increased keratinocytes proliferation, it is now believed that the disease is due to the pathogenic role of T cells and APCs.

Psoriasis is believed to be the result of pathogenic cross-talk between dendritic cells, partly activated through complexes of the antimicrobial peptide LL-37 cathelicidin and DNA in a TLR 9–dependent manner, and effector T cells (Nestle, FO. et al. N. Engl. J. Med. 361, 496–509 (2009)). After activation via surface TLRs, plasmacytoid dendritic cells produce IFN- α , activating myeloid dendritic cells in association with other key cytokines such as tumor

necrosis factor α (TNF- α), IFN- γ , interleukin-1 β (IL-1 β), and IL-6. Activated dendritic cells induce the differentiation of naïve T cells into T_H17 and T_H1 by secreting mediators such as IL-12 and IL-23. T-cells, recirculate, migrate into skin tissue where they secrete IL-17A, IL-17F, and IL-22 that activate keratinocytes, thus inducing proliferation of keratinocytes, production of antimicrobial peptides, proinflammatory cytokines (TNF- α , interleukin-1 β , and interleukin-6), chemokines (CXCL8, CXCL11 and CCL20), and S100 protein, leading to the vicious circle of inflammation (Nestle et al., 2009).

The antigens triggering psoriasis are still unknown, but it has been shown that after clinical resolution of psoriasis, there is a persistence of oligoclonal $\alpha\beta$ T cells in the skin, some of the TCR sequences of which are shared in between patients but not with controls, suggesting common that there are some common psoriasis antigens shared between patients (Matos et al., 2017).

Triggering factors

Multiple environmental or host-derived factors have been shown to trigger psoriasis: infections, in particular streptococcal and HIV infections can induce or aggravate psoriasis. Metabolic factors such as hypocalcemia can trigger the development of generalized pustular psoriasis. Multiple drugs, such as IFNs, b-blockers, antimalarials and lithium or a rapid taper of systemic corticosteroids can induce flares of psoriasis. Patients also commonly report psychogenic stress as a trigger of psoriasis. Alcohol consumption and smoking also have been associated with psoriasis.

A phenomenon known as Koebner phenomenon is the development of psoriatic lesions in injured body sites, either following a localized trauma such as a wound, or following more diffuse injuries such as sunburns or drug eruptions.

Psoriasis and microbiota

Psoriasis is a common chronic T-cell mediated disease thought to have a T_H17 signature. Compared to atopic dermatitis patients, there are not any 'microbial signature' in patients with psoriasis.

Still, skin and gut microbiome profiling in psoriasis patients have shown an increased representation of firmicutes such as *Streptococci* and *Staphylococci* in psoriasis lesions and in non lesional skin, while there could be a decrease in *Cutibacterium acnes* (formerly *P. acnes*), Chang HW et al, Microbiome 2018 (Chang et al., 2018; Gao et al., 2008; Tomi et al., 2005).

A specific form of psoriasis known as guttate psoriasis is associated with either a preceding or concurrent episode of tonsillitis with *Streptococci* (Owen et al., 2001); patients with the more common form, plaque psoriasis, have exacerbations of disease following Streptococcal throat infections (Gudjonsson et al., 2003). Peptidoglycan has been proposed as having an etiologic role in psoriasis, and polymorphisms in peptidoglycan recognition molecules have been implicated in the pathogenesis of psoriasis (Baker et al., 2006; Kainu et al., 2009). Additionally, fungal species have been isolated from psoriatic lesions, with *Malassezia* being implicated in scalp and facial psoriasis, whereas *Candida albicans* is frequently cultured from intertriginous psoriasis (Fry et al., 2013).

Additionally, pathways known to be driven by the microbiota are highly expressed in psoriatic lesions, such as IL-1 (Sims and Smith, 2010), IL-17 (Leonardi et al., 2012), IL-22 and IL-23 (Zheng et al., 2007). Going along with increase in various barrier defense related cytokines, psoriasis is also associated with an increase in AMPs (Harder and Schröder, 2005; Morizane and Gallo, 2012), which probably explains the decreased risk for infection in psoriatic patients (Ong et al., 2002).

Treatments

Psoriasis can be treated with topical or systemic therapies.

Topical treatments include corticosteroids, vitamin D3 analogues, retinoids, and anthralin.

Systemic treatments include photo(chemo)therapy, immunomodulators such as methotrexate, cyclosporine, retinoids, and more recently developed 'biologic therapies'. Efficacy of the latter further reinforces the major role of the immune system in the pathogenesis of psoriasis. Those include anti-TNF- α , anti-12/23, anti-IL-17A or IL-17 receptor, anti-IL-23. JAK inhibitors are also new players in the treatment of psoriasis, inhibiting the signal transduction downstream of cytokine-receptor.

5. Thesis aims

While it has been shown that immune responses to skin bacterial commensals are important for protecting the skin against further infections, very little is known about the role of adaptive immune responses to the skin microbiota during the development of inflammatory skin disorders. As T_H17 responses are crucial in the development of both psoriasis and for protection against fungal infection, we aimed to decipher the role of adaptive immune responses against fungal commensals in the pathogenesis of psoriasiform skin disorder in mice.

Material and Methods

Mice

C57BL/6 specific pathogen free (SPF) mice were purchased from Taconic Farms. Germ-free C57BL/6 mice were bred at Taconic Farms and maintained in the NIAID gnotobiotic facility. C57BL/6NTac-[KO]Abb/B2m, B6.SJL-Cd45a(Ly5a)Nai-[KO]RAG1 and C57BL/6-[TgH]EGFP:Foxp3 mice (FoxP3 reporter mice) were obtained through the NIAID-Taconic exchange program. B6.FVB-Tg(CD207-Dta)312Dhka/J (Lan-DTA) mice were obtained from Dr. Kaplan (University of Minnesota). Irf4fl/fl x Cd11cre+ mice were obtained by breeding B6.129S1-Irf4tm1Rdf/J (Irf4fl/fl) mice with B6.Cg-Tg(Itgax-Cre)1-1Reiz/J (Itgax-Cre) mice (both strains from the Jackson Laboratory). CARD9^{-/-} mice and Clec7^{-/-} mice were obtained from Dr. Lionakis (NIH, NIAID, Laboratory of Clinical Infectious Diseases, Fungal Pathogenesis Unit). All mice were bred and maintained under pathogen-free conditions at an American Association for the Accreditation of Laboratory Animal Care (AAALAC)-accredited animal facility at the NIAID and housed in accordance with the procedures outlined in the Guide for the Care and Use of Laboratory Animals. All experiments were performed at the NIAID under an animal study proposal approved by the NIAID Animal Care and Use Committee. Gender- and age-matched mice between 6 and 12 weeks of age were used for each experiment. When possible, preliminary experiments were performed to determine requirements for sample size, taking into account resources available and ethical, reductionist animal use. In general, each mouse of the different experimental groups is reported. Exclusion criteria such as inadequate staining or low cell yield due to technical problems were predetermined. Animals were assigned randomly to experimental groups.

Microbes

Unless stated otherwise, we used a *Candida albicans* strain that was isolated from the ear pinnae of IL22^{-/-} mouse. We also used the reference strain of *C. albicans* 5314 - ATCC® MYA-2876™ and another *C. albicans* isolated from human psoriatic skin (referred to as human *C. albicans*). Yeast locked *C. albicans* (hgc1Δ) and its control strain hgc1Δ+HGC1+ are kind gift

from Dr. M Lionakis. *C. albicans* strains were grown in YPD medium at 30°C with shaking for 18 h before the association.

M. furfur and *T. mentagrophytes* were purchased from ATCC (*M. furfur* ATCC® 14521™ and *T. mentagrophytes* ATCC® 18748™). *M. furfur* strain was cultured on CHROMagar™ *Malassezia* at 30°C. *T. mentagrophytes* was cultured on potato dextrose agar.

Conditioned medium

C. albicans was cultured aerobically in RPMI medium for 48 h at 37°C. Fungal culture was centrifugated at 3,200 g for 20 min. Supernatant was then filtered through 0.22 µm filter (Steriflip, Millipore-Sigma, MA). Sterile filtered supernatants were used immediately or frozen at -80°C for further experiments.

Topical association

For topical association of commensals, each mouse was associated with fungi by applying fungal suspension (approximately 10⁹/ml) across both sides of the ear surface using a sterile cotton swab. Application of fungal suspension was repeated every other day during 8 days. In experiments involving topical application of various fungal species or strains, cultures were normalized using OD600 to achieve similar fungal density (approximately 10⁹ c.f.u. per ml).

For some experiments, mice were infected intradermally in the ear pinnae with 10⁷ c.f.u. of *C. albicans*.

Imiquimod treatment

Mice between 6 and 12 weeks of age were treated daily for 5 days on each ear pinnae with 10 mg of 5% Imiquimod (IMQ) cream (Aldara Cream 5%; 3M Health Care, UK). Ear thickness was

measured before the first application, then daily during IMQ topical application and the day after the last topical application of IMQ with digimatic caliper (Mitutoyo, Japan).

In vivo treatment with monoclonal antibodies

For CD8⁺, CD4⁺, and $\gamma\delta$ T-cell depletion, mice were treated intraperitoneally (i.p.) with 0.5 mg of anti-CD8 antibody (clone 2.43), anti-CD4 antibody (clone GK1.5), anti- $\gamma\delta$ antibody (clone UC7-13D5) respectively, or rat IgG2b isotype control antibody (clone LTF-2, BioXCell) every other day.

Depending on the experiment (see text), the antibodies were started at day 13 after the first topical application of *C. albicans* or the day before re-challenge with *C. albicans* or control medium.

Tissue processing

Mice were euthanized 14 days after starting topical application of *C. albicans* on the ear, and/or the day after the last topical application of imiquimod. Single cell suspension from the skin draining lymph nodes or the ear pinnae were isolated as previously described (Naik et al., 2012). In brief, ear pinnae were digested in RPMI containing 100 U/ml penicillin, 100 μ g/ml streptomycin, 55 μ M β -mercaptoethanol, 20 μ M HEPES (HyClone), and 0.25 mg/ml Liberase TL (Roche Diagnostic Corp., Basel, Switzerland), or 2.5mg/ml collagenase D (Roche) and incubated for 1 hours 45 minutes at 37°C and 5% CO₂. Digested skin sheets were homogenized using the Medicon/Medimachine tissue homogenizer system (Becton Dickinson, NJ).

