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Évaluation et prévision des allergies aux produits chimiques et aux médicaments

Joelle Dagher

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Évaluation et prévision des allergies aux produits chimiques et aux médicaments

Assessment and prediction of drug and chemical allergies

Thèse de doctorat de l'Université Paris-Saclay et de l'Université Saint Joseph de Beyrouth

École doctorale n°569 Innovation thérapeutique : du fondamental à l'appliqué (ITFA)
Spécialité de doctorat: Toxicologie
Graduate School : Santé et médicaments
Réfèrent : Faculté de pharmacie

Thèse préparée dans l'unité de recherche, **Inflammation microbiome immunosurveillance
MI2 U 996 (Université Paris Saclay, Inserm)**
Sous la direction de **Marc PALLARDY**, Professeur des Universités,
la co-direction de **Hayat AZOURY**, Professeure des Universités,
et le co-encadrement de **Diane ANTONIOS**, Professeure Associée des Universités.

Thèse soutenue à Paris-Saclay,
le 30 juin 2023, par

Joelle DAGHER

Composition du Jury

Bernard MAILLÈRE Directeur de Recherche, HDR, Commissariat à l'énergie atomique et aux énergies alternatives (CEA), Saclay	Président
Aline MILANE Maître de Conférences, Lebanese American University, Byblos	Rapporteur & Examinatrice
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Discipline : Sciences Pharmaceutiques et Biologiques
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Titre : Évaluation et prévision des allergies aux produits chimiques et aux médicaments

Mots clés : Immunotoxicologie, Allergie, Allergisants cutanée, Médicaments

Résumé : Les allergies aux molécules chimiques et médicamenteuses sont considérées un problème majeur de santé publique. Ce travail de thèse vise à présenter trois modèles d'évaluation et de prévision de ces allergies. La première partie du projet consiste en une analyse rétrospective sur dix ans des réactions d'hypersensibilité médicamenteuse utilisés en ambulatoire dans la population libanaise. L'analyse a révélé que les bêtalactamines et les anti-inflammatoires non stéroïdiens étaient les médicaments les plus impliqués comme décrit dans la littérature. La deuxième partie du projet visait à construire une approche unique fondée sur une analyse bibliographique afin de prévoir le risque d'immunisation des nouveaux candidats médicaments. Cette approche analyse quatre paramètres prédictifs *in vitro* et *in silico* qui miment un événement clé dans l'immunisation et leur corrélation à deux paramètres cliniques.

La troisième et dernière partie du projet consistait à développer un nouveau modèle de coculture cellules dendritiques / lymphocytes T (LT) pour détecter et analyser la réponse des LT humains à six molécules chimiques impliquées dans la dermatite allergique de contact (DAC) chez des donneurs sains. L'analyse a révélé que des LT spécifiques ont été détectés pour toutes les molécules chimiques et chez la majorité des donneurs, et leurs fréquences moyennes ont été calculées. Les sous-populations de LT CD4+ ou CD8+ ont été majoritairement impliquées respectivement dans la réponse au cinnamaldéhyde et à l'acide 2,4-dinitrobenzène sulfonique. Pour la méthylisothiazolinone, les deux sous-populations ont été trouvés à égalité. Dans l'ensemble, ce travail de thèse a permis de faire progresser notre compréhension des allergies aux molécules chimiques et médicamenteuses et à développer de nouveaux outils et modèles pour prévoir et détecter ces réactions.

Title : Assessment and prediction of chemical and drug allergies

Keywords : Immunotoxicology, Allergy, Contact sensitizers, Drugs

Abstract : Drug and chemical allergies are a significant public health concern, affecting millions of people worldwide. This thesis project aims to present three models of assessment and prediction of drug and chemical allergies, each addressing a different aspect of the problem. The first part of the project involves a decade-long retrospective analysis of drug-induced hypersensitivity reactions in a Lebanese outpatient population. Our results were largely confirmatory of other worldwide studies. The analysis revealed that beta-lactams and non-steroidal anti-inflammatory drugs were the most implicated drugs. All patients were diagnosed based on detailed clinical history and the physician prescribed an allergy skin prick test when necessary for confirmation. The second part of the project aimed to build a one-of-a kind weight-of-evidence approach that predicts the immunization risk of new drug candidates based on what is known in the literature on a selection of pro-allergenic molecules.

This approach analyzes four *in vitro* and *in silico* predictive parameters that mimic an event that may contribute to immunization and their correlation to two clinical parameters. The third and final part of the project was the development of an improved dendritic cell/T-cell co-culture model to detect and analyze the human T cell response to six chemical sensitizers involved in allergic contact dermatitis in healthy donors. The analysis revealed that specific T cells were detected for all chemicals and in the majority of donors, and their mean frequencies were calculated. CD4+ and CD8+ T cells were mostly found in cinnamaldehyde and 2,4-dinitrobenzene sulfonic acid responding T cells, respectively. For methylisothiazolinone, both subpopulations were present equally. Overall, this thesis project seeks to advance our understanding of drug and chemical allergies and to develop new tools and models for predicting and detecting these reactions.

"Don't watch the clock, do what it does. Keep going."

- Sam Levenson

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ABBREVIATIONS

A

Aa: amino acids

ABC: abacavir

ACD: allergic contact dermatitis

ADH: aldehyde dehydrogenase

ADRs: adverse drug reactions

AGEP: acute generalized exanthematous pustulosis

AHSR: abacavir hypersensitivity reaction

AOP: Adverse Outcome Pathway

APC: antigen-presenting cells

ARFs: ADP-ribosylation factors

ATP: adenosine triphosphate

B

BAS: basophils

BAT: basophil activation test

BB: Bandrowski's base

BSA: bovine serum albumin

C

c-SMAC: central-supramolecular activation complex

CBZ: carbamazepine

cDC: conventional dendritic cell

CF: cystic fibrosis

CinA: cinnamaldehyde

CK2: casein kinase 2

CLIP: invariant chain peptide

CLR: C-type lectin receptors

CMV: cytomegalovirus

CoCl₂: cobalt chloride

CTLA-4: cytotoxic T lymphocyte-associated antigen-4

CYP: cytochrome P

D

DAMPs: damage-associated molecular patterns

DC: dendritic cells

DCIR2: dendritic cell immunoreceptor 2

DHRs: drug hypersensitivity reactions

DIHS: drug-induced hypersensitivity syndrome

DILI: drug liver injury

DN: double-negative

DNCB: dinitrochlorobenzene

DNP: dinitrophenol

DPRA: Direct peptide reactivity assay

DPT: drug provocation test

DRESS: drug reactions with eosinophilia and systemic symptoms

E

EBV: Epstein–Barr virus

EFS: Etablissement Français du Sang

ELISpot: enzyme-linked immunospot assay

ER: endoplasmic reticulum

ERGIC: ER-Golgi intermediate compartment

ESSCA: european surveillance service for contact dermatitis

F

FADD: Fas-associated death domain

FCS: fetal calf serum

FDE: fixed drug eruption

G

GM-CSF: granulocyte-macrophage colony-stimulating factor

H

HA: hyaluronic acid

h-CLAT: human cell line activation test

HHV6: human herpes virus 6

HIV: human immunodeficiency virus

HLA: human leukocyte antigen

HMGB1: high mobility group box one protein

HRs: hypersensitivity reactions

HSA-BP: human serum albumin - benzylpenicillin

HSA: human serum albumin

HSCs: hematopoietic stem cells

HSP: heat shock proteins

hTCPA: human T cell priming assay

I

IDT: intradermal tests

IFN- γ : interferon-gamma

Ig: immunoglobulin

IMDM: Iscove's Modified Dulbecco's Medium

IRF3: interferon-regulatory factor 3

IsoE: isoeugenol

J

JAK: janus kinase

K

KE: Key events

KLH: keyhole limpet hemocyanin

L

LC: langeran cells

LMW: low-molecular-weight

LPS: lipopolysaccharide

LTT: lymphocyte transformation test

M

MAIT: mucosal-associated invariant T cells

MC: mast cells

MDP: monocyte and DCs progenitor

MHC: major histocompatibility complex

MIT: methylisothiazolinone

Mo-DC: monocyte-derived dendritic cells

MPE: maculopapular exantheams

MRGPRX2: MAS-related G protein-coupled receptor X2

MyD88: myeloid differentiation factor 88

N

NAMPs: nanoparticle-associated molecular patterns

NAT2: N-acetyltransferases 2

NF- κ B: nuclear factor kappa B

NiSO₄: nickel sulfate
NKT: natural killer T cells
NLR: nod-like receptors
NLRP12: nucleotide-binding leucine-rich repeat-containing receptor 12
NMBA: neuromuscular blocking agents
NPV: negative predictive value
NQO1: NAD(P)H dehydrogenase (quinone 1)
Nrf-2: nuclear factor erythroid-2-related
NSAIDs: nonsteroidal anti-inflammatory drugs
NVP: nevirapine

O

OCD: occupational contact dermatitis
OECD: organization for economic co-operation and development

P

p-i: pharmacological interaction
PAF: platelet activation factors
PAMPs: pathogen-associated molecular patterns
PBMC: peripheral blood mononuclear cells
PD-1: programmed cell death 1
pDCs: plasmacytoid DCs
PHA: phytohemagglutinin
PKC: protein kinase C
PLC: peptide-loading complex
PPD: p-phenylenediamine
PPV: positive predictive value
PRRs: pattern recognition receptors

Q

QAC: quaternary ammonium compounds

R

RLR: RIG-like receptors

ROS: reactive oxygen species

S

SCARs: severe cutaneous adverse reactions

SI: stimulation index

SIRP α : signal regulatory protein- α

SJS: Stevens-Johnson syndrome

SMX: sulfamethoxazole

SPT: skin prick tests

SS: Sjögren's syndrome

Syk : spleen tyrosine kinase

T

TAP: transporters associated with antigen processing

T_{CM}: central memory T cells

TCR: T-cell receptor

T_{EM}: effector memory T cells

TEN: toxic epidermal necrolysis

Th: T helper

TL: T lymphocyte

TLR: Toll-like receptors

TNF- α : tumor necrosis factor-alpha

TNP: trinitrophenyl

TRIF: TIR-domain-containing adapter-inducing IFN- β

PREFACE

PREFACE

Drug and chemical allergies remind us of the intricate interplay between our bodies and the environment. The rise in the prevalence of these allergies in recent years underlines the urgent need for greater scientific understanding and more effective prevention strategies. We must strive towards a more sustainable and responsible approach to chemical and drug usage, one that considers the potential harm to human health. Ultimately, the study of drug and chemical allergies challenges us to consider the delicate balance between progress and caution and the impact of our choices on our collective well-being. For these reasons, this thesis work aims to develop new and improved models to better understand the mechanisms of drug and chemical allergies. We specifically focused on two types of models: assessment and prediction.

Our first objective was to better understand the epidemiology of drug allergies in Lebanon, as this was the first study tackling this subject. To achieve this, we studied the charts of all allergic patients in an allergy clinic in Lebanon between 2012 and 2021. These original works resulted in one article [submitted to the *Journal of Allergy and Clinical Immunology (JACI)* global, **article 1**].

Our second objective was to develop a predictive immunization score for new drug candidates. The different key events of drug immunization identified during the project allowed the calculation of an immunization score of known and unknown molecules, which will be the subject of a scientific publication.

Finally, our last objective was to better understand the pathomechanism of allergic contact dermatitis (ACD) to a panel of chemical sensitizers. This work resulted in preparing an article that will soon be submitted to a scientific journal (**article 2**).

INTRODUCTION

INTRODUCTION

CHAPTER 1: ALLERGIC REACTIONS TO DRUGS AND CHEMICALS

Adverse drug reactions (ADRs) are responsible for high morbidity and mortality rates, constituting a significant burden on healthcare resources (Mayorga et al., 2019). They are divided into different classes depending on whether their occurrence is expected (type A for augmented) or not (type B for bizarre) (Wilkerson, 2022). Type A reactions represent 85% of ADRs and are considered on-target reactions and are dose-related. They can include adverse effects dependent on the pharmacological mechanism of the drug, toxic effects (e.g: respiratory depression with opioids), and side effects (e.g: dry mouth associated with tricyclic antidepressants) (Pichler and Hausmann, 2016; Daly, 2016; Gupta et al., 2018; Warrington et al., 2018). Type B ADRs are idiosyncratic reactions that cannot be predicted from the known pharmacology of a drug. They can be further subdivided into immunological and nonimmunological ADRs. Drug hypersensitivity reactions (DHRs) are immune-mediated type B ADRs. They are unpredictable, dose-independent (Brockow et al., 2015; Wilkerson, 2022), and occur independently of the drug's pharmacological effect. They depend on the patient's genetic predispositions and environmental factors, which will be discussed in the next paragraph. These reactions are mediated by a specific adaptive immunological response (Demoly et al., 2014; Thong and Tan, 2011; Wheatley et al., 2015). However, in non-allergic (or pseudo-allergic) reactions, no adaptive immunological mechanism (drug-specific antibodies or T-cells) has been demonstrated.

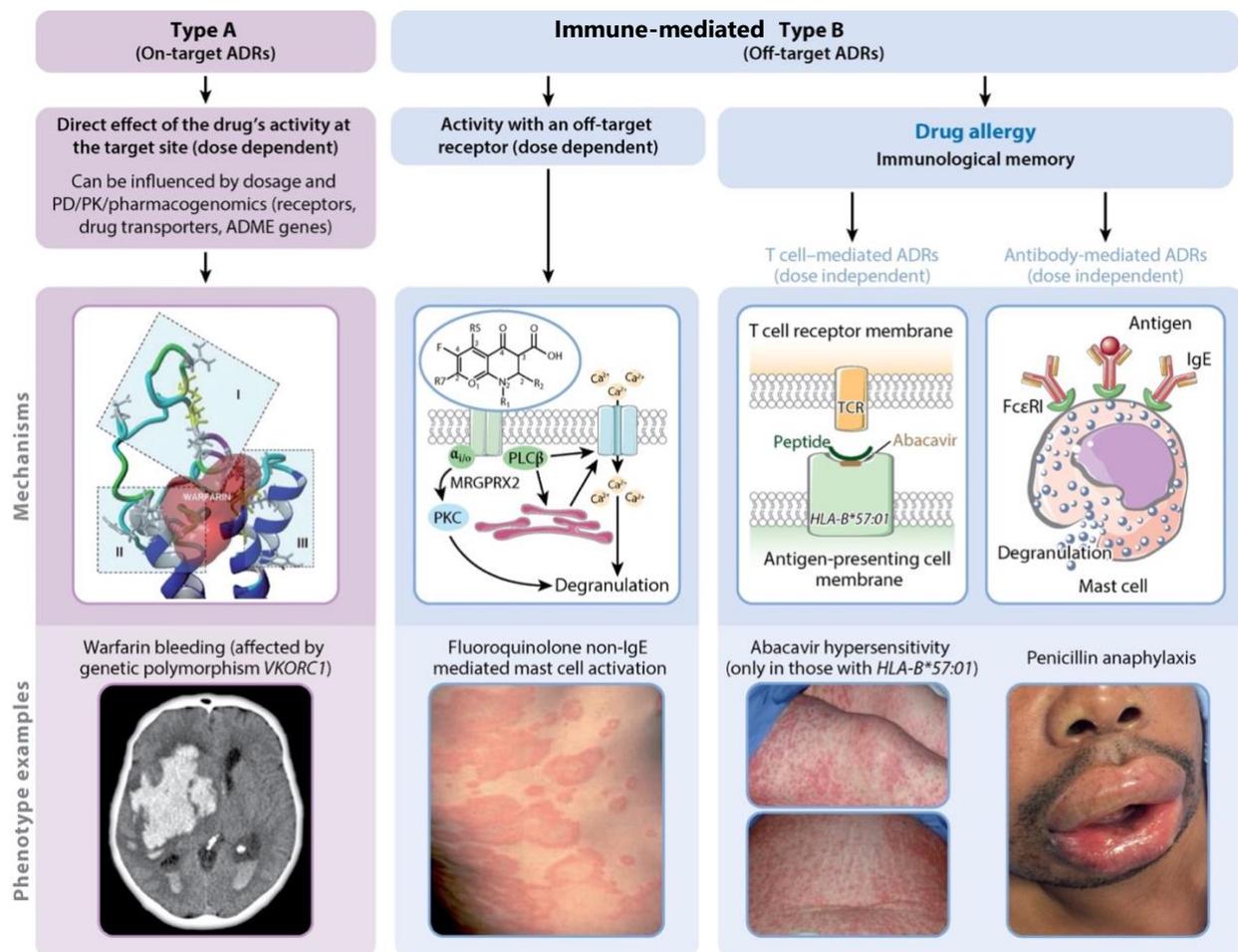


Figure 1. Classification of adverse drug reactions adapted from (Karnes et al., 2019) Adverse drug reactions can be categorized into type A (on-target effects) and type B (off-target effects). Type A reactions are associated with the drug's known pharmacological effect, such as warfarin-associated bleeding. In contrast, type B reactions are related to mechanisms that differ from the therapeutic effect or known toxicological reactions of the drug. Off-target adverse drug reactions can occur without the involvement of an adaptive immunological mechanism, such as fluoroquinolone-induced non-IgE-mediated mast cell activation. However, they can also be T-cell-mediated (e.g., abacavir hypersensitivity syndrome) or antibody-mediated reactions (e.g., beta-lactam anaphylaxis).