Histology

Mice were euthanized 19 days after starting topical application of *C. albicans* on the ear, and/or the day after the last topical application of imiquimod. The ears from each mouse were removed and fixed in phosphate buffered saline (PBS) containing 10% formalin. Paraffin-embedded sections were cut at 0.5 mm, stained with haematoxylin and eosin and examined histologically. Periodic Acid-Schiff (PAS) staining was performed with ear skin following 10% formalin fixation. Histological score was performed as reported previously: briefly, inflammation, neutrophil, mononuclear cells, edema, and epithelial hyperplasia were graded

from 0 to 4 as follows: 0, no response; 1, minimal response; 2, mild response; 3, moderate response; and 4, marked response (Nakajima et al., 2010).

Dendritic cell and T cell co-culture assay

CD45⁺ CD90.2⁺ CD4⁺ FoxP3⁻ CCR6⁺ effector T cells (>95% purity) were sorted by flow cytometry from the cervical lymph nodes of FoxP3-eGFP reporter mice 2 weeks after topical association with *C. albicans* following 5 days application of IMQ strain using a FACSAria cell sorter. For splenic dendritic cell (SpDC) purification, single-cell suspensions from the spleen of congenic wild-type, *Abb2/2* mice were magnetically enriched for CD11c⁺ cells by positive selection using CD11c MicroBeads and MACS separation columns (Miltenyi Biotec, Bergisch Gladbach, Germany). Purified SpDCs and CD4⁺ T cells were co-cultured at a 10:1 ratio (5 x 10³ CD4⁺ T cells) in a 96-well U-bottom plate in complete medium for 16 h at 37 °C in 5% CO₂. Brefeldin A (GolgiPlug) was added for the final 6h of culture. SpDC were previously incubated for 2 h with or without heat-killed *C. albicans* (*C. albicans*:SpDC ratio, 500:1) or *C. albicans* conditioned medium and washed before co-culture with CD4⁺ T cells. CD4⁺ T cell cytokine production following co-culture was assessed by flow cytometry after intracellular cytokine staining using the following antibodies: anti-CD4, anti-CD8b, anti-CD90.2, anti-TCR β , anti-IFN γ and anti-IL-17A.

Confocal microscopy of ear pinnae

Confocal microscopy was performed as previously described (Naik et al., 2015a). Ear pinnae were split with forceps, fixed in 1% paraformaldehyde solution (Electron Microscopy Sciences) overnight at 4°C and blocked in 1% BSA, 0.25% Triton X blocking buffer for 2 hours at room temperature. Tissues were stained with anti-H3 citrullinated histone monoclonal primary antibody (Ab-cam, donkey-anti-rabbit Alexa Fluor 555 (Life Technologies, CA) and with 4', 6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich). Ear pinnae images were captured on a Leica M205 stereomicroscope. Images were analyzed using Imaris Bitplane software.

Phenotypic analysis

Murine single-cell suspensions were surface stained with the indicated antibodies for 20-30min at 4°C. For intracellular staining, cells were fixed for 30 min with the eBioscience Fixation/Permeabilization kit as described by the manufacturer and stained intracellularly as recommended for at least 1hr. Antibodies for flow cytometry were purchased from BD Biosciences, BioLegend, or eBiosciences. Antibodies used were conjugated to FITC, AF488, PE, PerCP-Cy5.5, PCP-eFluor 710, PeCy7, Alexa Fluor 780, Pacific Blue, BV605, BV650, BV 510, BV785, eFluor 450, APC, Alexa Fluor 647, Pe Texas Red, PE-CF594. DAPI or Live/Dead fixable stain (Life Technologies) was used to exclude dead cells in all experiments.

Flow cytometric data acquisition were acquired on a LSR II or LSR Fortessa (BD Biosciences) and analyzed using FlowJo software (Tree Star).

Data and statistical analysis

Data are presented as mean \pm standard error of the mean or mean \pm standard deviation. Group sizes were determined based on the results of preliminary experiments. No statistical method was used to predetermine sample size. Mice were assigned at random to groups. Mouse studies were not performed in a blinded fashion. Generally, each mouse of the different experimental groups is reported. Statistical significance was determined with the two-tailed unpaired Student's t-test, under the untested assumption of normality. Within each group there was an estimate of variation, and the variance between groups was similar.

All statistical analysis was calculated using Prism software (GraphPad, CA). Differences were considered to be statistically significant when $P < 0.05$.

Fungal skin colonization drives a polarized IL-17A signature and exacerbates psoriasiform skin inflammation

How commensal fungi are sensed by the skin immune system has not been addressed. Using our previously described model of skin association (Naik et al., 2012), we topically applied highly prevalent human skin fungi including *Candida albicans* (*C. albicans*), *Malassezia furfur* (*M. furfur*) and *Trychophyton mentagrophytes* (*T. mentagrophytes*). Following *C. albicans* association, the fungi stably colonize the skin and are maintained at low colony forming units (CFU) that are maintained months post association (**Suppl. Fig. 1a**). Other fungi were also still detectable on the skin surface up to 2 months post association (data not shown). As we previously showed for bacterial commensals, topical application of a commensal fungi was not associated with tissue inflammation as evidenced by a lack of inflammatory cell recruitment, erythema, hyperkeratosis, or thickening of the skin (**Suppl Fig. 1b, 1c and 1d**). On the other hand, all three fungi promoted the accumulation of Th17 cells within the skin (but no other T helper subsets). *Candida albicans* also induced a significant accumulation of IL-17A producing CD8⁺T cells (Tc17) and *M. furfur*, the accumulation of IL-17A-producing $\gamma\delta$ T cells within the skin compartment (**Fig. 1a, Fig. 1b and Suppl. Fig. 1d and 1e**). This was in striking contrast with intradermal injection of *C. albicans*, which led to massive skin inflammation and a mixed polarized response dominated by Th1 responses (**Suppl. Fig. 1f**). Thus, cutaneous colonization with commensal skin fungi is associated with the highly polarized non-inflammatory type 17 skin responses.

Based on the clinical association between IL-17A and the etiology of psoriasis, we next assessed to which extent the pre-existence of commensal fungi-induced type 17 responses could contribute to skin inflammation. To this end, we utilized the experimental model of psoriasis associated with the topical application of imiquimod, a TLR7 (and TLR8 in humans) agonist. This regimen promotes a psoriasis-like skin inflammation, mediated by an IL-23/IL-17 axis (van der Fits et al., 2009). Using this model, we found that pre-exposure to each of the 3 skin fungi significantly exacerbated tissue inflammation compared to IMQ alone (**Fig. 1c**).

To gain mechanistic insights into this phenomenon, we next focused on *C. albicans* because of its genetic tractability. Increased tissue inflammation caused by *C. albicans* colonization prior to IMQ treatment was associated with enhanced hyperplastic epidermis, acanthosis, spongiosis, hyperkeratosis and enhanced inflammatory infiltrate (**Fig. 1d**) reflected by higher histology score in mice treated with *C. albicans*/IMQ compared to IMQ alone (**Suppl Fig. 1g**). Enhanced inflammation was not associated with significant changes in skin fungal load (**Suppl Fig. 1a**). Enhanced inflammation was associated with increased frequencies and absolute numbers of ROR γ t and IL-17A-producing CD4⁺ T cells (but not T_{reg} cells) in the skin of *C. albicans*/IMQ treated mice compared to all the other groups (**Fig. 1e, 1f and Suppl Fig. 1h**). The proinflammatory impact of *C. albicans* and Th17 increase were conserved among several strains of the fungi isolated from human or mouse skin (**Suppl Fig. 1i**).

We next addressed if the impact of *C. albicans* was direct or dependent on *C. albicans*-induced changes in the endogenous microbial community. As previously shown, mice raised in absence of live microbes (germ-free (GF)) have reduced number of IL-17A expressing T cells within the skin (Naik et al., 2015b). In GF mice, *C. albicans* alone was able to induce a small but significant increase in Th17. Similarly to SPF mice, the addition of *C. albicans* significantly enhanced tissue inflammation and Th17 response induced by IMQ (**Fig. 1g**). Thus, the impact of *C. albicans* on tissue inflammation and Th17 cells accumulation is dominant and independent of other members of the cutaneous microbiota.

While *C. albicans* is a normal constituent of the skin microbiome (Roth and James, 1988), this microbe can also cause active infection. As such, we next assessed if the ability of the microbe to promote inflammation resulted from an active infection rather than colonization. *C. albicans* is a dimorphic fungus, with a yeast form able to colonize the stratum corneum, and a pathogenic hyphal form able to invade the dermis and systemic organs (Gow et al., 2012). Periodic Acid Schiff (PAS) staining and electron microscopy imaging revealed that *C. albicans* remained in its yeast form localized on the stratum corneum in the context of IMQ induced inflammation (**Fig. 1h and Suppl Fig. 1i**). Long term colonization (up to 2 months) was also able to induce worsened inflammation after imiquimod treatment (**Fig. 1h**). Further, the yeast-locked *hgc1* Δ strain of *C. albicans*, which cannot make the transition from yeast-to-hyphae form, was also able to exacerbate IMQ-induced psoriasis-like dermatitis (**Fig. 1j**). Thus, *C.*

albicans exacerbated IMQ-induced skin inflammation as a yeast and the effect was not associated with active infection.

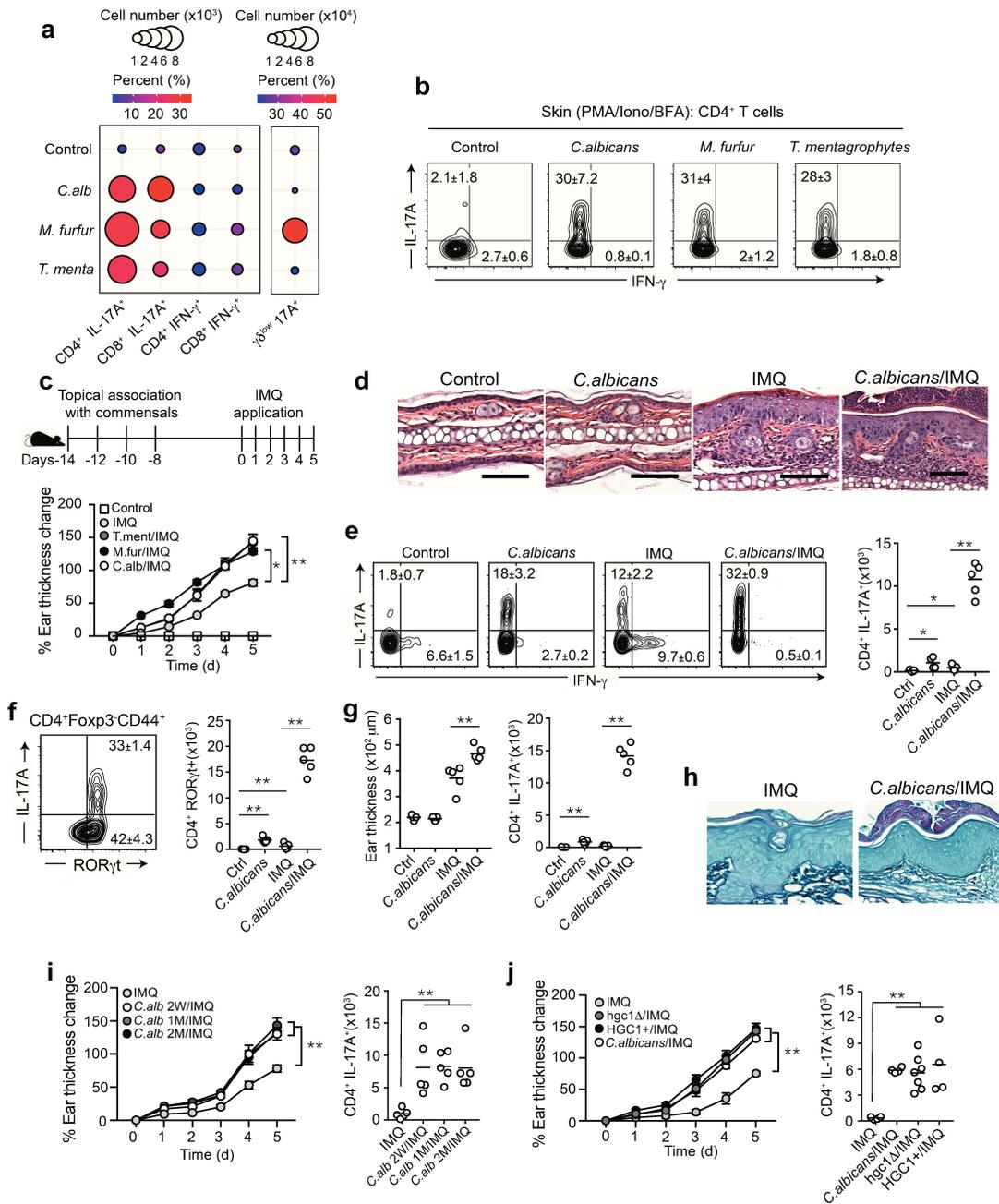


Figure 1

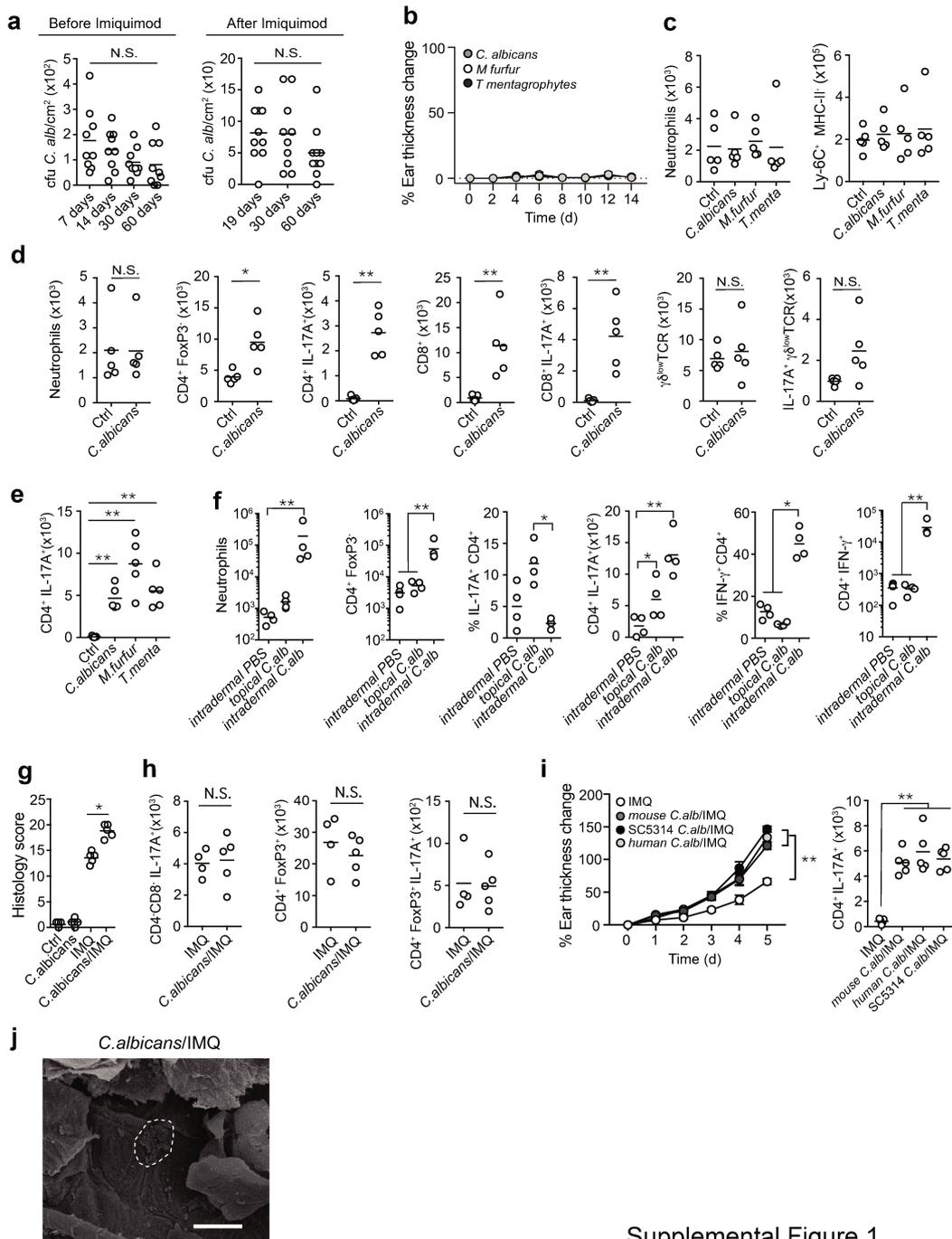
Figure 1. Skin colonized *C. albicans* exacerbates the inflammation of psoriasis-like dermatitis.

(a) Wild type mice under SPF condition were topically associated with control media or various commensal fungi every other day 4 times: *C. albicans* (*C. alb*), *Malassezia furfur* (*M. furfur*), *T. mentagrophytes* (*T. menta*). Mean of absolute numbers (represented by the size of the circles)

and frequencies (represented by the color of the circles) of live IL-17A-producing CD45⁺ CD90.2⁺ IL-17A- and IFN γ -producing CD45⁺ CD90.2⁺ TCR β ⁺ CD4⁺ and CD8⁺ cells (left) and $\gamma\delta$ TCR^{low} cells (right). Data were collected after in vitro restimulation with PMA and ionomycin in the presence of BFA. **(b)** Representative flow plots showing frequencies (mean \pm SEM) of IL-17A- and IFN γ -producing CD45⁺ CD90.2⁺ TCR β ⁺ CD4⁺ from the skin of control, *C. albicans*, *Malassezia furfur*, and *T. mentagrophytes* associated mice respectively. **(c)** Wild type mice under SPF condition were topically associated with control media or various commensal fungi every other day 4 times: *C. albicans* (*C. alb*), *Malassezia furfur* (*M. furfur*), *T. mentagrophytes* (*T. menta*) or media. Fourteen days after the initial commensal association, mice were treated with imiquimod (IMQ) cream 5 consecutive days or not (Control). Time course of percentage of ear thickness change relative to baseline at day 0. **(d)** Hematoxylin-and-eosin staining of ear skin from naïve (control), IMQ-treated (IMQ), *C. albicans*-associated (*C. albicans*), and IMQ-treated *C. albicans*-associated (*C. albicans*/IMQ) wild type mice treated for 5 days as in **c**. Scale bar; 100 μ m. **(e)** Representative flow plots showing frequencies of IL-17A⁺ and IFN- γ ⁺ CD4⁺FoxP3⁻ cells in the skin from control, IMQ, *C. albicans*, and *C. albicans*/IMQ mice under SPF condition. Absolute numbers of live CD45⁺ CD90.2⁺ TCR- β ⁺ CD4⁺ FoxP3⁻ IL-17A⁺ cells in the skin 5 days after IMQ treatment.

(f) Representative flow plot showing the frequency of IL-17A⁺ and ROR γ t⁺ in CD44⁺ CD4⁺ FoxP3⁻ effector T cells in the skin of *C. albicans*/IMQ treated mice under SPF condition. Absolute number of ROR γ t⁺ cells from CD44⁺ CD4⁺ FoxP3⁻ effector T cells in the ear skin from Control, Imiquimod, *C. albicans*, and *C. albicans*/IMQ mice under SPF condition. **(g)** Ear thickness and absolute numbers of CD45⁺ CD90.2⁺ TCR- β ⁺ CD4⁺ FoxP3⁻ IL-17A⁺ cells from control, IMQ, *C. albicans*, and *C. albicans*/IMQ mice under germ-free condition. **(h)** PAS staining of ear skin from IMQ and *C. albicans*/IMQ mice on day 5 of IMQ treatment. **(i)** Time course of percentage of ear thickness change relative to baseline at day 0 after start of Imiquimod in IMQ mice and in mice previously associated with *C. albicans* 2 weeks, 1 month, and 2 months ago respectively. **(j)** Ear-skin thickness change and absolute numbers of CD45⁺ CD90.2⁺ TCR- β ⁺ CD4⁺ FoxP3⁻ IL-17A⁺ cells of mice treated with imiquimod after no association (IMQ) or association with yeast-locked *C. albicans* (*hgc1* Δ /IMQ), control strain of yeast locked *hgc1* Δ +HGC1+ (HGC1+/IMQ) or our standard strain (*C. albicans*/IMQ).