1. Pathophysiology of allergic reactions: the Gell and Coombs classification and its modifications

Multiple classifications have been proposed for hypersensitivity reactions (HRs) throughout the years, depending on the latency of symptom onset, the mode of antigen recognition, or the immunological mechanism (Benno Schnyder and Brockow, 2015). A combination of these three classifications often defines HRs. In the 1960s, Gell and Coombs classified these reactions into four categories (I, II, III, and IV) based on the pathophysiology of the immune mechanism implicated and the key immune effector cells involved (Gell and Coombs, 1968). This classification became very suggestive in drug allergies as it links the clinical phenotype to the pathophysiological immune mechanism, reflecting the heterogeneity of the clinical presentation upon contact with the allergen. These reactions are mediated by specific antibodies (types I, II, and III) or specific T cells (type IV). Type I reactions are immediate reactions mediated by immunoglobulins (Ig) type E. Type II reactions are cytotoxic and involve IgG, IgM, and to a lesser extent, IgA antibodies. However, Type III reactions are IgG/IgM immune complexes-mediated. Finally, type IV reactions are delayed-type allergic reactions involving specific T cells (Pichler, 2004). Type IV has been recently subcategorized into four subtypes, IVa, IVb, IVc, and IVd, based on distinct cytokine profiles, types of immune cells involved, and pathogenesis (Pichler et al., 2010). Most drug allergies are type I or IV reactions; type II and III reactions are infrequently encountered (Schnyder and Pichler, 2009). Notably, this classification is crucial to develop appropriate diagnostic strategies for drug and chemical allergies and is still used to date (Demoly et al., 2014; Pichler, 2019; B. Schnyder and Brockow, 2015).

1.1 Type I

Type I HRs are standard IgE-mediated immediate reactions. Upon initial exposure, the chemical or drug, often a low-molecular-weight (LMW) antigen (or hapten), is captured by antigen-presenting cells (APC) such as dendritic cells (DCs), processed, and then presented via major histocompatibility complex (MHC) class II molecules to naïve T helper (Th) cells which are then primed and lead to the production of allergen-specific IgE antibodies by plasmacytes (Stone et al., 2014). These antibodies bind to their high-affinity receptor (FcεRI) on the surface of mast cells and basophils (Schnyder and Pichler, 2009). Upon further contact with the hapten (nth exposure), it specifically cross-links to cell-bound IgEs. This results in the activation of the mast cells leading to their degranulation and the release of several mediators in the following seconds (histamine, tryptase, bradykinin, and prostaglandins) (Jönsson and Daëron, 2012; Pichler et al., 2010). This causes vasodilation and increased capillary permeability, leading to edema and tissue matrix destruction while promoting eosinophil production and activation. The granules also contain leukotrienes (C4, D4, and E4) whose spasmogenic action leads to smooth muscle constriction, increased mucus production, angioedema, and amplification of the Th2 response to increase local inflammation and tissue damage. This is the effector phase (Jönsson and Daëron, 2012; Pichler et al., 2010).

Some reactions are triggered after the first contact with the allergen and do not require sensitization. In this case, a secondary cross-sensitization to another chemical is probable. One example is the cross-sensitization to neuromuscular blocking agents (NMBAs) following exposure to quaternary ammonium compounds (QAC) (a structure shared by all NMBAs) found in the opioid cough suppressant pholcodine. Moreover, specific IgEs and IgGs, as well as the release of platelet activation factors (PAFs), have been identified in subjects having experienced NMBA anaphylaxis (Brusch et al., 2014; Jönsson et al., 2020).

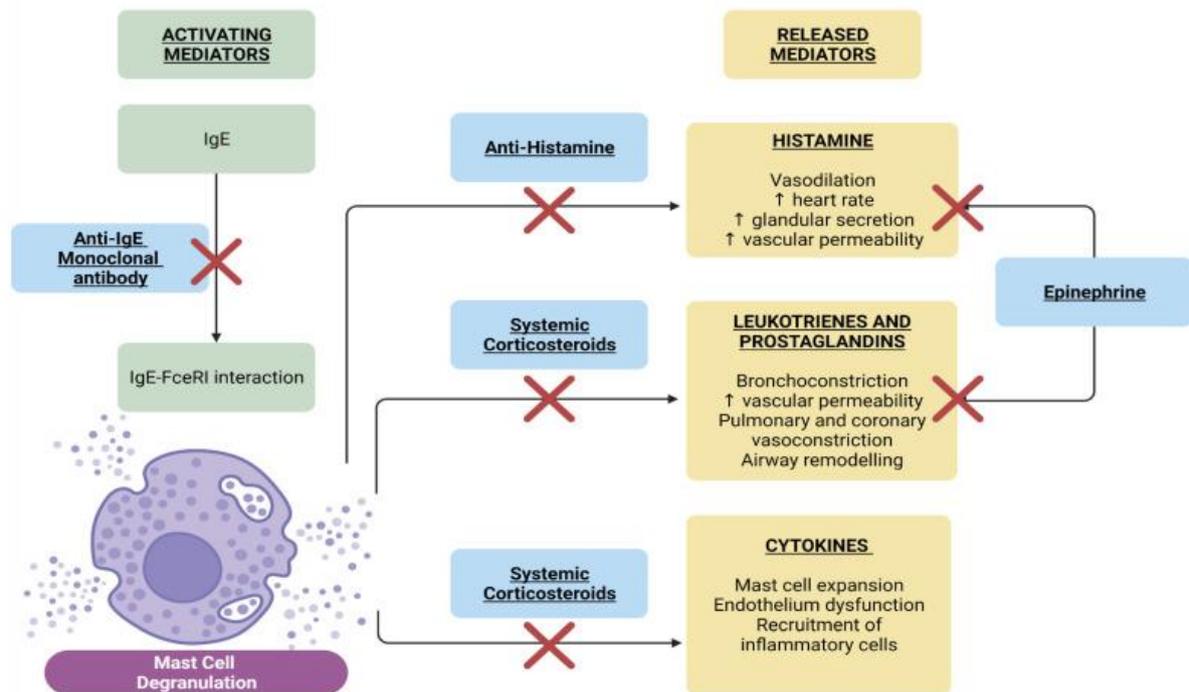


Figure 2. Mechanism of type I hypersensitivity reactions and targeted treatments

adapted from (Hung et al., 2022)

Type I reactions are mediated by immunoglobulin E (IgE) (or IgG for neuromuscular blocking agents), which induces mast cell activation. Cross-linking of the Fc receptor of IgE (FcεR) on the surface of mast cells, triggered by the interaction with the antigen, causes the degranulation of mast cells and, thus, the release of different mediators.

The clinical manifestations vary in severity ranging from mild cutaneous reactions such as urticaria, generalized erythema, or angioedema to moderate respiratory, cardiovascular, or intestinal symptoms to severe systemic anaphylaxis (Azevedo et al., 2019; Brown, 2004). The clinical manifestations and the severity of these reactions depend on the site of mast cell activation. Mast cells are tissue-fixed effector cells that reside in vascular and epithelial tissues. For instance, on the respiratory level, bronchoconstriction and increased mucus secretion occur; however, on the subcutaneous level, an increase in vascular permeability leads to inflammation and swelling (urticaria and angioedema). Finally, systemic mast cell activation increases capillary permeability, inducing hypotension, and smooth muscle contraction, all characteristics of systemic anaphylaxis (Valent et al., 2020). The most common drugs responsible for these reactions are predominantly beta-lactam antibiotics, quinolones,

NMBAs, and certain chemotherapies such as platinum-based agents (carboplatin, cisplatin, oxaliplatin) (Pagani et al., 2022; Schnyder and Pichler, 2009).

1.2 Type II

The mechanisms of Type II and III reactions are based on forming complement-IgG complex antibodies (IgG1, IgG3) or, less commonly, IgM. The two types differ in the targeted structures, thus resulting in different pathophysiological consequences.

Type II are delayed cytotoxic reactions in which the host cells are destroyed through complement-mediated reactions, antibody-dependent cell-mediated cytotoxicity, or antibody-mediated cellular dysfunction. Drug-specific antibodies cause cell lysis or sequestration. Erythrocytes, leukocytes, and platelets are among the target cells. By binding to Fc- or complement-receptors, the antibody-coated cells will be sequestered. Intravascular lysis caused by complement can rarely occur (Mondesir et al., 1985). This essentially translates into hemolytic anemias (penicillin, cephalosporin) and thrombocytopenia [heparin, sulfamethoxazole (SMX), quinine] (Dispenza, 2019; Pichler et al., 2010; Salmon, 2012).

1.3 Type III

Type III reactions are immune complex-mediated as they are triggered by tissue deposition of a significant number of small IgG or IgM-antigen immune complexes (which are challenging to remove from the circulation). These immune complexes accumulate in the vascular wall, trigger the complement cascade, and the recruitment and activation of other inflammatory cells, such as neutrophils. This results in inflammation and tissue injury. The symptoms are determined by the site of immune complex deposition and not by the type of antigen itself. They are usually formed in capillaries, renal glomeruli, and synovial joints, resulting in vasculitis, nephritis, and arthritis. However, the two most common conditions are vasculitis (SMX, allopurinol, clozapine) and serum sickness (rituximab, infliximab) (Pichler et al., 2010; Wilkerson, 2022).

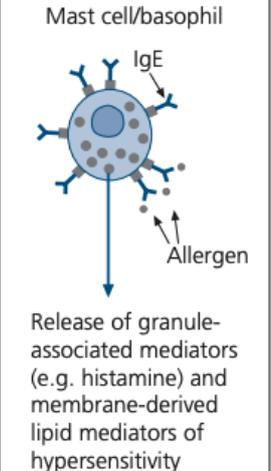
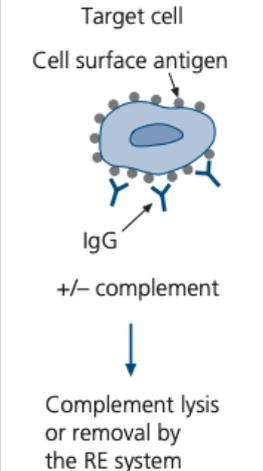
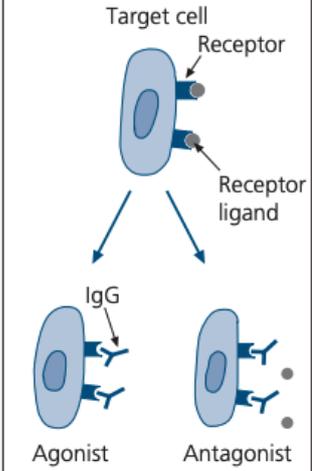
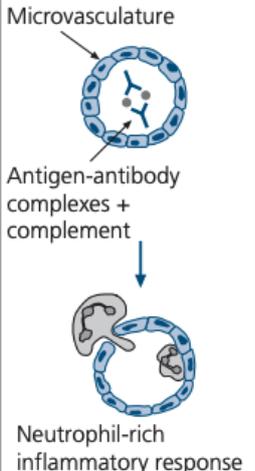
	Type I	Type II		Type III
		a	b	
Descriptive term	Immediate-type (IgE-dependent, or anaphylactic) hypersensitivity	Cytolytic, or cytotoxic, reactions	Cell-stimulating reactions involving altered cell function (or signaling)	Arthus type (or antigen-antibody complex) – often called ‘immune complex’ – hypersensitivity reaction
Initiating event	Antigen (allergen) interacting with mast cells or basophils passively sensitized by IgE	IgG antibody interacting with cell surface antigen	IgG cell-stimulating antibody interacting with cell surface receptors involved in cell signaling	Antigen-antibody complexes, in and around the microvasculature, which activate complement
Antigen	Soluble	Cell-associated	Cell-associated	Soluble
Simplified scheme of the proposed mechanism	 <p>Mast cell/basophil</p> <p>IgE</p> <p>Allergen</p> <p>Release of granule-associated mediators (e.g. histamine) and membrane-derived lipid mediators of hypersensitivity</p>	 <p>Target cell</p> <p>Cell surface antigen</p> <p>IgG</p> <p>+/- complement</p> <p>Complement lysis or removal by the RE system</p>	 <p>Target cell</p> <p>Receptor</p> <p>Receptor ligand</p> <p>IgG</p> <p>Agonist</p> <p>Antagonist</p>	 <p>Microvasculature</p> <p>Antigen-antibody complexes + complement</p> <p>Neutrophil-rich inflammatory response</p>

Figure 3. Characteristics of types I, II, and III hypersensitivity reactions proposed by Gell and Coombs adapted from (Kay, 2009)

1.4 Type IV

Type IV reactions do not implicate antibodies but involve the activation and expansion of T lymphocytes (TLs), which are essential in the delayed immunological mechanisms of allergic reactions. The revised classification of Gell and Coombs characterizes four subcategories taking into account the function and cytokine profile of each TL (Pichler, 2003). They are generally severe cutaneous adverse reactions (SCARs). However, other organs and tissues may be involved, such as the kidneys, liver, and heart (Brockow et al., 2019; Dykewicz and Lam, 2020; Pavlos et al., 2015). Table 1 showcases the characteristics of IVa, IVb, IVc, and IVd reactions.

1.4.1 Type IVa

Type IVa reactions are mediated by CD4 Type 1 helper cells (Th1). These cells lead to macrophage activation via the large production of primarily interferon-gamma (IFN- γ) and tumor necrosis factor-alpha (TNF- α), producing a pro-inflammatory response. Th1 cells are also known to promote the activation of CD8+ TLs (types IVa and IVc) in certain conditions such as allergic contact dermatitis (ACD) (Pichler, 2013; Pichler et al., 2010; Posadas and Pichler, 2007).

1.4.2 Type IVb

Type IVb reactions are mediated by Th2 CD4 T cells via the production of IL-4, IL-13, and IL-5, thus inducing the activation of mast cells and eosinophils (Pichler, 2003; Pichler et al., 2010). The most common clinical symptoms are drug reactions with eosinophilia and systemic symptoms (DRESS) and maculopapular exanthems (MPE) (Posadas and Pichler, 2007). DRESS, also known as drug-induced hypersensitivity syndrome (DIHS), has a long latency period of approximately three weeks to even three months after exposure (Miyagawa and Asada, 2021). It is first characterized by a severe morbilliform rash that often starts on the trunk and then spreads symmetrically to the extremities. This eruption can be complicated by more systemic symptoms such as fever, angioedema, adenopathy, hematologic abnormalities, and multiorgan system involvement (Criado et al., 2012). Mortality rates are reported at approximately 5% to 10%. The drugs most commonly associated with DRESS are aromatic anticonvulsants such as phenytoin, carbamazepine, and phenobarbital. However, other molecules have been implicated as causative agents, such as nonsteroidal anti-inflammatory drugs (NSAIDs) and antibiotics (Roujeau, 2005).

1.4.3 Type IVc

Type IVc reactions are mediated by CD8+ cytotoxic effector TLs that migrate to tissues and cause cell destruction, such as liver hepatocytes or skin keratinocytes. Indeed, cytotoxicity can be mediated by perforin and granzyme B release and the Fas-

Fas-ligand (FasL) interaction. The activation of T cell receptors releases cytotoxic granules containing perforin, granulysin, and granzyme, activating the caspase cascade and triggering cell apoptosis (Voskoboinik et al., 2015). Another cytotoxic mechanism used by CD8+ T cells is related to the binding of the Fas receptor with the FasL molecules expressed by CD8+ T cells. This leads to the recruitment of the Fas-associated death domain (FADD), which activates caspases 8 and 3, triggering cell apoptosis. (Nassif et al., 2002; Schnyder et al., 1998). Therefore, the main symptoms are related to cell lysis of keratinocytes in Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN) or ACD, or hepatocytes in drug-induced liver injury (DILI) (Pichler et al., 2010; Taylor et al., 2015).

1.4.4 Type IVd

Type IVd reactions are mediated by CD4 and CD8 T cells and neutrophil cooperation through the production of IL-8 and granulocyte-macrophage colony-stimulating factor (GM-CSF). T cells recruit neutrophils to the inflammation site via IL-8 release and prevent their apoptosis via GM-CSF release (Feldmeyer et al., 2016). This infiltration is responsible for the clinical symptoms of acute generalized exanthematous pustulosis (AGEP), which is characterized by the appearance of numerous non-follicular pustules on erythema (Roujeau, 2005). It is solely seen after exposure to a medication with a short latency period, usually within two days after the administration. These skin eruptions are accompanied by high fever, edema of the face and hands, and blisters located mainly on the trunk. The diagnostic feature of AGEP is hyperleukocytosis in the superficial part of the epidermis. The most implicated therapeutic molecules are amoxicillin, quinolones, and sulfonamide antibiotics (Sidoroff et al., 2007). The only recommended management is the discontinuation of the treatment. After skin desquamation of the lesions, the resolution is favorable and occurs spontaneously in less than fifteen days (Feldmeyer et al., 2016).

Table 1. Classification of type IV subcategories adapted from (Adair et al., 2021; Pichler, 2007)

Classification		T-cell type	Effector molecules	Pathomechanism	Clinical symptoms following drug or chemical exposure	Time from administration till the appearance of symptoms
IV T cell-mediated	IVa	CD4 Th1	IFN- γ , TNF- α	Monocyte/ Macrophage activation	ACD	1 to 21 days
	IVb	CD4 Th2	IL-4, IL-5, IL-13	Eosinophilic activation	MPE, DRESS	1 to multiple days for MPE, 3 weeks to 3 months for DRESS
	IVc	CD8 Cytotoxic T cells	Granzyme B, perforin, Fas ligand	Cell death mediated by CD8+ T-cells	DILI, SJS/TEN, ACD, FDE	1 to 2 days for FDE
	IVd	CD4-CD8 T cells mixed response,	IL-8, GM-CSF	Neutrophil recruitment and activation	AGEP	1 to 2 days typically but can be longer

Further categorization of type IV hypersensitivity reactions based on T-cell subset and effector molecules involved and their clinical manifestations. ACD: allergic contact dermatitis; AGEP: acute generalized exanthematous pustulosis; DRESS: drug reaction with eosinophilia and systemic symptoms; FDE: fixed drug eruption; IFN- γ : interferon-gamma; IL: interleukin; MPE: maculopapular exanthema; SJS/TEN: Stevens-Johnson syndrome/toxic epidermal necrolysis; Th: T helper; TNF: tumor necrosis factor.

1.5 Limitation of this classification

This classification does not consider the specific process by which drugs may stimulate T cells and only partially explains underlying immunological mechanisms. To better understand how drugs cause T-cell activation, three non-exclusive hypotheses have been presented: the hapten/prohapten model, the pharmacological interaction (p-i) hypothesis, and the altered peptide repertoire model (reference chapter 2). Furthermore, this classification rules out the role of double-negative (DN) T cells in the immune response. This T cell population are innate-like T cells that express semi-invariant $\alpha\beta$ TCRs and lack the CD4 and CD8 co-receptors. The main DN T cells subpopulations are natural killer T cells (NKT) and mucosal-associated invariant T cells (MAIT) (Velikkakam et al., 2022).

2. Epidemiology of allergic reactions to drugs and chemicals

Epidemiologic data on drug and chemical allergies are scarce and inconsistent, making it difficult to determine the incidence and prevalence of these reactions worldwide (Tanno et al., 2016). Each study differs according to the terminology, the population studied, the diagnostic methods used, and the drugs implicated. For example, a 2014 general population survey conducted in the United States showed an important difference in the prevalence of anaphylaxis by only changing the strictness of the diagnosis criteria (7.7% for a general definition of anaphylaxis to 1.6% if the definition included the involvement of two or more respiratory and/or cardiovascular symptoms and hospitalization); 35 % of these anaphylactic reactions were attributed to drug allergies. (Wood et al., 2014; Blumenthal et al., 2019).

However, hypersensitivity reactions are thought to represent 15-20% of ADRs and to affect more than 7% of the general population (Gomes and Demoly, 2005; Böhm et al., 2018; Fabrizio et al., 2019). In hospitalized patients, the frequency of these reactions is estimated between 1,8 and 4,2 reactions per 1000 hospitalizations (Thong and Tan, 2011). Most existing data on drug allergy prevalence in different populations originated from self-reporting. Surprisingly, up to 38% of adults reported having some drug allergy (Gomes and Kuyucu, 2017). Penicillins, sulfonamide antibiotics, opioids, NSAIDs, and NMBAs are the most frequently reported drugs, although there is a broad spectrum of possible culprits (Doña et al., 2012; Gomes and Kuyucu, 2017). Immediate reactions to penicillins are the most commonly reported (Albin and Agarwal, 2014; Joint Task Force on Practice Parameters et al., 2010; Patterson and Stankewicz, 2023). Sulfonamides are more likely to cause delayed reactions, which occur in about 3 to 8% of patients but can reach 50% in patients with HIV (Castrejon et al., 2010; Peter et al., 2019). On the other hand, allergic reactions to NMBAs represent the leading cause of peri-operative anaphylaxis (Mertes et al., 2011; Di Leo et al., 2018).

Due to the worldwide increase in drug allergies, it is a real challenge for physicians to properly diagnose and manage these reactions. A false allergy diagnosis based

solely on medical history may limit the patients' treatment options and lead to using less effective, more dangerous, or more expensive drugs (Chiriac and Demoly, 2014). Indeed, the sensitivity of current diagnostic tests is optimal for identifying the correct number of allergic patients but varies according to the type of DHR. The sensitivity of patch, prick, and intradermal tests vary from 2.6% for non-antibody-mediated reactions to 70% for antibody-mediated reactions (Castells, 2019). Numerous of these alleged drug allergies are non-immunological mediated ADRs; thus, the overuse of the "drug allergy" label in databases is a fundamental problem (Gomes and Kuyucu, 2017).

ACD is an allergic reaction to chemical sensitizers. It affects 15 to 20% of the general population in Europe (Diepgen et al., 2016). It is a leading cause of occupational skin disease, significantly affecting the lives of those exposed, mostly hairdressers, metal workers, healthcare workers, food industry workers, construction workers, cleaners, and painters (Kaplan et al., 2012; Peiser et al., 2012). These allergens are primarily organic molecules such as dinitrochlorobenzene (DNCB), cinnamaldehyde (CinA), isoeugenol (IsoE), or methylisothiazolinone (MIT), or inorganic molecules such as cobalt chloride (CoCl_2), and nickel sulfate (NiSO_4). In Europe, according to European Surveillance Service for Contact Dermatitis (ESSCA), the prevalence of ACD caused by metals is 18.1% for nickel and 5.9% for cobalt (Uter et al., 2017).

3. Risk factors of allergic reactions

It is well-documented that host-related and chemical-related risk factors contribute to the onset of allergic reactions.

3.1 Chemical-related factors

The chemical properties, route of administration, and molecular weight of the chemical are risk factors that can influence the occurrence of allergic reactions.

3.1.1 Route of administration and dosage

There is some evidence that the drug dosage and mode of administration influence sensitization (Thong and Tan, 2011). Evidence from clinical practice reported that intermittent and repeated administration of standard dosage is more sensitizing than interval exposure with high doses. This has been observed in patients with cystic fibrosis, where antibiotic prophylaxis is often given repeatedly (Ramesh, 2002; Roehmel et al., 2014). Moreover, oral administration appears less sensitizing than parenteral or topical administration (Guglielmi et al., 2006; Thong and Tan, 2011). Even though most studies on routes of administration and dosage are incomplete, valid confirmation through a comparison of allergology data for a particular medicine would undoubtedly be a convincing argument (Hammond et al., 2020).

3.1.2 Reactivity of the chemical or drug

It is difficult to predict the immunogenicity of a drug or chemical based on its chemical structure alone before its administration. Most LMW compounds (<1kDa) are not immunogenic alone. They act like haptens, forming hapten-protein adducts with intracellular and serum proteins, thus making them sufficiently immunogenic (Cho and Uetrecht, 2017) (reference chapter 2). Nevertheless, LMW drugs are not always directly reactive to proteins; they often undergo bioactivation to become protein reactive, as seen with SMX and dapsone (Uetrecht et al., 1988; Naisbitt et al., 1999; Alzahrani et al.,

2017). In addition, the formation of these bioconjugates does not necessarily confirm the onset of the immunization process and potentially the DHRs (Adair et al., 2021; Tailor et al., 2016), as these complexes are also found in non-allergic patients (Jenkins et al., 2009; Meng et al., 2016). For example, ethacrynic acids are chemically reactive and can covalently bind to proteins but never cause allergic reactions (Cho and Utrecht, 2017).

3.1.3 Danger signals

To overcome immunological tolerance and acquire an effector phenotype, APCs must receive activating signals, also known as "danger signals" (reference chapter 2) (Gallucci and Matzinger, 2001; Matzinger, 2002; Pallardy and Bechara, 2017a). These danger signals can be exogenous pathogen-associated molecular patterns (PAMP), such as lipopolysaccharide (LPS), or endogenous damage-associated molecular patterns (DAMP) from distressed and injured cells. Other danger signals include the inflammasome and pro-inflammatory cytokines such as the TNF- α and IL-1 (Tsan and Gao, 2004). For instance, it was evidenced that the active nitroso metabolite of SMX, SMX-NO, contributes to forming reactive oxygen species (ROS), known to cause cell damage. In addition, SMX-NO was shown to induce the expression of CD40 on in vitro-generated DCs (Elzagallaai et al., 2020; Sanderson et al., 2007a). Similarly, the reactive metabolite of nevirapine (NVP), 12-OH-NVP, which travels to the skin and forms a reactive benzylic sulfate, activates the inflammasome pathway and may involve danger signals (X. Zhang et al., 2013a).

3.2 Host-related factors

The heterogeneity of the immune response and the fact that some allergic reactions are more frequent in a particular population suggests the presence of other critical host-related factors such as genetics, concomitant infections, and associated

diseases. This section will outline additional factors that may explain why some individuals are more susceptible to allergic reactions.

3.2.1 Age, sex, and exposure

Based on the majority of epidemiological data, true chemical allergies are more common in adult women (Gomes and Demoly, 2005; Guglielmi et al., 2006; Kurt et al., 2011). Possible predisposing factors have included gender-related differences in pharmacokinetic, pharmacodynamic, immunological, and hormonal aspects as well as differences in how women and men are exposed to chemicals (Gomes and Kuyucu, 2017). For example, women are more frequently affected by nickel allergy due to a higher level of exposure to jewelry (Diepgen et al., 2016). This is also observed in the case of allergy to NMBAs, probably due to cross-sensitization with products containing QA in cosmetics or household products (Baldo and Fisher, 2009). Large-scale pediatric investigations revealed that the female gender is not a risk factor for DHRs in children. This is most likely because adult women and girls differ in metabolism and hormone secretion (Ponvert et al., 2011; Arikoglu et al., 2015; Tugcu et al., 2015; Gomes and Kuyucu, 2017).

One of the key risk factors for ACD is the occupational repeated exposure to the contact sensitizers, also known as occupational contact dermatitis (OCD). In the United States, an estimated 11 million workers may be exposed to substances that might lead to allergies such as OCD (Sasseville, 2008; Anderson et al., 2017). This exposure has been very well studied and hundreds of chemicals existing in nearly every industry have been identified, such as p-phenylenediamine (PPD) in hair dyes and methylisothiazolinone in cleaners, shampoos and conditioners affecting workers in the cosmetic and beauty sectors (Houle et al., 2021).

3.2.2 Genetic factors and polymorphisms

Several genetic factors are involved in the pathogenesis of chemical allergies, such as the polymorphism of HLA alleles, immune-related genes, and metabolism. These risk factors help explain the high susceptibility of specific populations to allergic reactions.

HLA alleles associations

Associations between HLA alleles and allergic reactions have been frequently detected, mostly with MHC class I molecules (Fan et al., 2017; Redwood et al., 2018), and some of those are linked to certain ethnicities (Ghattaoraya et al., 2017). Most of these reactions are mediated by T-cells and associated with severe cutaneous reactions such as SJS, TEN, and DRESS. Other HLA associations have been identified with organ-specific symptoms such as drug liver injury (DILI) with flucloxacillin and pancreatitis with azathioprine (Daly et al., 2009; Heap et al., 2014).

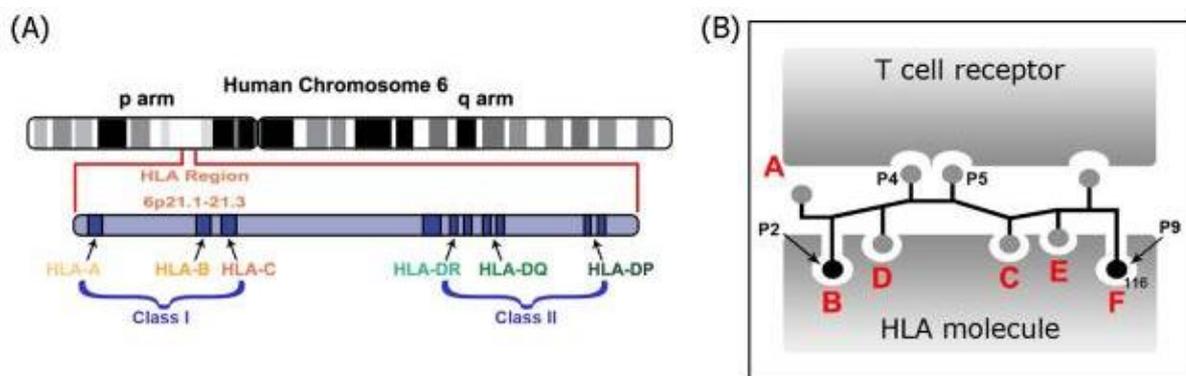


Figure 4. The human leukocyte antigen (HLA) adapted from (Redwood et al., 2018) (A) The HLA genes are considered the most polymorphic human genes found on the short arm (p) of the human chromosome 6. HLA-A, HLA-B, and HLA-C genes are encoded in class I regions, whereas HLA-DR, HLA-DQ, and HLA-DP genes are encoded in class II regions. HLA presents peptides to the T cell receptors on the surface of T cells. (B) Peptides bind to class I HLA alleles in the A, B, C, D, E, and F pockets of the peptide binding groove. The peptide's anchor residues P2 and P9 are bound by the B and F pockets, enabling binding specificity to a given HLA molecule.

The most robust and common association is between the HLA-B*57:01 allele and abacavir (ABC) hypersensitivity in Caucasian populations (Hughes et al., 2004; Mallal et al., 2002; Park et al., 2009). Other associations have also been described and shown in table 2. It is noteworthy that in addition to the specificity of the drug, multiple phenotypes in a single chemical can be associated with different alleles. For example, DRESS induced by carbamazepine (CBZ) was related to HLA-B*31:01; however, SJS/TEN to CBZ was also linked to HLA-B*15:02 (Chung et al., 2004; Khan, 2016). Very few of these associations have been considered solid enough to be the subject of screening tests in clinical use. For example, it is now mandatory to screen the HLA-B*57:01 allele in patients before ABC treatment to determine whether they are a carrier of this allele as the risk of developing SJS/TEN is much higher (with a negative predictive value of 100%). HLA-B*15:02 positive Han Chinese patients are advised to avoid carbamazepine for the risk of developing SJS/TEN (Tangamornsuksan et al., 2013; Khan, 2016).

Table 2. Examples of HLA associations with immune-mediated DHRs adapted from (Gibson et al., 2023)

Therapeutic class	Drug	DHR	HLA alleles	risk	PPV (%)	NPV (%)	Populations	References
Antibiotics	Dapsone	DRESS	B*13:01		7.8	99.8	Chinese	(F.-R. Zhang et al., 2013)
	Sulfamethoxazole	SCAR	B*13:01		4.05	99.92	Asian	(Wang et al., 2021)
		DRESS	B*13:01		3.64	99.92	Asian	(Wang et al., 2021)
	Vancomycin	DRESS	A*32:01		-	-	European ancestry	(Konvinse et al., 2019)
	Flucloxacillin	DILI	B*57:01		-	-	Australian	(Daly et al., 2009)
					-	-	United kingdom	(Daly et al., 2009)
Amoxicillin / Clavulanic acid	DILI	DRB1*15:01-DQB1*06:02		-	-	Caucasian	(Lucena et al., 2011)	
Antiretrovirals	Abacavir	AHSR	B*57:01		50	100	European, African	(Mallal et al., 2002; Saag et al., 2008)
	Nevirapine	Skin rash	B*35:05		-	-	Thai	(Chantarangsu et al., 2009)
		SJS/TEN	C*04:01		2.6	99.2	Malawian	(Carr et al., 2017)
Anticonvulsants	Carbamazepine	SJS/TEN	B*15:02		3	100 in Han Chinese	Han Chinese, Thai, Malaysian, Indian	(Mehta et al., 2009; Tangamornsuksan et al., 2013)
			A*31:01		-	-	Japanese, northern European, Korean	(McCormack et al., 2011; Genin et al., 2014)
		DRESS	A*31:01		0.89	99.98	Europeans	(Genin et al., 2014)
					0.59	99.97	Chinese	(Genin et al., 2014)

Therapeutic class	Drug	DHR	HLA alleles	risk	PPV (%)	NPV (%)	Populations	References
	Lamotrigine	SJS/TEN	B*15:02	-	-	-	Han Chinese	(Cheung et al., 2013)
	Phenobarbital	SJS/TEN	B*51:01	-	-	-	Japanese	(Kaniwa et al., 2013)
	Phenytoin	SJS/TEN	B*13:01	-	-	-	East Asian	(Su et al., 2019)
			B*15:02	33	100	Thai	(Locharernkul et al., 2008)	
Other drugs	Allopurinol	SJS/TEN, DRESS, MPE	B*58:01	3 in Han Chinese	3 in Han Chinese	100 in Han Chinese	Han Chinese, Thai, European, Italian, Korean	(Kang et al., 2011; Chiu et al., 2012; Gonçalo et al., 2013; Jarjour et al., 2015)
	Sulfasalazine	DRESS	B*13:01	-	-	-	Han Chinese	(Yang et al., 2014)

DHR: drug hypersensitivity reaction; DIHS: drug-induced hypersensitivity syndrome; DILI: drug-induced liver injury; DRESS: drug reaction with eosinophilia and systemic symptoms; HLA: human leucocyte antigen; AHSR: abacavir hypersensitivity reaction; MPE: maculopapular exanthema; NPV: negative predictive value; PPV: positive predictive value; SJS/TEN, Stevens-Johnson syndrome.