Results are representative 2-3 independent experiments. **P* < 0.05, ***P* < 0.01.



Supplemental Figure 1

Supplemental Figure 1.

(a) Colony forming unit numbers of *C. albicans* per ear 7, 14, 30 and 60 days respectively after topical association with *C. albicans* (left) and 19, 30 and 60 days after first association with *C. albicans* in Imiquimod treated mice (right). (b) Time course of percentage of ear thickness

change relative to baseline at day 0 from *C. albicans* (*C. alb*), *Malassezia furfur* (*M. furfur*) and *T. mentagrophytes* (*T. menta*) associated mice. **(c)** Absolute numbers of neutrophils and MHC-II⁻ inflammatory monocytes in the skin of Control, *C. albicans* (*C. alb*), *Malassezia furfur* (*M. furfur*) and *T. mentagrophytes* (*T. menta*). **(d)** Absolute number of live neutrophils, CD4⁺ FoxP3⁻, CD4⁺ FoxP3⁻ IL-17A⁺, CD8⁺, CD8⁺ IL-17A⁺, $\gamma\delta$ TCR^{low} and IL-17A⁺ $\gamma\delta$ TCR^{low} cells in the ears of control (Ctrl) and *C. albicans* associated mice. **(e)** Absolute numbers of IL-17A-producing CD45⁺ CD90.2⁺ TCR β ⁺ CD4⁺ FoxP3⁻ from the skin of control, *C. albicans*, *Malassezia furfur*, and *T. mentagrophytes* associated mice respectively. **(f)** Absolute number of neutrophils, CD4⁺ FoxP3⁻, CD4⁺ FoxP3⁻ IL-17A⁺, CD4⁺ FoxP3⁻ IFN- γ ⁺, and percentages of CD4⁺ FoxP3⁻ IL-17A⁺ and CD4⁺ FoxP3⁻ IFN- γ ⁺ cells 7 days after intradermal PBS, *C. albicans* topical association or intradermal *C. albicans* in the ears. **(g)** Total histology score evaluated by H&E staining section of the ear of control, IMQ, *C. albicans*, and *C. albicans*/IMQ mice under SPF condition. **(h)** Absolute numbers of CD4⁻ CD8⁻ IL-17A⁺, CD4⁺ FoxP3⁻, and CD4⁺ FoxP3⁻ IL-17A⁺ in IMQ and *C. albicans*/IMQ mice under SPF condition. **(i)** Time course of percentage of ear thickness change relative to baseline at day 0 and absolute number of live CD45⁺ CD90.2⁺ TCR- β ⁺ CD4⁺ FoxP3⁻ IL-17A⁺ cells from imiquimod-treated mice, after association or not with mouse *C. albicans* (mouse *C. alb*/IMQ), human *C. albicans* (human *C. alb*/IMQ) and reference strain of *C. albicans* (SC5314 *C. alb*/IMQ). **(j)** Ear surface image of representative *C. albicans*/IMQ mouse obtained by scanning electron microscope. Dashed white line circle indicate the colonization of yeast type fungi on the surface of the ear. Scale bar; 100 μ m

C. albicans promotes tissue inflammation in a T_H17 dependent manner

As previously shown, *C. albicans* promoted the selective accumulation of both CD4 and CD8 IL-17A expressing T cells (**Fig. 1a** and **1e**). Nonetheless, because V γ 4⁺ TCR $\gamma\delta$ ^{low} have been shown to contribute to IMQ-induced pathology, we assessed the potential contribution of this subset to enhanced pathology (Gray et al., 2013). Interestingly, there was no difference in frequencies and absolute numbers of IL-17A⁺ TCR $\gamma\delta$ ^{low} cells in mice associated with *C. albicans* prior to IMQ (**Fig. 2a**). Additionally, increased inflammation was still observed in mice lacking V γ 4⁺ TCR $\gamma\delta$ ⁺ cells (**Fig. 2b**). Further, depletion of CD8⁺T cells had no impact on tissue inflammation (**Fig. 2c**) supporting the idea that under these settings, Th17 may be the main mediator of enhanced pathology.

To further evaluate the direct contribution of Th17 cells in our model, we used mice depleted of ROR γ t in $\alpha\beta$ T cells (LCK^{cre}^{+/+}xROR γ t^{fl/fl} mice). As expected, there was no significant change in $\gamma\delta$ T cells in these mice (**Fig. 2d**). Notably, *C. albicans* did not enhance tissue inflammation (and neutrophil recruitment) provoked by IMQ in LCK^{cre}^{+/+}xROR γ t^{fl/fl} mice compared to controls (**Fig. 2d**). These results highlighted a non-redundant role for Th17 cells in the promotion of skin inflammation induced by fungi colonization.

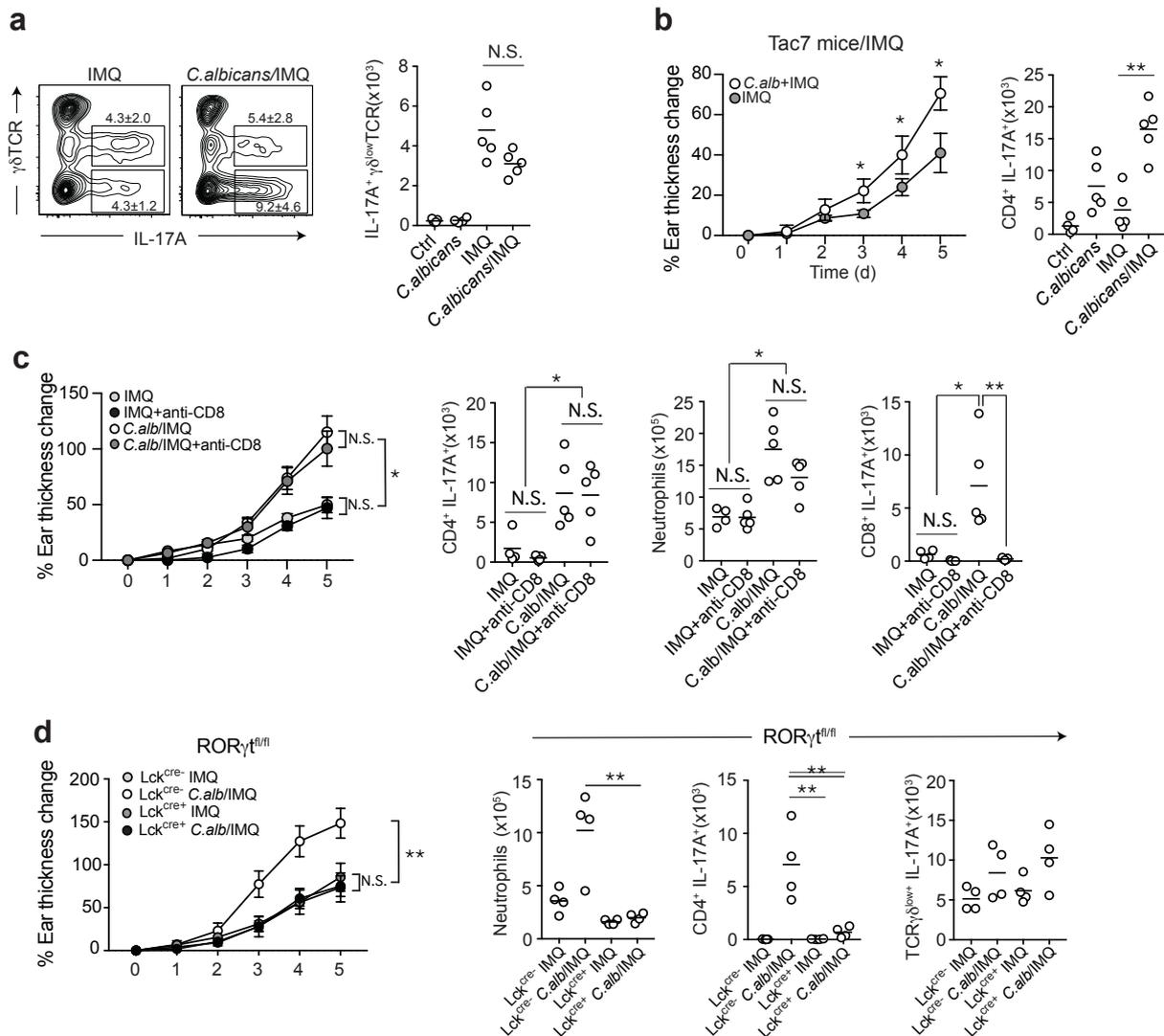


Figure 2

Figure 2. *C. albicans* colonization selectively promotes IL-17A production from CD4⁺ effector T cells.

(a) Representative flow plots showing frequency of IL-17A⁺ $\gamma\delta$ TCR^{low} and IL-17A⁺ $\gamma\delta$ TCR⁺ cells in the skin of IMQ and *C. albicans*/IMQ mice (left). Absolute numbers of IL-17A⁺ $\gamma\delta$ TCR^{low} cells in the skin of Control, Imiquimod, *C. albicans*, and *C. albicans*/IMQ mice (right). (b) Time course of the percentage of ear-skin thickness change of IMQ and *C. albicans*/IMQ in B6.SJL-Cd45a(Ly5a)/Nai (Tac 7) mice (left). Absolute number of IL-17A⁺ CD4⁺ FoxP3⁻ effector T cells in the skin of Control, Imiquimod, *C. albicans*, and *C. albicans*/IMQ Tac 7 mice (right). (c) Ear-skin thickness change of IMQ or *C. albicans*/IMQ mice after depletion of CD8 T cells with anti-CD8 monoclonal antibody (left). Absolute numbers of live CD4⁺ FoxP3⁻ IL-17A⁺ T effector cells, neutrophils, and CD8⁺ IL-17A⁺ T effector cells (right) of IMQ and *C. albicans*/IMQ cells treated

or not with CD8 depleting monoclonal antibody. **(d)** Time course of the percentage of ear-skin thickness change of IMQ treated and *C. albicans*/IMQ in *Lck^{cre+}ROR γ t^{flox/flox}* mice versus in *Lck^{cre-}ROR γ t^{flox/flox}* mice (left). Absolute numbers of live neutrophils, live CD4⁺ FoxP3⁻ IL-17A⁺ T effector cells and IL-17A⁺ $\gamma\delta$ TCR^{low} cells (right) of IMQ treated and *C. albicans*/IMQ in *Lck^{cre+}ROR γ t^{flox/flox}* mice versus in *Lck^{cre-}ROR γ t^{flox/flox}* mice (right).

Results are representative of 2-3 independent experiments. **P* < 0.05, ***P* < 0.01.

C. albicans colonization promotes neutrophil infiltration and NETs formation in the context of skin inflammation

Among numerous other functions, IL-17 can have a profound effect of neutrophil generation recruitment and function (Ouyang et al., 2008). Neutrophils also contribute to the etiology of psoriasis and lesions are characterized by neutrophils infiltrates, with spongiform pustules in the stratum spinosum and Munro's micro abscesses in the stratum corneum (Schön et al.). As assessed via flow cytometry, pre-exposure to *C. albicans* in IMQ treated mice significantly increased neutrophil and inflammatory monocyte infiltrate compared to IMQ alone (**Fig. 3a** and **3b**). On the other hand, no differences were detected in the number of eosinophils, basophils and mast cells compared to mice treated with IMQ alone (**Suppl. Fig. 3a**).

Electron microscopy imaging of the candida-exacerbated inflamed skin revealed web-like structures compatible with neutrophil extracellular traps (NETs) at the surface of the epidermis in *C. albicans*/IMQ mice (**Fig. 3c**). NETosis is a defined process of cell death where neutrophils extrude DNA which extends into web-like threads (Papayannopoulos, 2017). Of particular relevance to our observation, NETs have been associated with response to large pathogens and in particular *C. albicans* (Branzk et al., 2014; Byrd et al., 2013; Urban et al., 2006).

NETosis is typically associated with activation of peptidylarginine deiminase 4 (PAD4) – a nuclear enzyme that is primarily expressed in neutrophils – which leads to histone H3 citrullination and chromatin decondensation (Wang et al., 2004). To confirm the presence of NETs, we quantified histone H3 citrullination using confocal microscopy. Imaging of whole skin pinnae revealed that imiquimod (but not *C. albicans* alone) induced histone H3 citrullination. H3 citrullination was significantly increased in mice associated with *C. albicans* prior to IMQ treatment compared to IMQ alone (**Fig. 3d, 3e** and **Suppl Fig. 3b and 3c**).

Of relevance to our observation, the amount of NETs in the peripheral blood of psoriasis patients correlated with psoriasis disease severity (Hu et al., 2016; Lin et al., 2011). To test the

possibility that enhanced NETosis may contribute to the enhanced inflammation caused by *C. albicans* pre-exposure, mice were treated with DNase, an approach previously used to suppress NETs formation (Brinkmann et al., 2004b). DNase treatment had no significant impact on the pathogenesis of IMQ alone. On the other hand, treatment with DNase significantly reduced inflammation induced by *C. albicans* prior to IMQ (**Fig. 3f**).

Altogether, these results support the idea that in the context of inflammatory settings, previous colonization of the skin with a commensal fungus can promote aberrant neutrophil activation/death thereby exacerbating tissue inflammation.