TCR associations

Some individuals do not develop a clinical reaction while carrying the risk allele. This shows that other factors are implicated besides polymorphism in HLA alleles. Indeed, CBZ-reactive LTs isolated from HLA-B*15:02 allergic patients were characterized by a particular VB11 T cell receptor (TCR) sequence (Ko et al., 2011). In addition, studies have previously indicated that nickel-specific memory T cells from allergic patients preferentially express distinct TCRV β chains, and it was demonstrated that the overexpression of TRBV19 genes was correlated with the patient's reactivity to nickel (Moulon et al., 1995; Vollmer et al., 1997; Bechara et al., 2019b). Furthermore, a polymorphism in the gene coding for TNF- α has been proposed as a marker for sensitization to multiple metals (Colagiovanni et al., 2016).

Other genes

Many chemicals undergo metabolism to generate reactive metabolites capable of binding to proteins and eliciting an immune response (Cao et al., 2021; Cho and Utrecht, 2017; Tailor et al., 2019). It has been suggested that specific genetic polymorphisms of genes coding for proteins involved in drug metabolism can increase the risks of DHRs. For example, a link between the polymorphism in cytochrome P (CYP) 2C9 and phenytoin-mediated skin reactions has been evidenced at therapeutic doses (Franco and Perucca, 2015). Similarly, slow N-acetylation by N-acetyltransferases 2 (NAT2), which is the main detoxification pathway for sulfonamides, has been more frequently reported in patients with SMX and sulfasalazine hypersensitivity (Rieder et al., 1991; Spielberg, 1996; Wadelius et al., 2000; Soejima et al., 2007).

3.2.3 Underlying diseases and viral infections

The patient's health status can be considered a predisposing factor for DHRs. For instance, in patients with impaired renal excretion, the elimination of allopurinol is reduced, causing the accumulation of its reactive metabolite, oxypurinol, thus increasing the risk of DHRs (Chung et al., 2015; Yun et al., 2013). Another classic example would be the incidence of piperacillin hypersensitivity in patients suffering from cystic fibrosis (CF) (Whitaker et al., 2012). It is suggested that frequent infections in CF patients may increase innate immune activity, raising the likelihood of hypersensitivity (Whitaker et al., 2012). Other systemic autoimmune diseases are reportedly associated with a high frequency of drug allergy. In particular, observational studies showed a link between Sjögren's syndrome (SS), characterized by lymphocytic infiltration and inflammation of the exocrine glands, and penicillin and sulfamide drug allergies (Watanabe and Yamaguchi, 2022).

Generally, drug allergic reactions are closely linked to concomitant viral infections (Pichler and Brügger, 2023) by decreasing the threshold of co-stimulation required to induce lymphocyte activation (Elsheikh et al., 2010). Several viruses are known to be involved mainly: human immunodeficiency virus (HIV), Epstein–Barr virus (EBV), human herpes virus 6 (HHV6), and cytomegalovirus (CMV). Patients with HIV who are more susceptible to developing drug allergies are a well-known example of this relationship (Castrejon et al., 2010). This is the case of SMX allergy, which occurs in about 3% of patients but can reach 30% in HIV-infected patients administering the drug either as prophylaxis or for the treatment of pneumocystis pneumonia (Castrejon et al., 2010). Similarly, patients with infectious mononucleosis due to Epstein–Barr virus (EBV) showed an increase in the frequency of allergic cutaneous reactions with amoxicillin administration (Wheatley et al., 2015).

The interplay between allergic reactions and viral infections depends on the mechanisms of drug antigen recognition and T-cell activation (Pichler and Brügger, 2023). The drugs can either act like haptens where the protein-molecule (or metabolite) complex is captured by APCs, digested into haptenized peptides, and

presented to the T cells (Pichler, 2004; Martin et al., 2010a), or follow the pharmacological interactions with immune receptors (p-i) concept where antigen metabolism and priming by APCs are not required, and these molecules can interact with the MHC-TCR complex via labile non-covalent bonds (*the detailed mechanisms will be described in chapter 2*) (Schnyder et al., 1997; Pichler et al., 2011).

- For hapten-acting drugs: the danger signals and the pro-inflammatory environment, and the release of cytokines produced by a viral infection, leads to the increase in the uptake, processing, and presentation of the antigen, therefore, providing the first and second signals leading to direct T-cell activation (**Figure 5**).
- For p-i acting drugs, the enhanced expression of HLA and adhesion molecules on tissue cells leads to more TCR per cell and the enhanced binding of the complex TCR-drug-HLA (**Figure 6**).
- The presence of cross-reactivity with viral-specific memory T cells.

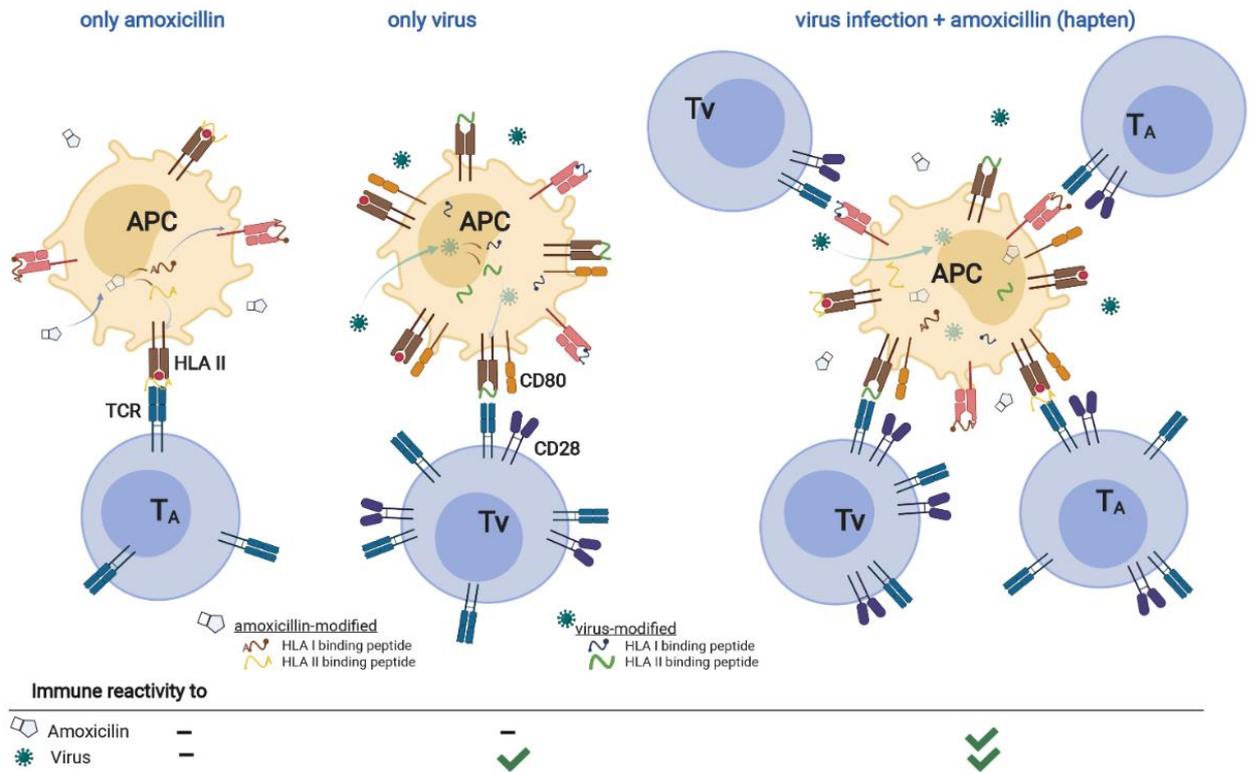


Figure 5. Virus infection and hapten (amoxicillin) stimulation adapted from (Pichler and Brüggem, 2023)

Drug-modified peptides reach the HL-A-class I and class II antigen presentation pathways when haptens, such as amoxicillin, bind via covalent bonds to numerous proteins in the peripheral blood and/or tissue. However, no immune response is observed, most likely due to the absence of co-stimulatory signals. However, the expression of immunological receptors (TCR, HLA) and co-stimulatory molecules (CD80, CD86, and CD40) on the APCs is increased following viral infection. In addition, the same cells presenting viral peptides may also present drug-modified peptides and co-stimulatory molecules when the patient administers the drug during a viral stimulation. As a result, T cells specific to the drug are also activated in the presence of the necessary co-stimulation. APC, antigen-presenting cell; HLA, human leukocyte antigen; TCR, T-cell receptor; T_A, T-cell recognizing amoxicillin; T_v, T-cell recognizing virus-modified HLA.

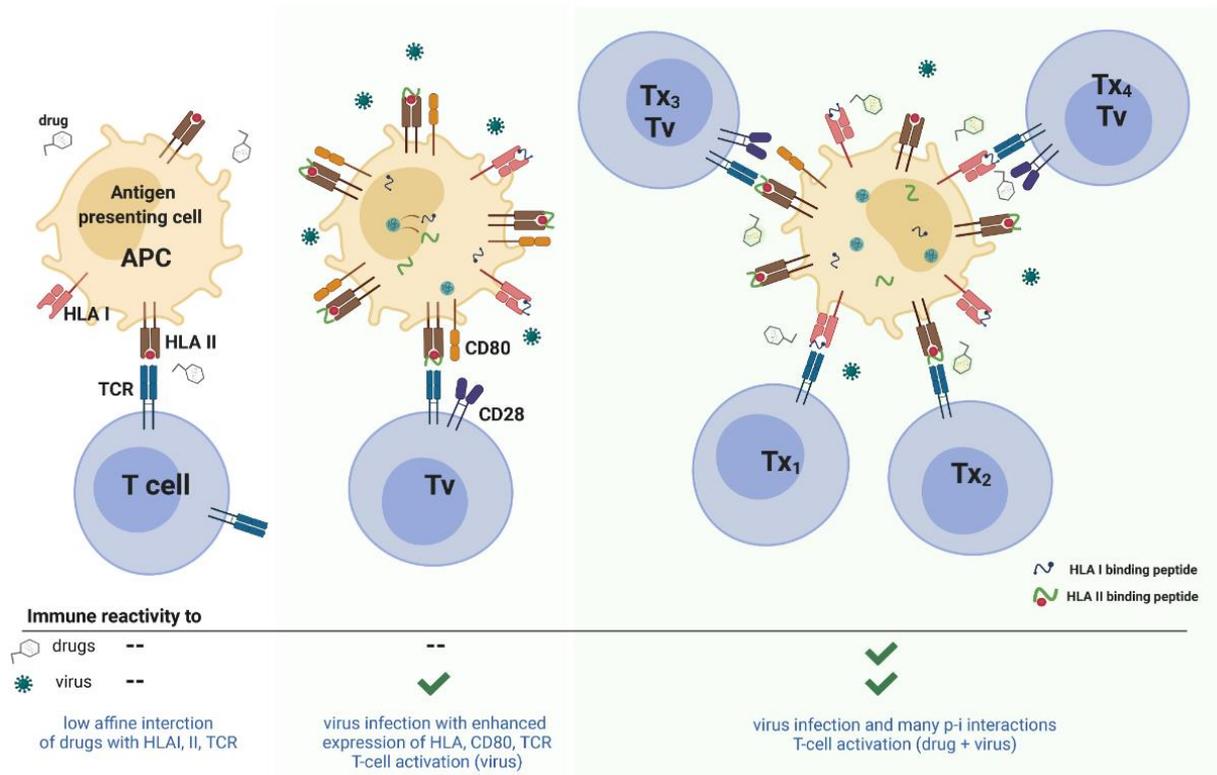


Figure 6. Virus infection and the pharmacological interaction (p-i) mechanism of T-cell activation adapted from (Pichler and Brügger, 2023)

P-i-acting drugs non-covalently and reversibly bind to proteins, including immunological receptor proteins. This low-affinity binding is insufficient to cause T-cell activation. However, during viral infections, tissue cells overexpress immunological receptors. As a result, p-i binding may become functionally important and activates T cells. HLA, human leukocyte antigen; TCR, T-cell receptor; Tv, T-cell recognizing virus-modified HLA; Tx, drug-reactive T cell; some Tx react with drug/HLA (Tx1, Tx2), other T cells react with the drug and with viral peptides (Tx3, Tx4/Tv).

In addition to the concept of heterologous immunity, some allergic reactions are associated with viral **reactivation**. In DRESS, drug hypersensitivity precedes the detection of the viremia of endogenous herpes viruses (HHV6, CMV, and EBV). It was recently shown that azathioprine therapy increased the occurrence of reactivation of endogenous latent herpesviruses (Ingelfinger et al., 2023). This viremia can coincide with the acute phase of the DHR but is most commonly found 3 to 6 weeks later after the stop of the treatment (Seishima et al., 2006; Shiohara et al., 2007; Tohyama et al., 2007). Often the viremia remains asymptomatic; however, severe complications are described mainly for CMV (Mizukawa et al., 2020).

3.2.4 Associated treatments example of immune checkpoint inhibitors

The principle of diminishing suppressive/regulatory/tolerance signals has been recently exploited with the use of immune checkpoint inhibitors [cytotoxic T-lymphocyte associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1) and programmed cell death ligand 1 (PD-L1)] in oncology successfully (Okazaki and Honjo, 2007; Lee et al., 2019). Although these therapies are effective in allowing the immune system to be more aggressively towards tumors through lowering the threshold for immune activation (Guram et al., 2019), they also have autoimmune side effects (Postow et al., 2018). Perturbation of the PD-1 and CTLA-4 pathways has been shown to enhance priming of NO-SMX *in vitro* (Gibson et al., 2014, 2017). Similarly, *in vivo* models with reduced inhibitory pathway functions have shown potential in enhanced modelling of hypersensitivity to drugs such as nevirapine (Mak and Uetrecht, 2015; Metushi et al., 2015). There is evidence for relevance of such findings with the suggestion of increased rates of hypersensitivity observed in patients co administering tyrosine kinase inhibitors with immune checkpoint inhibitors (Johnson et al., 2013). Whether this represents a true hypersensitivity reaction resulting from the downfall of immunological tolerance, or simply cumulative pharmacological interaction is a question that still needs to be addressed (Hammond et al., 2020, 2022).

4. Diagnostic methods of drug and chemical allergies

The current diagnostic approach for drug allergies is based firstly on a thorough clinical history which is often inconclusive (Demoly et al., 2014). This eventually leads to a complete allergy work-up, ideally 4 to 6 weeks after the resolution of the reaction, with standardized skin tests, biological, and occasionally *in vitro* T-cell tests, and finally, drug provocation tests or DPTs, which is the last step for accurate recognition of drug allergies (Bellón, 2019; Rive et al., 2013). These diagnosis methods help identify the molecule involved and better understand the physiopathology of these reactions to implement preventive measures (Chiriac and Demoly, 2014; Mayorga et al., 2019). It is noteworthy that diagnostic methods differ according to the region; for example, Europe and the United States follow different guidelines (Fernandez and Doña, 2018).

4.1 Diagnosis of IgE-mediated immediate reactions

In vivo, skin prick tests (SPT) and intradermal tests (IDT) are recommended and widely used for immediate reactions such as anaphylaxis or immediate skin reactions. They are utilized to identify the culprit molecule (Chiriac and Demoly, 2014). These skin tests should be performed as soon as possible (within the 4 to 6-week window) to avoid a decrease in sensitivity over time (Aun et al., 2017). Sensitivity and positive or negative predictive values are variable and drug dependent: high rates are reported for beta-lactams and NMBAAs (Fernandez and Doña, 2018). However, skin tests might also detect non-allergic reactions or (pseudo-allergies), mediated by the release of mediators from basophils and mast cells but not triggered by IgE cross-linking (Farnam et al., 2012). One of the mechanisms described, notably with fluoroquinolones and NMBAAs, is the direct binding to the MAS-related G protein-coupled receptor X2 (MRGPRX2) on the surface of mast cells. This triggers their degranulation and the release of mediators, such as tryptase and histamine, responsible for the onset of the reaction (Spoerl et al., 2017; Subramanian et al., 2016). A negative skin test, however,

does not entirely rule out the occurrence of specific IgE with other substances (Warrington et al., 2018), and a positive result suggests some sensitivity to the drug.