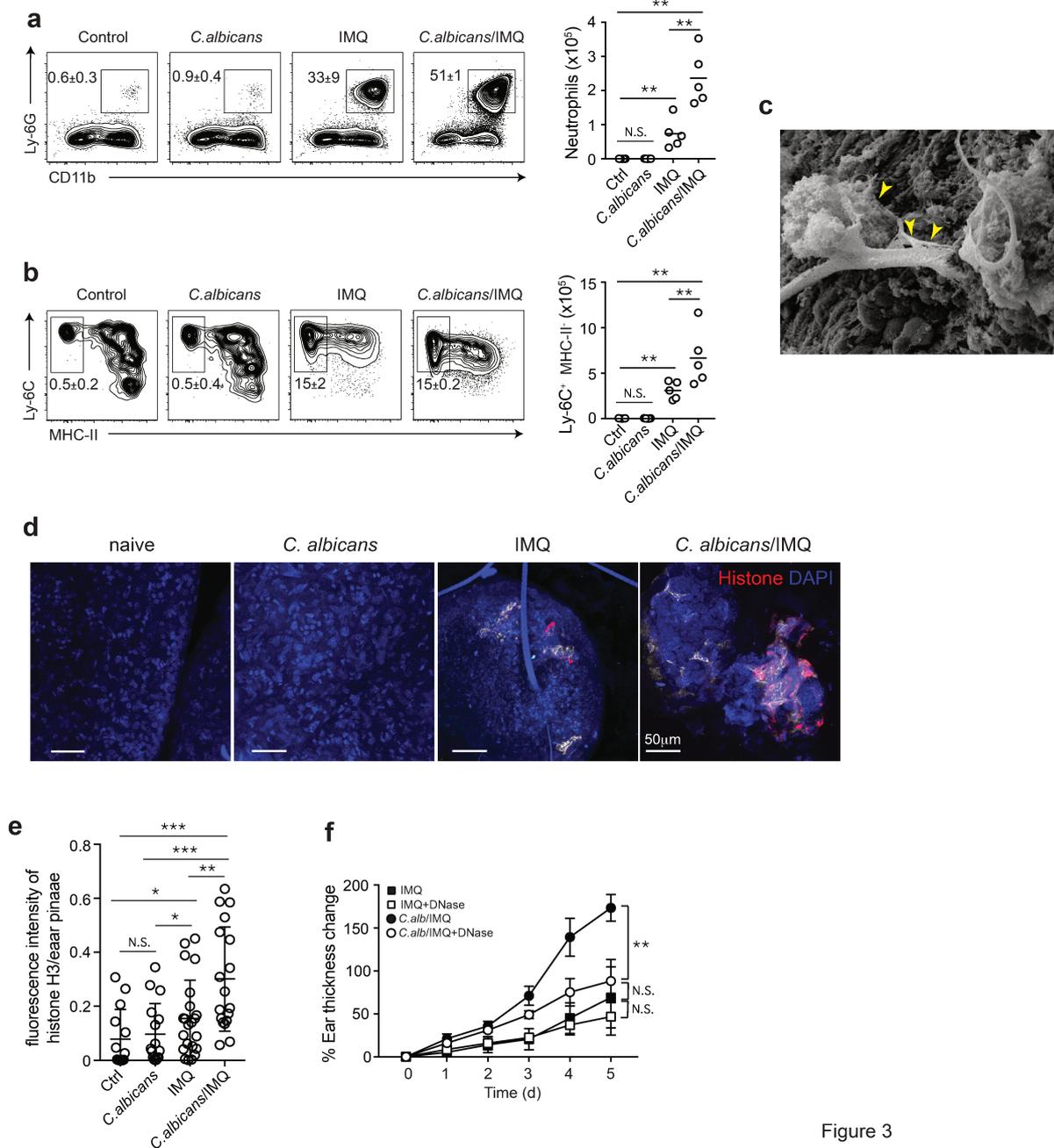


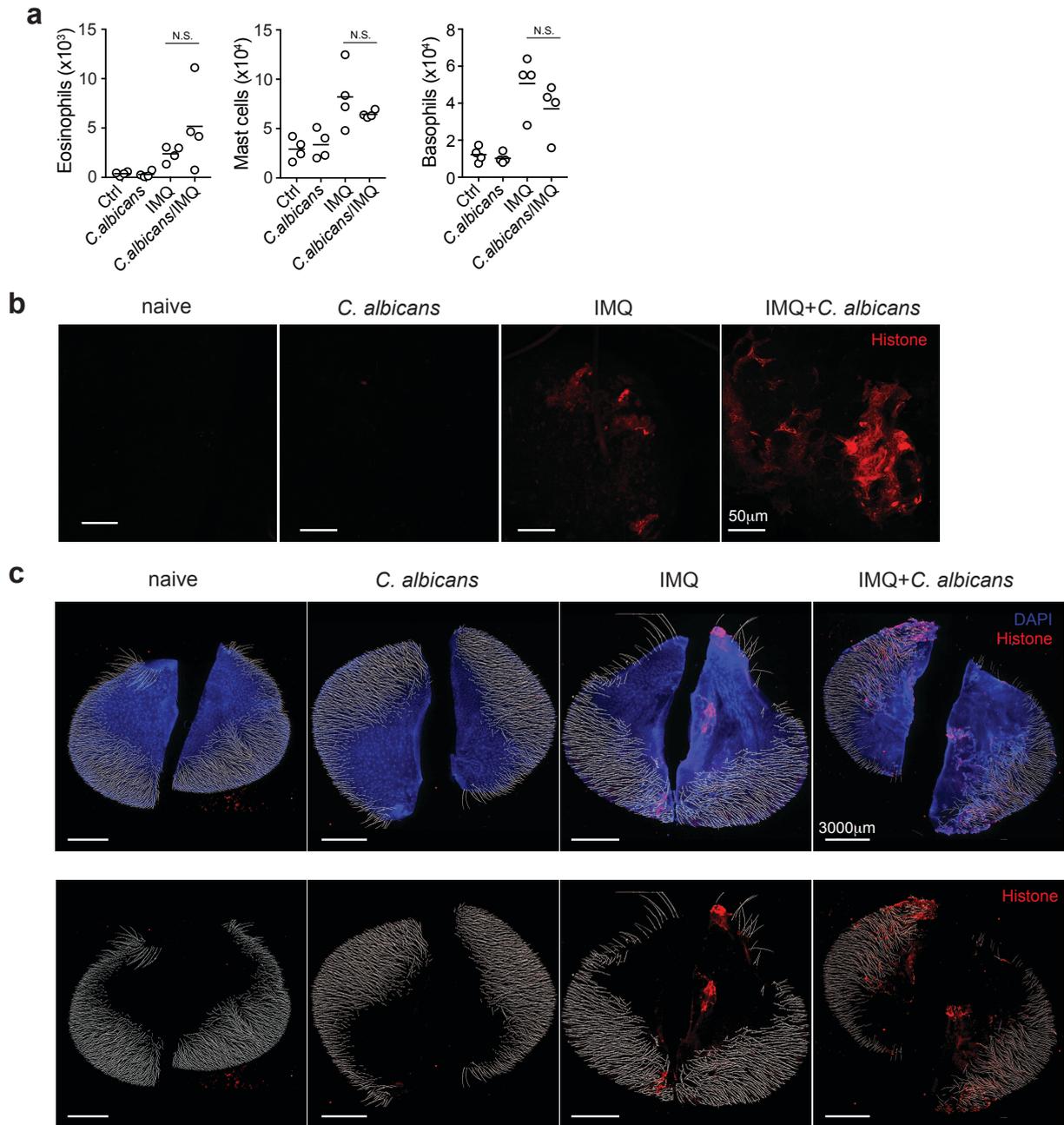
Figure 3

Figure 3. *C. albicans* colonization promotes neutrophil infiltration and NETosis formation.

(a) Representative flow plots showing frequency of neutrophils in the skin of Control, Imiquimod, *C. albicans*, and *C. albicans*/IMQ mice (left). Absolute numbers of neutrophils in the skin of Control, Imiquimod, *C. albicans*, and *C. albicans*/IMQ mice (right). (b) Representative flow plots showing frequency of inflammatory monocytes in the skin of Control, Imiquimod, *C. albicans*, and *C. albicans*/IMQ mice (left). Absolute numbers of MHC-II⁺ inflammatory monocytes in the skin of Control, Imiquimod, *C. albicans*, and *C. albicans*/IMQ

mice (right). (c) Ear surface image of representative *C. albicans*/IMQ mouse obtained by scanning electron microscope. Scale bar: 100 μ m. Arrows indicate web like structure on the ear. (d) Representative imaging of high magnification of whole mount ear section from Control, Imiquimod, *C. albicans*, and *C. albicans*/IMQ mice showing DAPI and citrullinated histone H3⁺ cells. Scale bars: 50 μ m. (e) NETs frequency evaluated per fluorescence intensity of citrullinated histone H3/ear from Control, Imiquimod, *C. albicans*, and *C. albicans*/IMQ mice. (f) Time course of percentage of ear thickness change relative to baseline at day 0 from IMQ, IMQ/DNase, *C. albicans*/IMQ, and *C. albicans*/IMQ/DNase (DNase 1,000IU twice daily intraperitoneal injection from day -1 to day 4 of IMQ treatment).

Results are representative of 2-3 independent experiments. N.S. not statistically significant, NS: not statistically significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.



Supplemental Figure 3

Supplemental Figure 3.

(a) Absolute number of eosinophils, mast cells and basophils in the ears of Control, *C. albicans*, Imiquimod, and *C. albicans*/IMQ mice. (b) Representative imaging of high magnification of whole mount ear section from Control, Imiquimod, *C. albicans*, and *C. albicans*/IMQ mice showing DAPI and citrullinated histone H3⁺ cells. Scale bars: 50 μ m. (c) Representative imaging of whole ear from Control, Imiquimod, *C. albicans*, and *C. albicans*/IMQ mice, showing NETs, as demonstrated by positive citrullinated histone H3⁺ staining. Scale bars: 3000 μ m.

Langerhans cells are indispensable to induce *C. albicans* derived exacerbation of the inflammation

In a murine model of skin infection, the yeast form of *C. albicans* induced Th17 cell responses in a Langerhans cells dependent manner (Kashem et al., 2015). In agreement with this observation, skin association with *C. albicans* did not promote Th17 responses in the skin of Langerhans deficient mice (Langerin-DTA (Kaplan et al.)) as opposed to WT mice (**suppl. Fig. 4a**). Further, *C. albicans* was no longer able to enhance IMQ induced inflammation, neutrophil recruitment or Th17 accumulation (**Fig. 4a, 4b and Suppl. Fig. 4b**). In contrast *C. albicans* still promoted inflammation and cellular infiltrates in IMQ treated mice lacking dermal dendritic cells (BATF3^{-/-} and CD11c^{cre+}IRF4^{flox/flox}) (**Fig. 4a, 4b and Suppl. Fig. 4b**).

The fungi-responsive C-type lectin receptors (CLRs) play a central role in fungal detection during infection (Marakalala et al., 2013; Taylor et al., 2007). Notably, the CLR Dectin-1 (encoded by *Clec7a* gene in mice) recognizes *C. albicans* yeast cells but not hyphae through binding to surface β -glucan (Kashem et al., 2015). While, *C. albicans* association did not alter the number and frequency of three main skin DC subsets (**Suppl. Fig. 4c**), the intensity of Dectin-1 expression was significantly increased in LC (but not other subsets) from IMQ and *C. albicans*/IMQ mice (**Fig. 4c**). Further, enhanced inflammation associated with pre-exposure to *C. albicans* was abolished in mice deficient in Dectin 1 (*Clec7a*^{-/-}) and in mice deficient in its downstream adaptor (*CARD9*^{-/-}) mice (**Fig. 4d and 4e**).

These results support the idea that a Langerhans cells/ Dectin-1 axis contributes to the priming of Th17 cells in response to *C. albicans* and enhanced pathology associated with these responses.

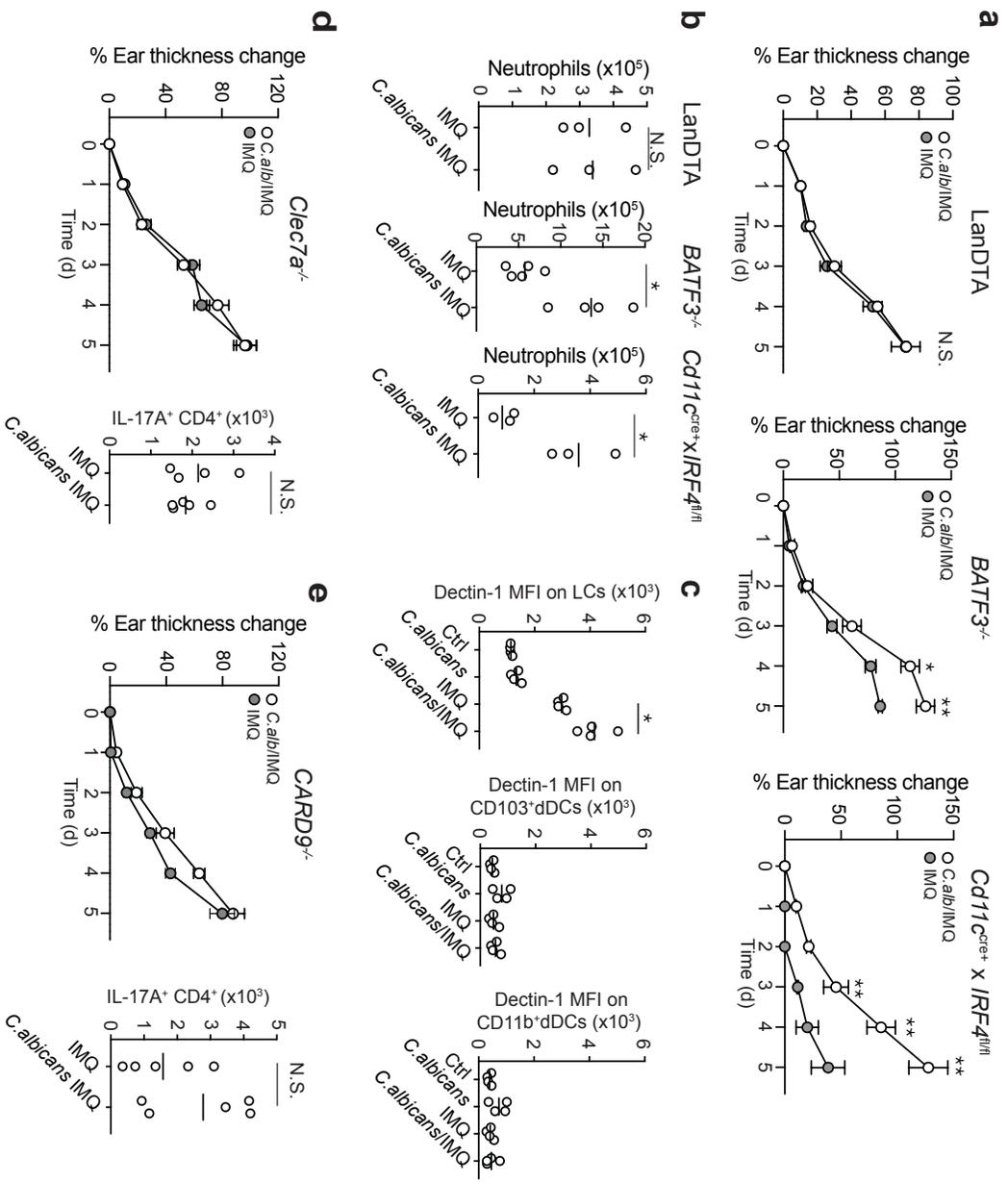
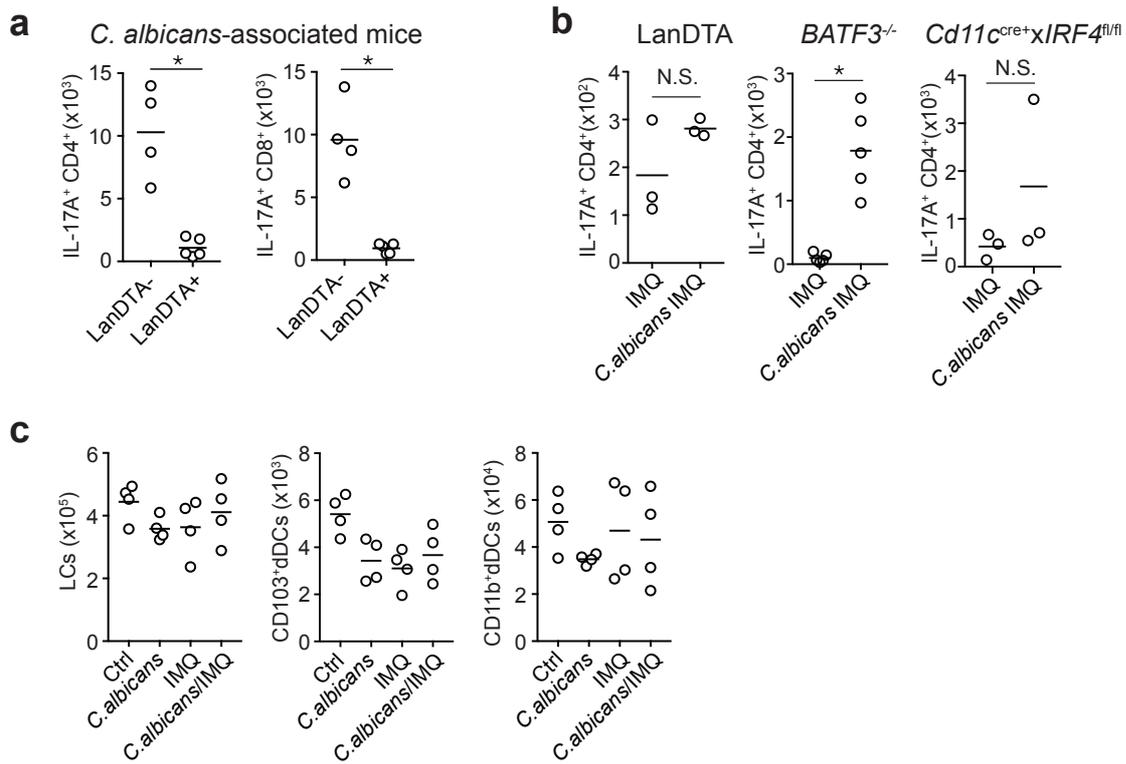


Figure 4

Figure 4. Distinct dendritic cell subset promotes the commensal-derived immune responses.

(a) Percentage of ear thickness change relative to baseline at day 0 from IMQ and *C. albicans*/IMQ, from *Langerin-DTA* (*LanDTA*), *BATF3*^{-/-}, and *CD11c*^{cre+}*IRF4*^{flox/flox} mice. (b) Absolute numbers of neutrophils from IMQ and *C. albicans*/IMQ, from *Langerin-DTA* (*LanDTA*), *BATF3*^{-/-}, and *CD11c*^{cre+}*IRF4*^{flox/flox} mice. (c) Dectin-1 mean fluorescence intensity (MFI) of skin DC subsets; LCs, CD103⁺dDCs, and CD11b⁺dDCs. (d) Percentage of ear thickness change relative to baseline at day 0 with IMQ or *C. albicans*/IMQ and absolute numbers of IL-17A⁺ CD4⁺ FoxP3⁻ T cells from *Clec7a*^{-/-} mice. (e) Percentage of ear thickness change relative to baseline at day 0 with IMQ or *C. albicans*/IMQ and absolute numbers of IL-17A⁺ CD4⁺ FoxP3⁻ T cells from *CARD9*^{-/-} mice. Results are representative 2-3 independent experiments. **P* < 0.05. N.S., not significant.



Supplemental Figure 4

(a) Absolute number of IL-17A⁺ CD4⁺ FoxP3⁻ T cells and IL-17A⁺ CD8⁺ T cells after *C. albicans* association in LanDTA⁻ and LanDTA⁺ mice. (b) Absolute numbers of IL-17A⁺ CD4⁺ FoxP3⁻ T cells from IMQ and *C. albicans*/IMQ, from *Langerin-DTA* (*LanDTA*), *BATF3*^{-/-}, and *CD11c*^{cre+}*IRF4*^{fl/fl} mice. (c) Skin dendritic subsets: Langerhans cells (LCs), CD103⁺dermal dendritic cells (dDCs), and CD11b⁺dDCs, in the ear of naïve, *C. albicans*, IMQ or *C. albicans*/IMQ SPF mice.

Memory responses to *C. albicans* can be sufficient to induce disease flare

We next assessed if the Th17 cells recruited in the skin in the context of *C. albicans*/IMQ were *C. albicans*-specific (as opposed to bystander). To this end, we used an *in vitro* recall assay with *C. albicans* derived antigens. Splenic dendritic cells (DCs) from wild-type mice were loaded with or without heat-killed *C. albicans* or *C. albicans* conditioned medium (a filter-sterilized supernatant from *C. albicans* stationary-phase culture). To enrich in ROR γ t expressing effector T cells, CD4⁺T cells were purified as CD4⁺Foxp3⁻CCR6⁺ T cells. Exposure of CCR6⁺CD4⁺T cells purified from the regional lymph nodes of *C. albicans*/IMQ treated mice to DCs loaded with *C. albicans*-derived antigens promoted their production of IL-17-A (**Fig. 5a** and **Fig. 5b**). *In vitro* recall was abolished when DCs were deficient in major histocompatibility complex II (MHC II) supporting the idea that this response was *C. albicans* specific. Together, these results support the idea that pre-existing adaptive immunity to a skin commensal fungus can directly promote tissue inflammation.

We next investigated the long-term consequences of this exacerbated inflammation caused by commensal specific T cells. Notably, we explored the possibility that inflammation-induced aberrant responses to a skin commensal may predispose to flares induced by the rechallenge with this microbe. To address this point, mice pre-associated with *C. albicans* before IMQ treatment were then observed until resolution of the inflammation and were then re-exposed to the fungal commensal or to control medium (**Fig. 5c**). Remarkably, in *C. albicans*/IMQ treated mice, re-challenge with the fungi was sufficient to promote inflammation, with re-increase in ear thickness, psoriasiform skin histology, and inflammatory Th17, Tc17 cells and neutrophils (**Fig. 5c**). As $\gamma\delta$ T cells have been shown to drive stronger secondary responses to the same stimulus, we explored if these cells had a role in the establishment of the rechallenge response to the skin commensal. Treatment of mice with $\gamma\delta$ depleting antibody demonstrated that $\gamma\delta$ T cells did not contribute to this response (**Fig. 5d**). On the other hand, depletion of CD4⁺T cells (and further of both CD4⁺ and CD8⁺ T cells) prior to commensal re-challenge abrogated disease flare (**Fig. 5e**). Together these results support the idea that inflammation

can permanently alter the relationship of the host with its microbiota and that acute insult predispose to subsequent tissue inflammation triggered by adaptive immunity to the microbiota. Previous work demonstrated that tissue maintain a memory of inflammatory insults associated with epigenetic of skin epithelial stem cells (Naik et al., 2017). While there is a clear role of IL-17 and IL-22 in the activation of keratinocytes in psoriasis, inducing proinflammatory cytokines and proliferation respectively (Chiricozzi et al., 2016; Dainichi et al., 2018), how such epithelial remodeling predispose to microbiota induced flare remains unclear. The tissue itself may contribute to the cyclic flares triggered by the adaptive immune responses to the microbiota as a result of permanent scarring after the original injury. To dissect the role of the epithelial cells compared to the one of the immune cells, IMQ was applied on the left ear only after topical association with *C. albicans*, and the rechallenge a month later was done on both ears. Interestingly, while there was no enhanced inflammation at distant site during the inflammatory trigger, there was an inflammatory immune response during the re-challenge, both at the site of initial inflammation and at the distant site, as shown by an increase in the distal ear thickness as well as in the inflammatory cells (**Fig. 5f**). This results was concordant with previous studies showing that IMQ sensitization of epithelial stem cells in the skin was only local and was not transmitted to distant sites (Naik et al., 2017).