Other *in vitro* assays can also be indicated when skin tests are contraindicated or to increase the diagnostic sensitivity, as they allow the evaluation of mediators and cells involved in the acute phase of the disease or to identify the molecules involved after the resolution of the illness (Mayorga et al., 2016). The two most commonly used assays are the **detection of specific IgE** to the culprit and the **basophil activation test (BAT)** (Decuyper et al., 2017). The limited availability of commercial drugs restricts BAT use, and the sensitivity is variable (Decuyper et al., 2017; Vultaggio et al., 2009). It is low for BL (22-55%) (Sanz et al., 2002; Torres et al., 2004, 2017) and variable for NMBAs (64-90%) (Ebo et al., 2006, 2011; Torres et al., 2017). On the other hand, BAT does not require *in vivo* challenge as it detects cellular surface markers (mostly CD63, CD203c, and histamine release) but might detect pseudo-allergies as basophils can be activated through a non-IgE mediated mechanism (Leysen et al., 2011; Thinnes et al., 2018).

4.2 Diagnosis of T-cell-mediated delayed reactions

For delayed reactions, a **patch test** or **delayed-reading IDT** is used instead (Balakirski and Merk, 2017). The sensitivity of patch tests is approximately 64% for DRESS, 58% for AGEP, and 24% for SJS/TEN (Chiriac and Demoly, 2014). The diagnosis of ACD is usually established by a positive patch test (Esser and Martin, 2017). Cell-based assays also require *in vitro* stimulation by suspected molecules, such as the **enzyme-linked immunospot assay (ELISpot)**, which measures cytokine production, and the **lymphocyte transformation test (LTT)**, which measures the proliferation of memory T cells and identifies the culprit drug. This is mainly important for patients on multidrug therapy (Polak et al., 2013). Even though the majority of authors believe that the LTT is most useful in delayed-type reactions (type IV), its successful use in IgE-mediated immediate type reactions has also been demonstrated along with the involvement of CD4+ T cells in these reactions (Romagnani et al., 1991; Maggi and

Romagnani, 1994; Pichler and Tilch, 2004; Torres et al., 2006; Azoury et al., 2018a). These tests are not routinely done as they require a trained researcher in cell culture techniques and depend on the accessibility of isolated peripheral blood mononuclear cells from (PBMC) patients (Rozières et al., 2009). In other cases, such as severe cutaneous adverse reactions (SCARs), histopathology and tissue analysis of the cellular infiltrate from a skin biopsy would be favored (Mayorga et al., 2016).

4.3 Drug provocation test

The drug provocation test (DPT) is the gold standard for diagnosing allergic reactions accurately. It can be performed as a last resort after the results of the other tests (*in vivo* and *in vitro*) are negative (Chiriach and Demoly, 2014; Garvey and Savic, 2019; Mayorga et al., 2016). However, in mild reactions, a direct challenge without prior skin testing may be considered (Macy et al., 2023). It is time-consuming and involves a risk of serious, life-threatening complications that require high surveillance in a hospital setting and may be contraindicated, such as in severe drug reactions (Decuyper et al., 2017).

4.4 Algorithm of diagnosis

The evaluation of DHRs still heavily relies on diagnostic tests (**Figure 7**) (Demoly et al., 2014; Khan et al., 2022). Recent recommendations from the American Academy of Allergy Asthma and Immunology, the European Association of Allergy and Clinical Immunology, and the European Network of Drug Allergy on the drug allergy work-up specifically include pragmatic alternative pathways under conditions of the impending drug need or when the pretest probability is low, using minimal diagnostic procedures, and relying more on direct drug challenges (Jeimy et al., 2020; Khan et al., 2022; Romano et al., 2020; Torres et al., 2021).

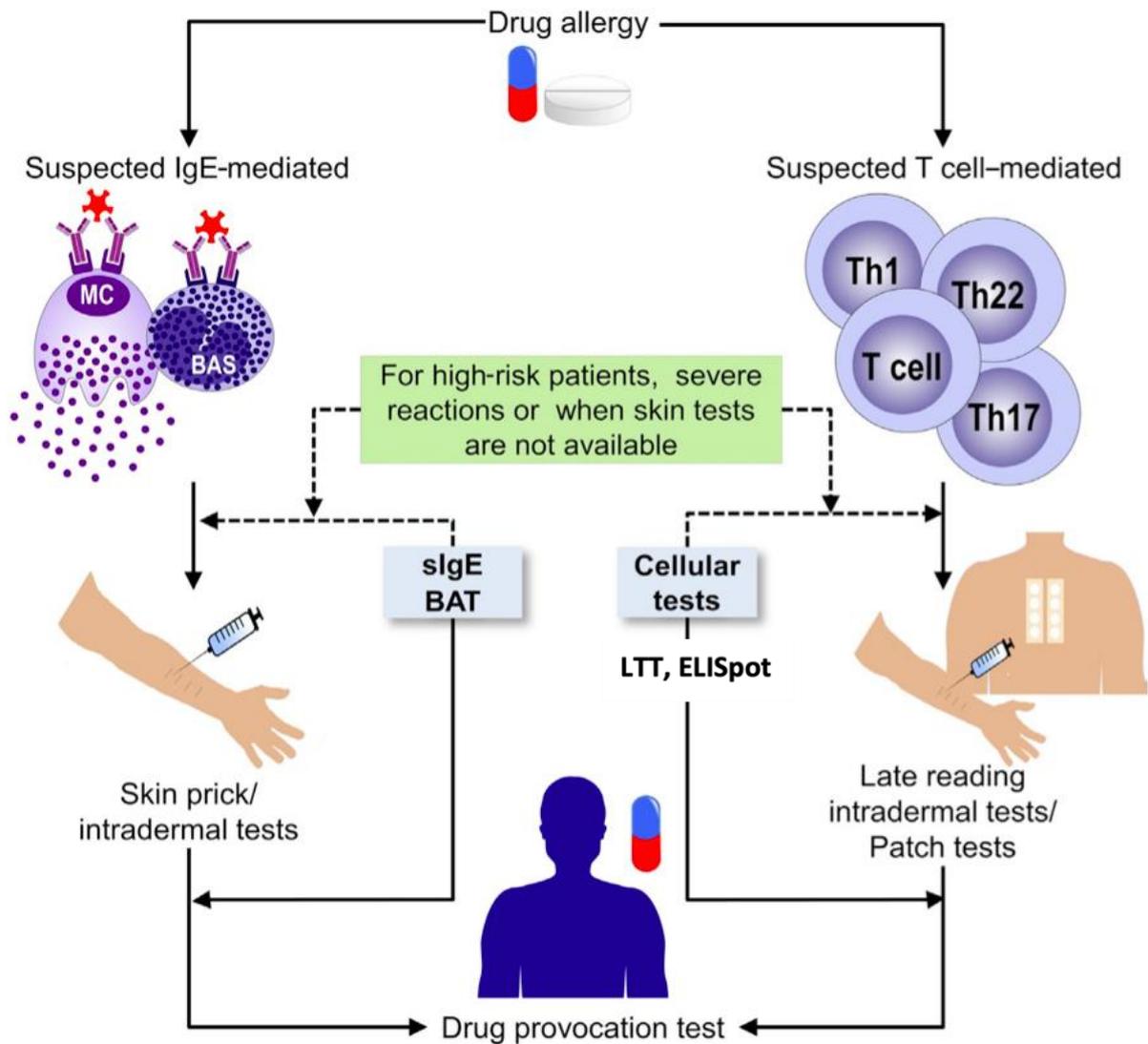


Figure 7. Allergology work-up algorithm for suspected drug hypersensitivity

adapted from (Mayorga et al., 2019)

BAS, basophils; BAT, basophil activation test; LTT: lymphocyte transformation test; ELISpot: enzyme-linked immunospot assay; MC, mast cells; sIgE, specific IgE.

5. Example of methods of detection of T-cell activation:

5.1 The lymphocyte transformation test

Numerous researchers have studied LTT as a diagnostic tool for immediate but mainly delayed hypersensitivity reactions (Pichler and Tilch, 2004; Torres et al., 2006; Polak et al., 2013). The patient's lymphocytes are isolated from PBMCs and cultured for 5 to 7 days with pharmacological concentrations of the suspected medication. The stimulation index (SI), calculated using radioactive thymidine (H-thymidine) uptake, measures the T-cell response. Typically, a SI > 2 is required for the positivity of the test (Pichler and Tilch, 2004). This test has resulted in favorable outcomes in clinical contexts and with several implicated medicines (Rive et al., 2013). However, the LTT approach for diagnosis has received very little attention in studies over the past ten years, except for case reports or small case series (Kim et al., 2013; Cabañas et al., 2014; Dias de Castro et al., 2015; Tomida et al., 2016). Indeed, apart from the intricate and time-consuming laboratory procedures, the use of radioactivity, and specialized tools, make it challenging to use the LTT as a diagnostic tool (Pichler and Tilch, 2004; Glässner et al., 2022). LTT has been reported to have a sensitivity of 27% (Porebski et al., 2013) to 74% (Nyfeler and Pichler, 1997) and a specificity of 85% (Nyfeler and Pichler, 1997; Rozieres et al., 2009) to 100%. (Porebski et al., 2013; Porębski et al., 2015).

5.2 The human T-Cell Priming Assay (hTCPA)

All the diagnostic tools listed above are performed after the onset of the reaction. However, an assay that could predict drug immunogenicity is still considered an unmet goal. In the last 20 years, new approaches have been emerging towards developing *in vitro* assays capable of predicting DHRs for new drug candidates. These methods are based on the detection of T-cell proliferation and cytokine release and aim to be considered as alternatives to animal testing to identify chemicals that present risks to human health (Hernandez-Jaimes et al., 2022).

The antigen-specific T-cell response to hapteneized proteins is the endpoint for chemical or drug sensitization. To measure the hapten-specific T-cell response brought on by test chemicals, the human T Cell Priming Assay (hTCPA) has been developed (Dietz et al., 2010a; Richter et al., 2013) using immature moDCs (monocyte-derived dendritic cells) and naive T cells from healthy donors. The immature MoDCs are treated with a non-cytotoxic concentration of the test drug or chemical to ensure their maturation and the presentation of the antigen. LPS is then added to speed up the maturation process. The matured MoDCs are then co-cultured with isolated naïve T cells for 5 to 10 days. Following the priming phase, the T cells are stimulated again using matured chemical-loaded MoDCs, unloaded MoDCs (background control), or cells loaded with an inert chemical for specificity control. The readout is performed by measuring T-cell proliferation, cytokine production, or the expression of cell surface markers (Martin et al., 2010b; Richter et al., 2013). Since then, multiple strategies have been implemented to enhance the sensitivity of the assays, including the depletion of immune-regulatory cells (CD56+ and CD25+ cells) and the use of CD1a+ MoDCs as effective antigen-presenting cells (Vocanson et al., 2014).

Much significant progress has been made in refining and restructuring the hTCPA. However, additional optimization and standardization are needed to use the assay to effectively and accurately assess the sensitization potential. Detecting weak and moderate sensitizers is still challenging; a high inter-donor variability is observed, and the assay is time-consuming (Vliet et al., 2018).

5.3 Repeated stimulation test

Over the past 10 years, the researchers in our laboratory have been working on the development and application of a new *in vitro* functional T cell assays for T cell repertoire identification of small molecules. This assay is highly sensitive *in vitro* mo-DC and autologous T-cell co-culture model to detect human naïve and total TL specific to drugs and chemicals and semi-quantify their frequency in healthy donors (Nhim et al., 2013a). The population of T cells studies varies between naïve CD4+ T cells, naïve CD8+

T cells, total CD4⁺ T cells and total CD8⁺ T cells, depending on the desired outcome. This assay has also been previously used for detection of naïve T-cells to biotherapeutics (Delluc et al., 2010, 2011).

Monocytes were isolated from PBMCs by positive antibody anti-CD14 magnetic sorting and differentiated into DCs at day 4 in the presence of GM-CSF and IL-4. Percentages of monocyte purity (CD14^{high}), and DC differentiation (DC-SIGN^{high}, CD1a^{high}) were evaluated by flow cytometry. T cells were then retrieved from CD14 negative cells of the same donor by magnetic sorting using the appropriate microbeads. T cells were co-cultured with autologous DCs previously loaded overnight (18h) with the drug or chemical antigen. The co-culture was conducted in the presence of IL-6 and IL-12 for the orientation towards a Th1 response. The particularity of this assay is that T cells are restimulated weekly for three rounds on days 7, 14, and 21 with autologous chemical or drug-loaded DCs. This allows the expansion of the specific T cells, usually present at low frequencies, and facilitates their detection. On day 28, an IFN- γ ELISpot was performed as the final readout (Nhim et al., 2013a). The specificity of the T cells response was confirmed if the spot count was at least two-fold higher in the presence of the chemical compared to unloaded DCs, and a minimum of 30 spots per well was required for the validity of the analysis.

Over the years, the protocol has been slightly enhanced and multiple drugs and contact sensitizers have been tested. A naïve T-cell repertoire has been recorded for benzylpenicillin (Nhim et al., 2013a; Azoury et al., 2015; Bechara et al., 2019a), nickel and cobalt (Bechara et al., 2019b). It suggested that some naïve T-cells can recognize these molecules bound to peptides presented through HLA-II but not that these T-cells were “specific” for these molecules.

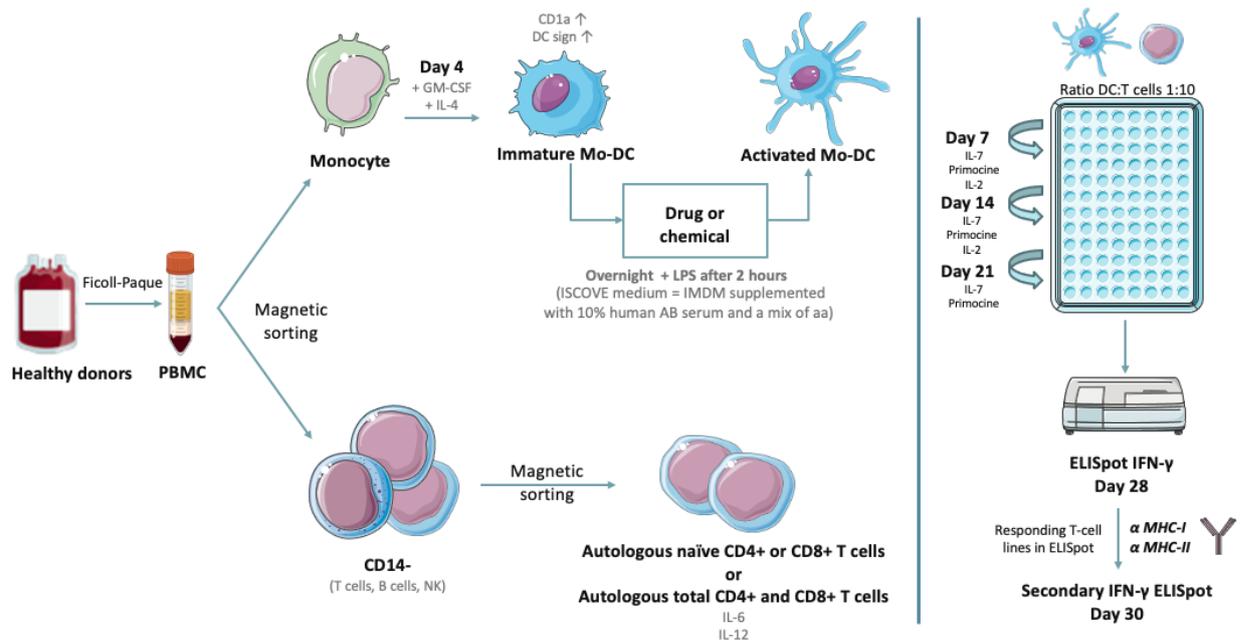


Figure 8. The repeated T-cell stimulation test

ELISpot: enzyme-linked immunosorbent spot assay; PBMC: peripheral blood mononuclear cells; GM-CSF: Granulocyte-macrophage colony-stimulating factor; IL: interleukin; Mo-DC: monocyte-derived dendritic cells; α-MHC: anti-major histocompatibility complex antibodies; NK: natural killers.

CHAPTER 2: MECHANISMS OF MOLECULE RECOGNITION BY IMMUNE CELLS

The immune system involves activated cells and mechanisms following exposure to foreign antigens such as bacteria, fungi, parasites, viruses, cancer cells, drugs, and chemicals. The immune system has two distinct defense mechanisms that trigger two types of immunity: innate and adaptive immunity. Innate immunity is the first line of defense against antigens and is activated immediately after exposure. It mainly involves monocytes and polynuclear cells. It is antigen-independent (non-specific) and lacks immunologic memory. However, adaptive immunity is antigen-dependent and specific, thus granting lasting protection. This chapter will outline the various cellular and molecular mechanisms involved in adaptive immunity following exposure to chemicals and drugs. If one of these steps is lacking, the probability of generating an effective adaptive immune response is reduced.

Drug or chemical HRs are diverse and comprise various effector cells and mediators. Multiple pathways are typically used to explain how these molecules can activate the immune system and cause clinical symptoms. The following are non-exclusive mechanisms involving a drug or chemical-specific adaptive immune response: the "hapten" model, the "pharmacologic interaction with immune receptors (p-i)" model, and the "altered self-repertoire" model.