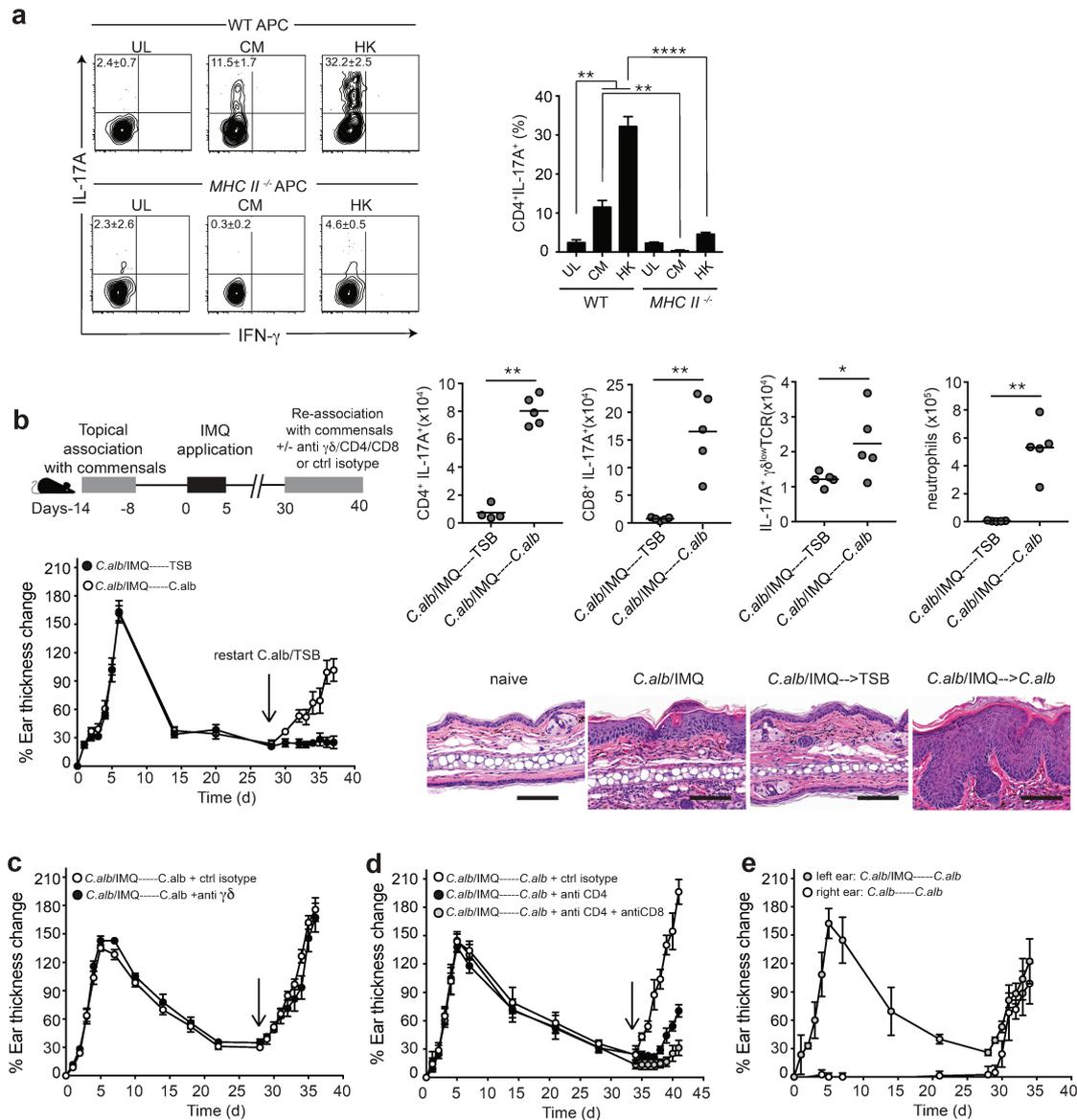


Figure 5. Commensal-memory CD4⁺ T cell responses can be sufficient to induce disease flare.

(a) CD4⁺ FoxP3⁻ CCR6⁺ T cells from the skin draining LNs of *C. albicans*/IMQ treated mice were co-cultured with *Rag2*^{-/-} (WT) or *MHC class II*^{-/-} splenic dendritic cells (APC) untreated (UL) or pre-incubated with *C. albicans* conditioned media (CM) or heat killed *C. albicans*. Flow plots illustrate the frequency of IL-17A⁺ CD4⁺ T cells in overnight co-cultures (left). Frequency of IL-17A production in CD4⁺Foxp3⁻ T cells (right). (b) Wild type mice under SPF condition were treated with *C. albicans*/IMQ, then no treatment was done during the following 3 to 4 weeks to allow for resolution of the inflammation. *C. albicans* or control medium was then re-started every day for 10 days. Time course of percentage of ear thickness change relative to baseline

at day 0. Arrow indicated the day *C. albicans* or control medium was restarted (left). Absolute numbers of IL-17A⁺ CD4⁺ Foxp3⁻ T cells, IL-22⁺ CD4⁺ Foxp3⁻ T cells, IL-17A⁺ CD8⁺ T cells, IL-17A⁺ $\gamma\delta$ TCR^{low} and neutrophils after the last association (right). (c) The day before rechallenge with *C. albicans*, anti- $\gamma\delta$ monoclonal antibody or control isotype was started. Time course of percentage of ear thickness change relative to baseline at day 0. (d) The day before rechallenge with *C. albicans*, anti-CD4⁺ or anti-CD4⁺ and anti-CD8⁺ monoclonal antibody or control isotype was/were started. Time course of percentage of ear thickness change relative to baseline at day 0. (e) After topical association with *C. albicans*, IMQ was applied on the left ear only. A month later, *C. albicans* was restarted on both ears and ear thickness was evaluated every day during rechallenge.

Results are representative 2-3 independent experiments. ** $P < 0.01$, **** $P < 0.0001$.

Conclusion

While psoriasis is considered an autoimmune disease, the specific antigens driving this disease have not yet been identified. The skin microbiota represents a major source of foreign antigens and in recent years, previous works have uncovered that the vast majority of the interface immune system - microbial interactions happen with the symbiotic microbiota. These responses have been shown to be mostly beneficial to the host, promoting antimicrobial defenses and tissue repair (Linehan et al., 2018a; Naik et al., 2015a). Here we show that aberrant reactivity to commensal fungi can promote a pathogenic response when associated with a pro-inflammatory trigger. Further, commensal-induced IL-17 CD4⁺ T cells developed in this inflammatory setting are able to traffic throughout the body and to promote psoriasiform skin inflammation during re-encounter with the specific commensal at distant site.

We approached the present study focusing on the immune response to skin commensal in an inflammatory milieu using the imiquimod model. We previously showed that skin bacterial commensals induce commensal-specific adaptive immune responses that vary depending on the commensals (Linehan et al., 2018b; Naik et al., 2015a) but are usually associated with an increase in the production of IFN γ or IL-17A, without inducing an inflammatory state. Using this approach, we observed that the universal host immune response to skin fungal commensal was an increase in the production of IL-17A from CD4⁺ and CD8⁺ T cells. This was interesting to us because of the importance of IL-17 in the pathogenesis of psoriasis. Contrarily to other inflammatory skin disorders, such as atopic dermatitis, there is not a clear skin microbial signature in the skin of patients with psoriasis. Indeed, in atopic dermatitis, there is a clear association between the flares of the disease with temporal shifts in the skin microbiota, with notably an increase in *S. aureus* during flares of the disease and it has been hypothesized that *S. aureus* and *Corynebacterium bovis* could trigger the inflammatory flares of the disease and not only be a consequence of the skin inflammation (Kobayashi et al., 2015). In psoriasis, while the microbiota has an essential role as shown by a decrease of psoriasiform inflammation after IMQ or IL-23 injection in germ-free mice (Zákostelská et al., 2016; Zhu et

al., 2017), there is no evidence of an altered response to a specific microbe or group of microbes in the induction of flares of the disease. In our model, immune responses to skin fungal microbes were clearly exacerbating tissue inflammation.

Interestingly, while Imiquimod model of psoriasis has been widely used since its description due to the easiness, reproducibility, and resemblance of the model with human psoriasis (Conrad and Nestle, 2006; Gilliet et al., 2004; van der Fits et al., 2009), the most important difference with human psoriasis is its dependence on IL-17A produced by $\gamma\delta^{\text{low}}$ T cells while IL-17A production in human psoriasis comes from CD4⁺ T cells. Our model better recapitulated the features of human psoriasis as it induces a massive Th17 immune response, these Th17 cells being specific for *C. albicans* antigens. This was confirmed by the use of genetic inhibition of Th17 cells. In these mice, no enhanced skin inflammation was observed following *C. albicans*/IMQ compared to IMQ alone. On the other hand, genetic abrogation of V γ 4 T cells, the dominant source of IL-17A in IMQ-induced inflammation in mice (Pantelyushin et al., 2012), did not alter the promotion of the inflammation following *C. albicans*/IMQ. In addition, NETs have been found to be increased in skin and blood of psoriasis patients and the amount of NETs in the peripheral blood of psoriasis patients correlates with psoriasis disease severity (Hu et al., 2016; Lin et al., 2011). Studies also suggest that psoriatic NETs may contain antimicrobial peptides such as cathelicidin (LL-37) (Lin et al., 2011; Skrzeczynska-Moncznik et al., 2012), and yet LL-37 antimicrobial protein levels are elevated in psoriasis skin, leading both to a high protection against bacterial infections and to contribution to disease pathogenesis because LL-37 ligates to DNA, generating complexes that may promote interferon- α production through TLR9 stimulation in psoriatic plasmacytoid dendritic cells (Kahlenberg and Kaplan, 2013; Lande et al., 2007). Therefore, it is possible that in patients with psoriasis, NETosis leads to the secretion of LL37-DNA complexes, which may activate plasmacytoid dendritic cells to produce interferon- α , resulting in psoriasis initiation and exacerbation. Stimuli responsible for inducing NET formation in psoriasis patients are currently unknown. In our model, we observed that *C. albicans* skin colonization followed by IMQ was a trigger for NETs formation and that the NETs could exacerbate psoriasis skin inflammation.

Another key finding of our study is the identification of *C. albicans* pathologic sensing in an inflammatory context as a key driver for inducing flares when re-encountering the fungi even at distant sites. This could be of importance in several human inflammatory disorders. It has been shown that *C. albicans* is the individual microbe leading to the strongest Th17 response in humans (Bacher et al., 2016) and *C. albicans*-specific Th17 cells are present in virtually every healthy volunteer (Acosta-Rodriguez et al., 2007). Interestingly, it has been recently shown that *C. albicans* specific T cells are expanded in patients suffering of Crohn's disease compared to healthy volunteers (Bacher et al.). In addition, most of the Th17 cells reactive to other fungi commensals or pathogens in humans are also cross-reactive to *C. albicans*, and they are the ones increased and contributing to inflammatory pathology at distant sites (Bacher et al.). This demonstrates the importance of immune responses to *C. albicans* in the development and flares of inflammatory disorders. In the future, it would also be interesting to see how the cells in our model circulate and promote pathology in other organs such as the joints, Indeed, psoriatic arthritis is a pathology affecting up to 40% of patients with psoriasis (The Australo-Anglo-American Spondyloarthritis et al., 2011). Of interest, mice with ZAP-70 mutation develop IL-17-dependent psoriatic-like arthritis after dectin-1 mediated fungal infection or beta-glucan injection (Ruutu et al., 2012; Yoshitomi et al., 2005), showing the importance of fungi antigens again for enhancing IL-17 derived inflammation. Our model recapitulates the features of human psoriasis and enables further mechanistic elucidation of human psoriasis pathogenesis and may allow for the development of tailored clinical interventions aiming at controlling skin microbiota and/or aberrant responses to these microbes.

Together, our results suggest that highly conserved anti-fungal commensal responses can in certain contexts become pathogenic and suggest that 'autoimmunity' could sometimes be triggered by a loss of tolerance to our own microbiota.

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