1. The hapten concept

Based on studies in the early 20th century, the hapten theory was considered the classical explanation of how APCs can take up LMW antigens and trigger an immune response. It is defined as a relationship between the reactivity of the antigens with proteins and their ability to elicit an immune response. Indeed, drugs and chemicals (haptens) are generally electrophilic LMW compounds (Chipinda et al., 2011) unrecognized by the immune system and therefore do not trigger an immune

response. To become immunogenic, they must bind to larger soluble or cellular proteins or peptides, thus forming a chemical-protein complex that will be captured by APCs, processed, and drug-modified peptides will be presented to T cells (Cho and Utrecht, 2017). Therefore, a chemical's allergic potential is directly related to its ability to form these complexes or bio-conjugates (Martin et al., 2010b). The hapten concept was first described by Landsteiner and Jacobs, who determined that chemicals are capable of binding to animal proteins, triggering the immune system (Landsteiner and Jacobs, 1935). This occurrence was then referred to as "haptentation" ("to fasten" in Greek). Haptens are potent electrophiles that form covalent bonds with the nucleophilic residues of proteins, particularly lysines, cysteines, and histidines (Posadas and Pichler, 2007; Pichler and Hausmann, 2016).

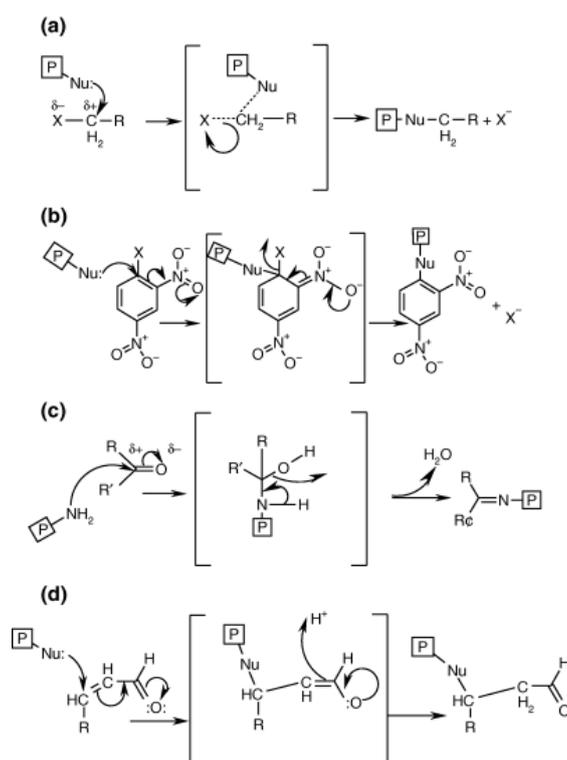


Figure 9. Examples of haptentation mechanisms adapted from (Divkovic et al., 2005) (a) Nucleophilic substitution on a saturated center, (b) nucleophilic substitution on an unsaturated center, (c) Schiff base formation, (d) 1,4-Michael addition. X, leaving group; Nu, nucleophile; P, protein.

1.1 Types of haptens

Four types of haptens are classified: classic haptens, pre-haptens, pro-haptens, and non-classical haptens.

1.1.1 Classic haptens

Classic haptens are reactive molecules that bind directly to proteins without prior metabolism or transformation (Pichler et al., 2011). Drugs and chemicals can bind to extracellular and intracellular proteins; however, haptentation with intracellular proteins is much less studied in the literature (Pickard et al., 2007; Sánchez-Gómez et al., 2017). Albumin is the most abundant protein in the human serum and is considered the primary target for drugs and chemicals (Fan et al., 2022).

Although several electrophilic (reactive) drugs behave like haptens, haptentation is best described for β -lactam antibiotics. BLs are a typical example of classic haptentation as they exhibit direct reactivity through the opening of their β -lactam ring (Ariza et al., 2015; Castells et al., 2019), which undergoes nucleophilic attacks, generally with the lysine residues of proteins (Stone et al., 2014). BLs bind to extracellular proteins, mainly albumin, transferrin, and Ig light and heavy chains (Ariza et al., 2014; Martín-Serrano et al., 2020). This haptentation has been exhibited with many BLs, including amoxicillin, benzylpenicillin (BP), piperacillin, flucloxacillin, and aztreonam (Jenkins et al., 2009; Meng et al., 2011; Ariza et al., 2012; Nhim et al., 2013b; Jenkins et al., 2013; Azoury et al., 2018b; Bechara et al., 2019a).

Furthermore, multiple chemicals were also found to be directly protein-reactive such as the strong human contact sensitizer DNCB or its water-soluble form DNBS (Karlberg et al., 2008). DNCB and DNBS covalently bind to nucleophilic residues of extracellular skin proteins such as albumin, cytokeratin 14, and cofilin by nucleophilic substitution of its aromatic ring. This results in the formation of stable dinitrophenol (DNP) adducts often used to characterize the haptentation of this molecule (**Figure 9**)

(Aleksic et al., 2008). However, DNCB and DNBS can also haptenize intracellular proteins (Pickard et al., 2007; Megherbi et al., 2009; Dietz et al., 2010b). The intra and extracellular haptentation of DNBS was evaluated in our work.

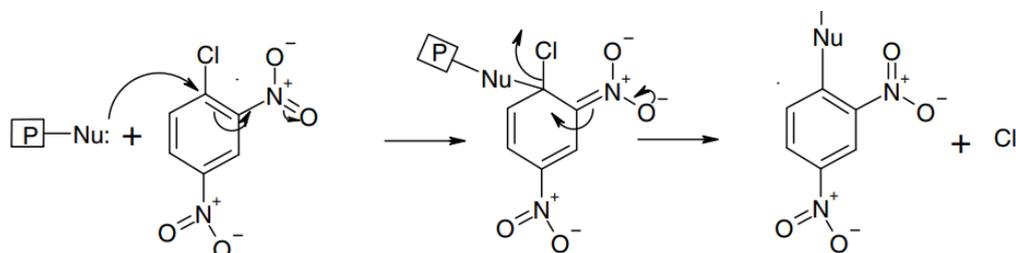


Figure 10. Mechanism of haptentation of 2,4-dinitrochlorobenzene (Aleksic et al., 2008)

Nu, nucleophile; *P*, protein.

1.1.2 Pro-haptens

Contrary to classic haptens, pro-haptens require in vivo metabolism to form reactive metabolites. These reactions are driven by metabolizing enzymes in the skin, the intestinal, or the pulmonary barriers. Most commonly, phase I enzymes trigger the metabolism, primarily alcohol or aldehyde dehydrogenase (ADH) and CYP450 (Kaplan et al., 2012).

Numerous drugs are considered pro-haptens, such as SMX, CBZ, and NVP. Indeed, SMX is the most described pro-hapten and is metabolized in the liver by CYP2C9, or myeloperoxidase, into SMX-hydroxylamine (SMX-NHOH). The latter undergoes spontaneous oxidation into nitroso-SMX (SMX-NO), which then binds to soluble or cellular proteins (Naisbitt et al., 1999; Ogese et al., 2015). Moreover, CBZ and NVP are predominantly metabolized to produce different epoxide/arene oxide reactive metabolites with cysteine and histidine residues of human serum albumin (HSA) (CBZ, NVP) and hemoglobin (NVP) (Lu et al., 2008; Caixas et al., 2012; Meng et al., 2013; Faulkner et al., 2014). CBZ is primarily metabolized by CYP 3A4/2C19/3A5/3A7/1A2 and myeloperoxidase (Pearce et al., 2005, 2008), while NVP appears to be metabolized by CYP 3A4/2B6 in the liver and then sulfated at the cutaneous level (Sharma et al.,

2013a, 2013b). Other contact sensitizers, such as eugenol and cinnamic alcohol, are also known to act like pro-haptens (Gerberick et al., 2009; Lee et al., 2018).

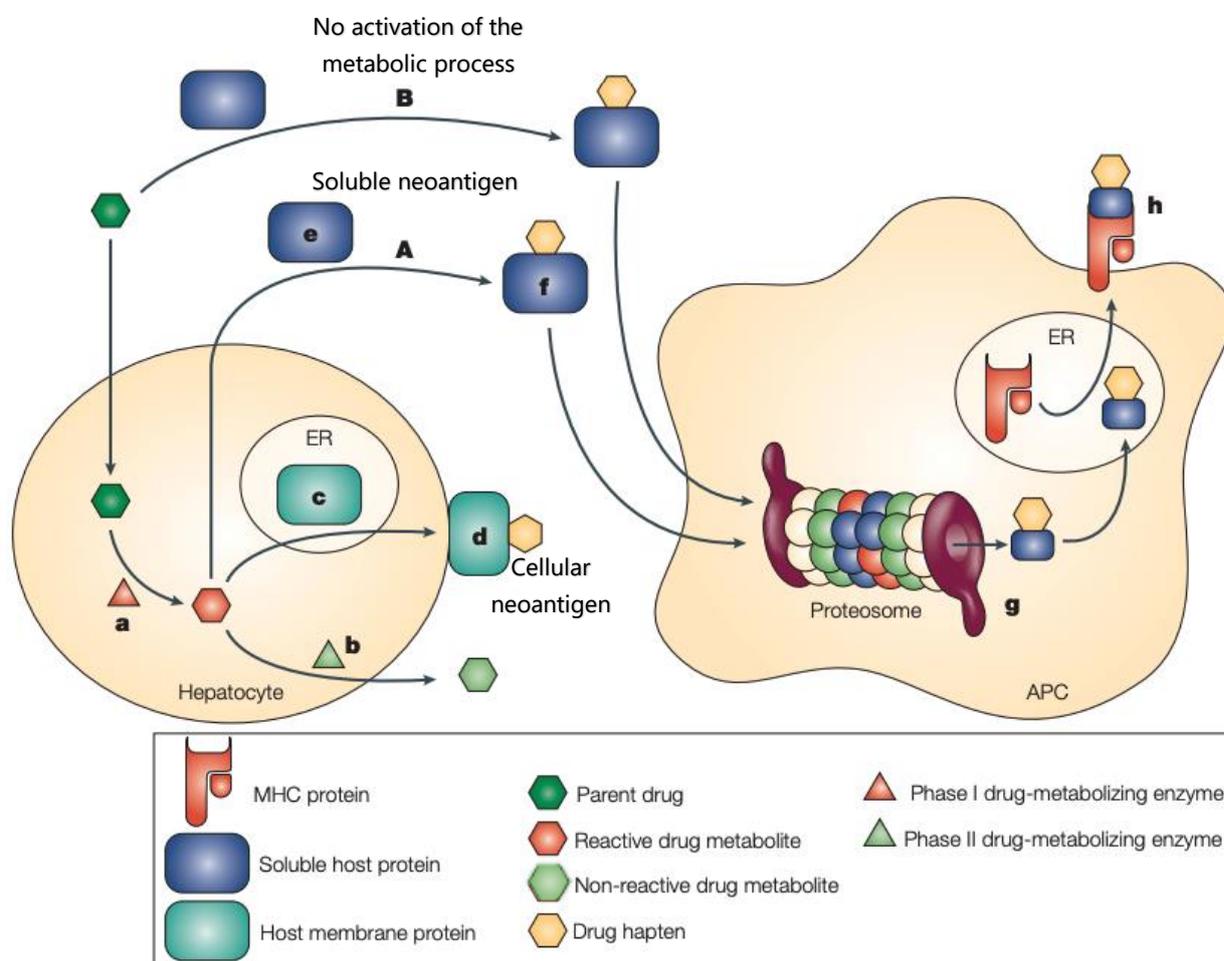


Figure 11. Formation of haptens and pro-haptens are necessary for antigen capture and presentation by antigen-presenting cells adapted from (Bugelski, 2005)

1.1.3 Pre-haptens

Pre-haptens require a chemical non-enzymatic transformation to become reactive haptens and bind to proteins (Kaplan et al., 2012). Most commonly, the mechanisms involved are oxidation by atmospheric oxygen or photo-activation by an ultraviolet light (Esser and Martin, 2017). Most pre-haptens are non-drug chemical molecules,

such as PPD, used in hair dyes and colorants. It undergoes auto-oxidation in the skin, forming a reactive electrophilic quinone diimine (Pot et al., 2013). The intermediates then undergo oxidation-conjugation reactions before forming a Brandowski base, which can bind to cysteine residues of proteins (Jenkinson et al., 2010; Faulkner et al., 2014). Other molecules described are hydroquinone and isoeugenol (Gerberick et al., 2009).

1.1.4 Non-classical haptens

Unlike classical haptens, the haptentation of non-classical haptens (metals) does not result in covalent bonds but reversible coordination complexes with proteins. (Thierse et al., 2005). Thus metals such as nickel, cobalt, or chromium can shape particular configurations when bound to proteins (Zhang and Wilcox, 2002). This configuration diversity might explain the difficulty in accurately and adequately characterizing the epitopes involved in metal allergies (Thierse et al., 2005).

Nickel is the most frequent cause of contact allergy worldwide. It is found in metal form when in contact with the skin. It only becomes sensitizing when it is converted to Ni²⁺ ions by air corrosion, making it reactive and allowing it to form coordination complexes with proteins (Franken et al., 2015). Furthermore, the sensitizing power of nickel is increased with its degree of oxidation (Artik et al., 1999). The presence of the HSA-nickel complexes with *in vitro* co-cultures has been shown to trigger a nickel-specific lymphocyte response that may be responsible for the allergic reactions observed in patients (Martin et al., 2006).

1.2 Hapten capture and processing by dendritic cells

The first step of an adaptive immune response requires antigen presentation by APCs to T cells through the MHC class I and II molecules. In this section, we will detail the role of DCs in priming haptens and the pathways of antigen presentation to T cells.

1.2.1 The role of dendritic cells in antigen presentation

DCs play a significant role in initiating the immune response (Hilligan and Ronchese, 2020). Their primary function is capturing, priming, and transporting antigens to lymphoid organs for presentation to naïve T cells (Eisenbarth, 2019). DCs capture endogen or exogen antigens in peripheral tissues and migrate to the draining lymph nodes. They present the antigen to CD4+ or CD8+ T cells through MHC class II and I molecules, respectively. Depending on their activation and cellular microenvironment, they can trigger a tolerogenic or inflammatory response (Théry and Amigorena, 2001; Hilligan and Ronchese, 2020).

DCs are derived from hematopoietic stem cells (HSCs), which differentiate into monocyte and DCs progenitor (MDP) and then into common DCs progenitor (CDP) (Merad et al., 2013; Matsuo et al., 2021). DCs are divided into two main subpopulations based on their ontogeny: myeloid conventional DCs (cDC1, and cDC2) that can be further subdivided into resident and migratory, and plasmacytoid DCs (pDCs) that are thought to be of lymphoid or myeloid origin (Rodrigues et al., 2018; Dress et al., 2019; Eisenbarth, 2019; Cabeza-Cabrerizo et al., 2021). Monocyte-derived DCs (moDCs) and langeran cells (LCs) are also considered other subtypes that arise independently of DC-ontogenic pathways. All these subpopulations are distinguished by their phenotype and functions (**Figure 12**) (Hilligan and Ronchese, 2020).

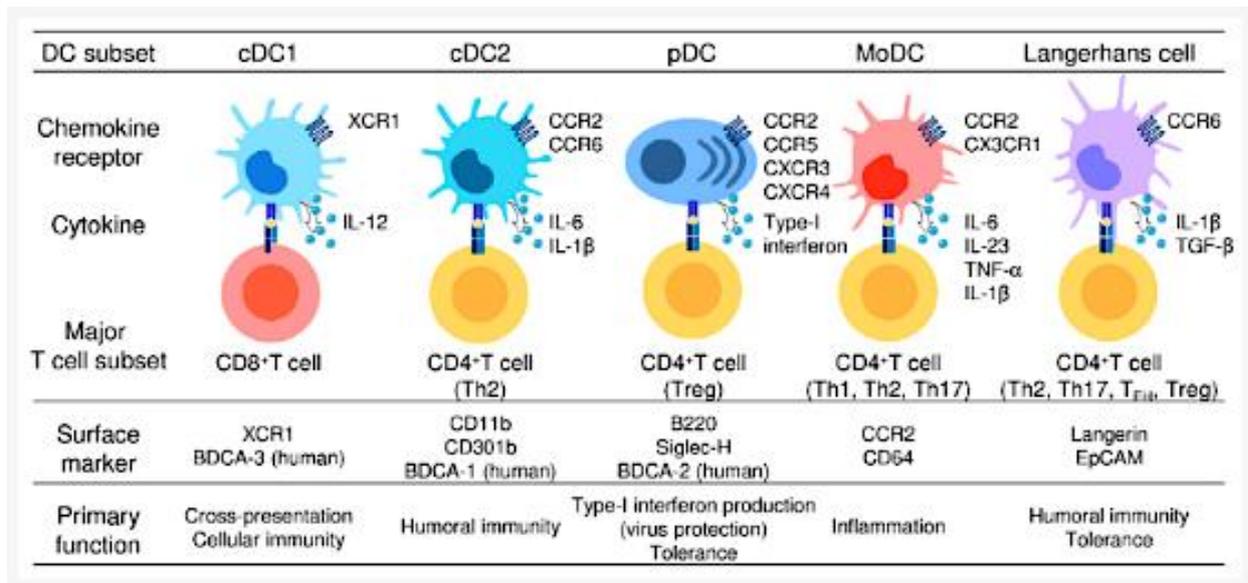


Figure 12. Different subsets of dendritic cells adapted from (Matsuo et al., 2021)

Conventional DCs, or cDCs, are the most common population of DCs. They are divided into two subtypes: cDC1 and cDC2 (Cabeza-Cabrero et al., 2021). The cDC1 express XCR1 (XC receptor 1) and BDCA3 molecules and have been described to cross-present antigens to CD8⁺ T cells. The cDC2s are identified by the expression of CD11b, DCIR2 (DC immunoreceptor 2), and SIRP α (signal regulatory protein- α). Unlike cDC1, the latter is described as the primary activating cells of naive CD4⁺ T cells. The cDC1 and cDC2 are further subdivided into resident and migratory cells depending on their localization and how they detect antigens (Eisenbarth, 2019).

On the other hand, pDCs produce considerable amounts of type I interferons, have been associated with numerous autoimmune and inflammatory disorders, and are essential for antiviral immunity (Ye et al., 2020). MoDCs originated from monocytes. They constitute a unique subset of DC implicated in inflammation and infection. They are commonly used in cell culture as APCs after differentiation from circulating monocytes (Jeras et al., 2005; Marzaioli et al., 2020). While CDP are the source of cDCs and pDCs, LCs develop from erythromyeloid precursors that seed in the epidermis. LCs

are intraepithelial subpopulation of DCs in part characterized by the expression of the C-type lectin receptor langerin (Wysocki and Shlomchik, 2013; Ono and Kabashima, 2016; Tanne and Bhardwaj, 2017).

1.2.2 Mechanisms of antigen capture

APCs should internalize antigens before presentation to T cells. Three primary endocytic internalization mechanisms are identified based on the solubility of the antigen: receptor-mediated endocytosis, macropinocytosis, and phagocytosis (**Figure 13**) (Roche and Furuta, 2015). Soluble antigens are non-specifically captured along with extracellular fluid by macropinocytosis. Indeed, DCs are characterized by a high basal level of macropinocytosis, allowing them to ingest large amounts of fluid into macropinosomes. This process is actin-dependent and requires the involvement of several intracellular molecules, including GTPases such as Rab5 or ADP-ribosylation factors (ARFs) (Jones, 2007; Lim and Gleeson, 2011). Insoluble antigens are preferentially internalized via receptor-mediated endocytosis, such as lectins-C receptors, mannose receptors, CD209 receptors, and langerin receptors into small vesicles called early endosomes (Guermonprez et al., 2002). These early endosomes ultimately fuse with endosomal-lysosomal antigen-processing compartments where the proteolysis and peptide-MHC class II complex formation occurs.

On the other hand, phagocytosis allows DCs to internalize larger antigens, apoptotic cells, and opsonized pathogens by an actin-dependent membrane reorganization around the particle, which enters the cells in membrane-derived phagosomes. This process can be receptor-independent and triggered by activating surface molecules such as scavenger receptors (MARCO, CD36) (Roche and Furuta, 2015).

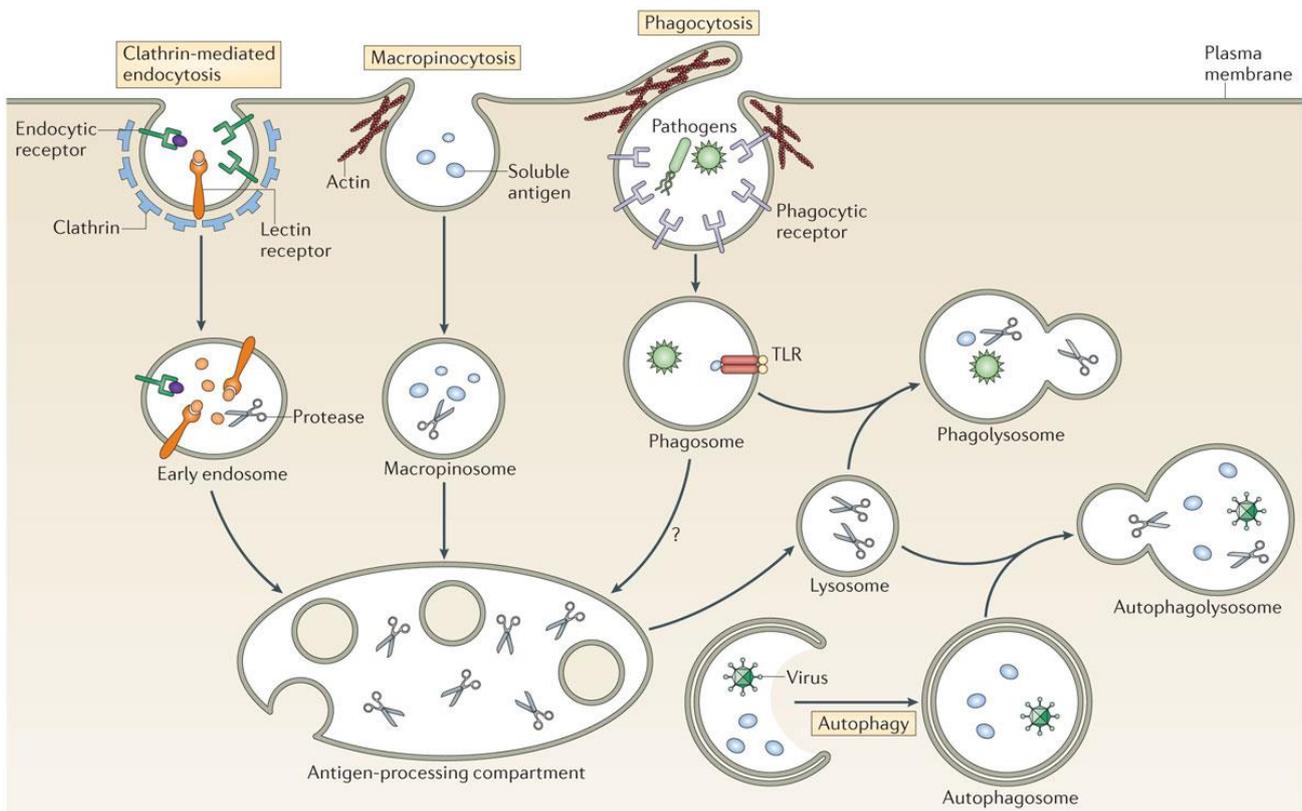


Figure 13. Antigen internalization pathways in dendritic cells adapted from (Roche and Furuta, 2015)

Exogenous molecules are internalized by dendritic cells via macropinocytosis, phagocytosis, or endocytosis.

1.2.3 Mechanisms of antigen processing and presentation

The presentation of haptenedated peptides requires the peptide to travel through all phases of antigen processing, MHC loading, and transportation to the cell surface before the T-cell contact (White et al., 2014).

i. Major histocompatibility complex molecules

MHC molecules (or HLA) are membrane glycoproteins whose primary function is to bind to self or antigen-derived peptides and display them on the cell surface for recognition by the specific T cells (Charles A Janeway et al., 2001). They are subdivided into three gene classes: I, II, and III. However, only MHC class I (HLA A, B, C) and II (HLA DP, DQ, DR) molecules present antigenic peptides to CD8+ and CD4+ T cells, respectively (Holland et al., 2013). The two classes have different structures (**Figure**

14). MHC class I molecules contain a large transmembrane α heavy chain that has three extracellular domains $\alpha 1$, $\alpha 2$, and $\alpha 3$ associated with a smaller invariant $\beta 2$ -microglobulin ($\beta 2m$) light chain. However, MHC class II molecules are formed by two α and two β chains, each with two extracellular domains. Unlike MHC class I molecules that are ubiquitous on all nucleated cells, MHC class II molecules are only expressed on professional APCs (Holland et al., 2013; Mak et al., 2014).

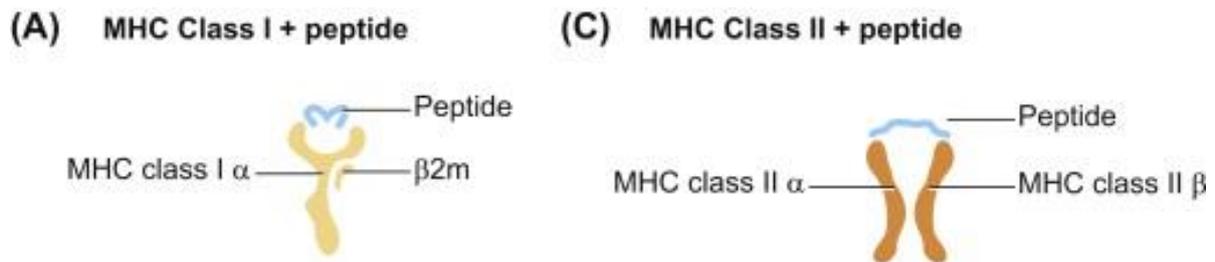


Figure 14. Presentation of antigen peptides on MHC Class I and II molecules

adapted from (Mak et al., 2014)

(A) MHC class I molecules are formed by a heavy chain with three extracellular domains. $\alpha 1$ and $\alpha 2$ form a closed groove in which the antigenic peptide of 9 amino acids (aa) is located on average (8-13 aa). The $\alpha 3$ domain is associated with an invariant light chain $\beta 2$ -microglobulin ($\beta 2m$). **(B)** MHC class II molecules are formed by two α and two β chains, each with two extracellular domains. The $\alpha 1$ and $\beta 1$ domains limit the presentation groove to about 15 aa (12-20 aa).

ii. [Antigen presentation](#)

The origin of the antigen dictates the orientation of antigen processing toward the exogenous pathway (for extracellular antigens) or the endogenous pathway (for intracellular antigens) **(Figure 15)**. DCs internalize extracellular antigens into early endosomes that undergo acidification and trigger proteolysis by endosomal proteases (Neefjes et al., 2011; Heijmans et al., 2020). Simultaneously, MHC II molecules are synthesized in the endoplasmic reticulum (ER) and associated with the class II-associated invariant chain peptide (CLIP), which protects their anchoring pockets. The final step is driven by HLA-DM and HLA-DO molecules and involves releasing the CLIP protein to allow binding to an antigenic peptide (Kobayashi and van den Elsen, 2012; Jurewicz and Stern, 2019). The MHC II-peptide complex formed is then directed to the

plasma membrane for presentation to the T-cell receptors (TCR) of CD4⁺ T cells (Trowsdale and Knight, 2013; Holland et al., 2013). This is the case of chemical or drug molecules that act like haptens and where antigen priming would play an important role in the activation of specific T cells.

On the other hand, intracellular antigen processing and presentation of peptides is done by MHC I molecules and involve the proteasome (Kobayashi and van den Elsen, 2012). The proteasome is a complex of approximately 30 proteins that cluster into two subunits: the catalytic core and the regulatory complexes. These subunits form the 26S proteasome, which is responsible for most non-lysosomal protein degradation within cells (Kotsias et al., 2019; Heijmans et al., 2020). During pro-inflammatory conditions exposing cells to IFN- γ , proteasome subunits (including LMP2) form the immunoproteasome that generates antigenic peptides (Kotsias et al., 2019). The peptide-loading complex (PLC) then transports the antigenic peptides through transporters associated with antigen processing (TAP), endoplasmic reticulum resident protein 57 (ERp57), and calnexin-calreticulin to the ER for binding to MHC I molecules. The MHC I-peptide complex is then directed to the Golgi for externalization and presentation to CD8⁺ T cells (Neefjes et al., 2011; Holland et al., 2013; Heijmans et al., 2020). While some chemicals and therapeutic molecules have been shown to interact with the MHC class I or II molecule, there is no comprehensive list of drugs and therapeutic molecules that are specifically presented on MHC class I or II to CD8⁺ or CD4⁺ T cells.

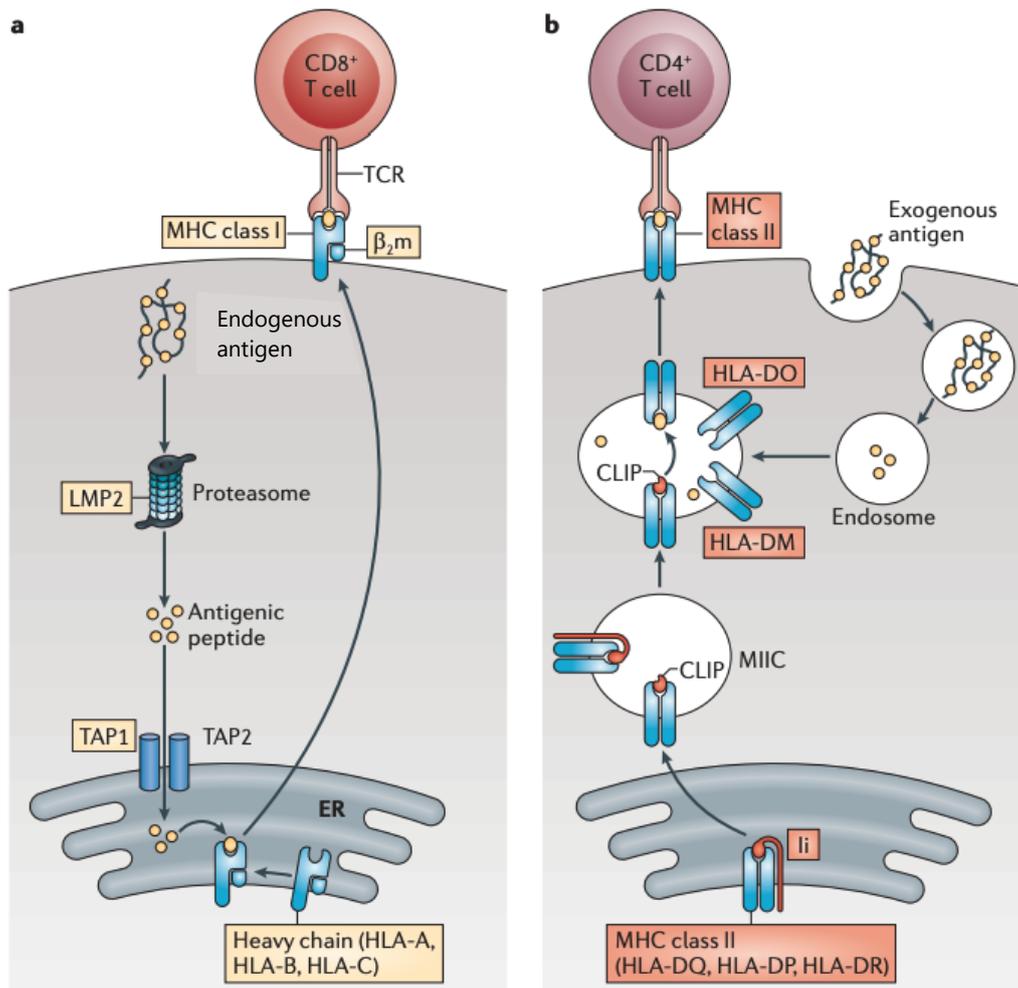


Figure 15. Presentation of intracellular and extracellular antigens to CD4+ and CD8+ T cells adapted from (Kobayashi and van den Elsen, 2012)

(a) The immunoproteasome, formed by several subunits, including LMP2, converts intracellular antigens into peptides. These peptides are transported into the endoplasmic reticulum (ER), loaded in the MHC class I complex groove, and presented to CD8+ T cells.

(b) Endo-lysosomal enzymes convert extracellular antigens into peptides. These peptides bind to the MHC class II binding groove by dislocating the class II invariant chain-associated peptide (CLIP). HLA-DO and HLA-DM direct the antigen-loading process. The MHC class II complex presents antigens to CD4+ T cells. TAP, transporter associated with antigen processing; TCR, T-cell receptor; MIIC, MHC class II compartment.

iii. Mechanisms of cross-presentation

MHC I molecules can occasionally present extracellular antigens to trigger a CD8+ cytotoxic response. This mechanism is called "cross-presentation" (Amigorena and Savina, 2010; Veerappan Ganesan and Eisenlohr, 2017). DCs predominantly drive cross-presentation; however, several other APCs can also cross-present. cDC1s have low amounts of endosomal proteases, which favors the formation of early endosomes (Eisenbarth, 2019). These are characterized by slow acidification and a low capacity to degrade extracellular antigens, which preferentially directs toward the cross-presentation (Joffre et al., 2012; Merad et al., 2013; Alloatti et al., 2016). There are two pathways of cross-presentation: the cytosolic pathway and the vacuolar pathway **(Figure 16)**.

The cytosolic pathway is the most common pathway where the antigen is released into the cytosol directly after being internalized for priming by the proteasome. The peptides produced can join the classical pathway by being transported to the ER through TAP, where they can be conjugated to MHC I molecules. The binding could also take place in the phagosome. Indeed, the SEC22b protein localized at the ER-Golgi intermediate compartment (ERGIC) interacts with syntaxin 4 in the endo/phagosome and allows the recruitment of different ER components, including TAP. This pathway is sensitive to proteasome inhibitors (Joffre et al., 2012; Alloatti et al., 2016). In the vacuolar pathway, the antigen is degraded within the phagosome compartment in a proteasome-independent, thus not sensitive to proteasome inhibitors but to endosome alkalinizing molecules such as chloroquine (Joffre et al., 2012).

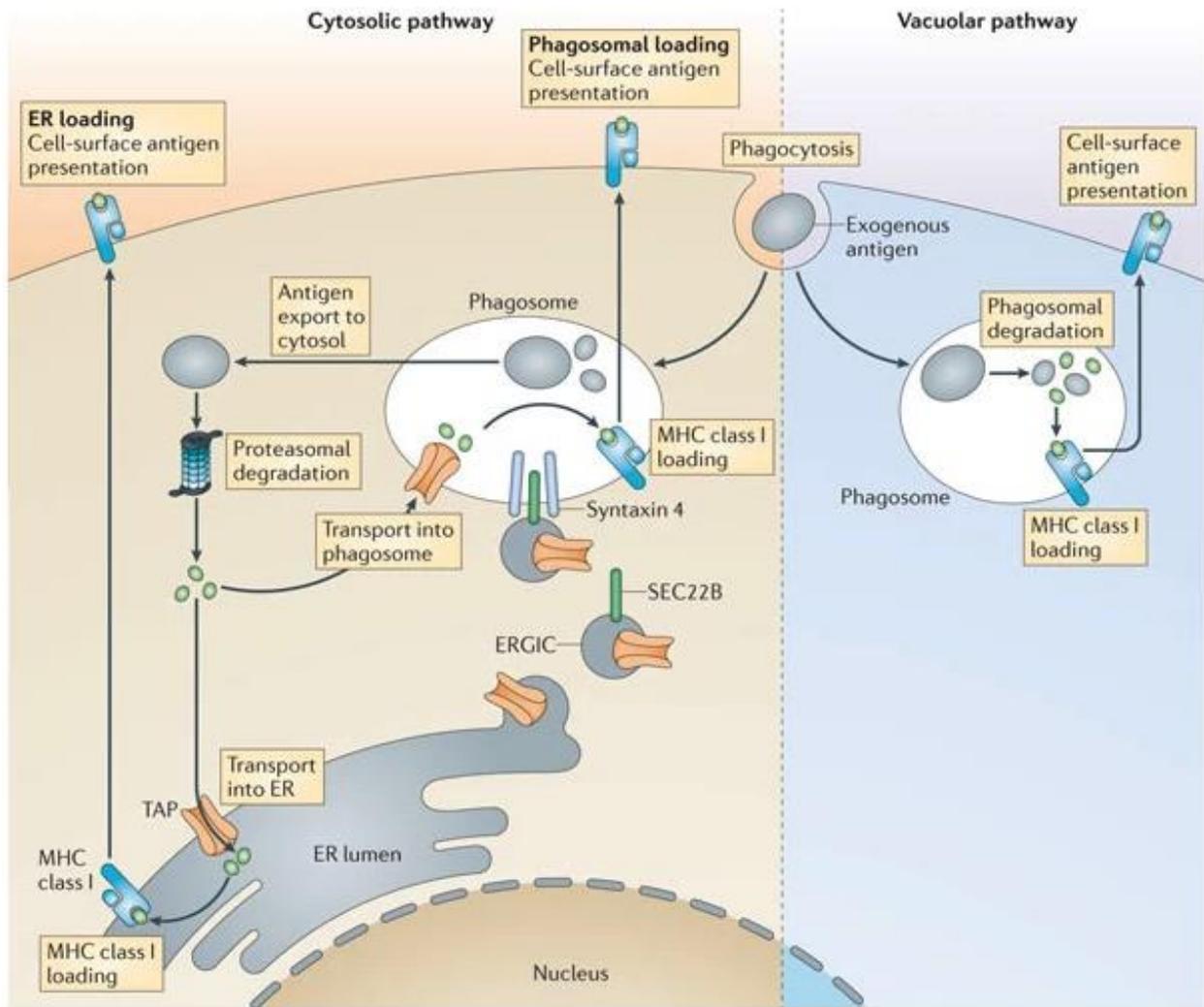


Figure 16. Intracellular pathways for cross-presentation in dendritic cells adapted from (Joffre et al., 2012)
TAP: Transporter associated with antigen processing; ERGIC: ER-Golgi intermediate compartment.

1.3 Immunological and chemical danger signals.

Antigen capture, processing, and presentation by DCs are insufficient to elicit an immune response. DCs require the presence of so-called "danger signals" to trigger their maturation and allows efficient presentation of the antigen to T cells (Gallucci and Matzinger, 2001).

Danger signals are necessary to overcome immune tolerance barriers that otherwise may suppress the immune response (Li and Uetrecht, 2010; Pirmohamed et al., 2002). They induce the maturation of DCs (Matzinger, 1994; Gallucci and Matzinger, 2001; Matzinger, 2002), thus alerting the DCs to eliminate the detected antigen. Consequently, DCs gain a mature (or effector) phenotype (Matzinger, 1994, 2002), losing their ability to internalize other antigens in favor of their migration, priming, and presentation of the specific antigens.

1.3.1 Types and mechanisms of danger signals

There are two significant categories of danger signals: "pathogen-associated molecular patterns" (PAMPs), usually expressed by exogenous pathogens, or "damage-associated molecular patterns" (DAMPs), which are endogenous danger signals secondary to cellular damage (Matzinger, 2002; Kaplan et al., 2012). PAMPs are formed by pathogens such as bacteria, parasites, viruses, or fungi. They include nucleic acids, fungal wall components (β -glucan and α -mannan), bacterial proteins (flagellins), or bacterial wall components such as lipopolysaccharide (LPS) (Kaplan et al., 2012). DAMPs are intracellular molecules excreted into the extracellular compartment due to stress or cell death. DAMPs include proinflammatory cytokines such as TNF- α and IL-1 β , uric acid, nucleic acids, adenosine triphosphate (ATP), ROS, high mobility group box one protein (HMGB1) secreted by necrotic cells, heat shock proteins (HSP), and interferon-gamma (IFN- γ) (Tang et al., 2012; Schaefer, 2014). A new family of danger signals has recently been introduced: the nanoparticle-associated molecular patterns (NAMPs) (Gallo and Gallucci, 2013; Farrera and Fadeel, 2015; Pallardy and Bechara, 2017b; Feray et al., 2020).

DCs detect danger signals via pattern recognition receptors or PRRs (**Figure 17**). These receptors can be expressed on the cell surface [Toll-like receptors (TLR), C-type lectin receptors (CLR)] or intracellularly [TLR; Nod-like receptors (NLR), inflammasome, and RIG-like receptors (RLR)] (Schaefer, 2014). The most common and best-characterized PRRs are the TLRs. Ten TLRs are found in humans, all expressed in

cDCs (Sato and Akira, 2016). All TLRs are located on the cell membrane except for 3, 7, 8, and 9, expressed at the endosomal membrane. TLRs are classified according to the type of danger signal they recognize. TLRs 1, 2, 4, and 6 recognize lipids and lipopeptides; TLRs 3, 7, 8, and 9 recognize nucleic acids, while TLR 5 recognizes proteins, particularly bacterial flagellin (Brennan and Gilmore, 2018; Sadeghalvad et al., 2022). In particular, TLR4 is known to recognize LPS, a significant component of the wall of gram-negative bacteria (Sato and Akira, 2016; Brennan and Gilmore, 2018).

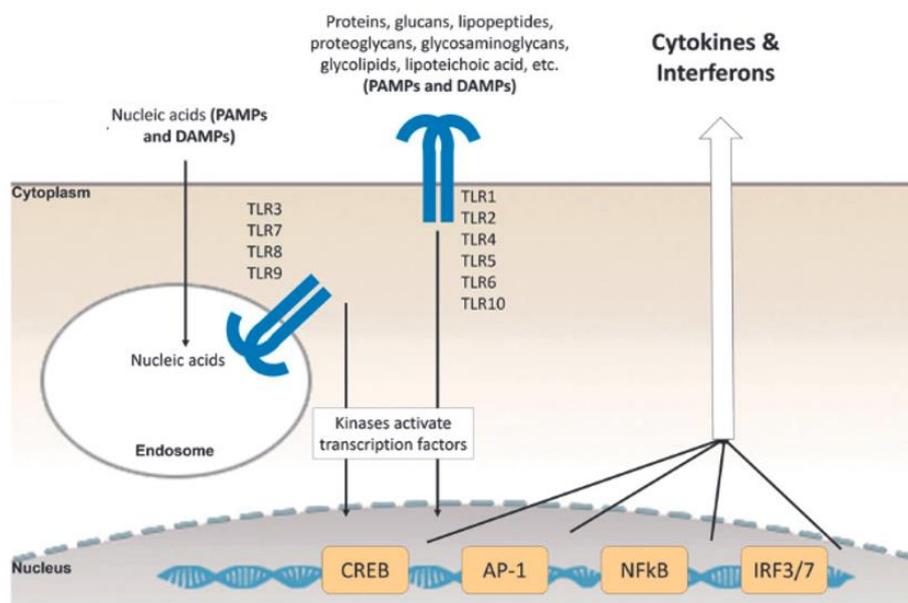


Figure 17. Danger recognition by the innate immune system adapted from (D'Arpa and Leung, 2017)

Activation of a TLR, except for TLR3, triggers a MyD88 (myeloid differentiation factor 88) adaptor-dependent pathway resulting in the activation of MAPKs (mitogen-activated protein kinases) such as p38, JNK and ERK, and the NF- κ B (nuclear factor kappa B) transcription factor pathway (Kaplan et al., 2012). This activation triggers the secretion of proinflammatory cytokines such as IL-1 β (Yu et al., 2010). TLR3 passes through the TRIF (TIR-domain-containing adapter-inducing IFN- β), which triggers the

activation of the transcription factors IRF3 (IFN-regulatory factor 3) and NF- κ B, resulting in the secretion of type I IFN (**Figure 17**) (Lim and Staudt, 2013; Mohammad Hosseini et al., 2015; De Nardo, 2015). These cellular pathways ensure the maturation of DCs and the secretion of proinflammatory cytokines.

1.3.2 Maturation of dendritic cells

Once the DCs detect a danger signal, they initiate a maturation process that allows them to prime the antigen into antigenic peptides that will be conjugated to MHC molecules for presentation on the cell surface (Neeffjes et al., 2011; Holland et al., 2013). This process is a prerequisite for initiating the lymphocyte response (Bakdash et al., 2013).

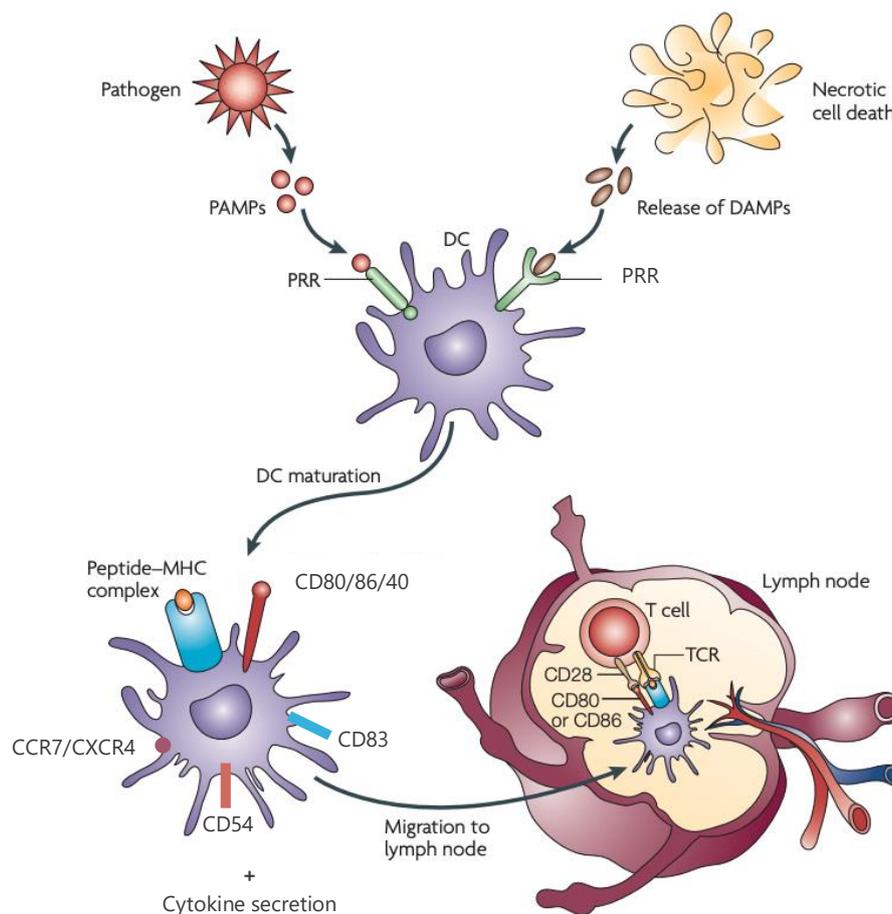


Figure 18. Maturation markers of dendritic cells adapted from (Kono and Rock, 2008)

As DCs transition from an immature to an activated state, they lose their ability to uptake and process new antigens instead, they migrate to present antigenic peptides to antigen-specific naïve T cells in the lymph nodes (Steinman et al., 1997; Wang et al., 2020). DCs will thus lose the expression of E-cadherin molecules that allow them to adhere to epithelial cells, and some chemokine receptors (CCR1, CCR2, CCR5, and CXCR1) (Seyfizadeh et al., 2016) and acquire the expression of proteolytic enzymes like metalloproteinases (MMP2, MMP9) and other chemokine receptors that allow them to migrate from the epidermis to the dermis (case of LCs) and from peripheral tissues to lymph nodes (Seyfizadeh et al., 2016). Thus the migration of different subtypes of DCs are ensured by different chemokines. The couple CXCL12/CXCR4 will be responsible for LC migration from the epidermis to the dermis (Ouweland et al., 2008). On the other hand, several chemokines are involved in DC migration to lymph nodes. Mature DCs acquire expression of CCR7: receptor for CCL21 and CCL19 (Kabashima et al., 2007; Bakdash et al., 2013). In addition to chemokines, an important role of the nucleotide-binding leucine-rich repeat-containing receptor 12 (NLRP12) in DC migration to lymph nodes without affecting their maturation has recently been demonstrated (Seyfizadeh et al., 2016).

The expression of MHC molecules on the surface of DCs is increased, as well as the expression of other molecules such as adhesion molecules (CD54), activation molecules (CD83), and co-stimulatory molecules to activate T-cells (CD40, CD80, and CD86) (**Table 3**) (Ryan et al., 2007). This maturation also triggers the secretion of pro-inflammatory cytokines allowing for T-cell activation and polarization, which we will detail in the following sections (Brennan and Gilmore, 2018).

Table 3. Phenotype differences between immature and mature dendritic cells

adapted from (Hardonnière et al., 2022)

Immature dendritic cells		Mature dendritic cells
FcR Mannose receptor E-cadherine ++ Langerin ++	← Uptake	FcR Mannose receptor - E-cadherine +/- Langerin +/-
CCR7 -	Migration →	CCR7 ++
MHC II + CD40 - CD83 - CD86 +/-	Antigen presentation co-stimulation →	MHC II ++ CD40 ++ CD83 ++ CD86 ++

1.3.3 Examples of chemicals and drugs

The phenotypic changes enable DCs to migrate from peripheral tissues to regional lymph nodes and to promote clonal expansion of specific T cells. Organic molecules such as DNCB or trinitrochlorobenzene (TNCB), eugenol, CinA, and metals such as nickel and cobalt have all been shown to upregulate the expression of one or more activation and co-stimulatory molecules (CD40, CD80, and CD86), CD83, CD54 and HLA-DR. Nickel and DNCB are also capable of inducing IL-6, IL-1, IL-8, TNF- α , and IL-12p40 production in human DCs (Coutant et al., 1999; Tuschl et al., 2000; Aiba et al., 2003; Boislève et al., 2005; Antonios et al., 2009, 2010; Rachmawati et al., 2013; de Bourayne et al., 2017). However, the mechanism by which drugs induce danger signals remains poorly documented (Séguin and Uetrecht, 2003; Galbiati et al., 2016). SMX and its reactive metabolite SMX-NO induce CD40 expression without altering CD80, CD83, and CD86 in human DCs (Sanderson et al., 2007b). Danger signals significantly increase SMX-protein adducts in DCs *in vitro* (Lavergne et al., 2009). Most recently, researchers showed that MAPKs and NF- κ B are involved in amoxicillin (AX)-induced DC phenotype modifications in mo-DCs from AX-delayed allergic patients (Lopez et al., 2015). Additionally, some penicillins can also induce DC maturation *in vitro*, identified by an increased expression of IL-1 β (BP, penicillin V) and genes involved in

oxidative stress such as NAD(P)H dehydrogenase (quinone 1) (NQO1) (BP, penicillin V, and ampicillin) (Sebastian et al., 2012).

It has been well described in the literature that the DC phenotype following activation by chemicals or drugs is regulated by a network of intracellular signaling pathways such as the TLR activation previously described (MAPK, NF- κ B and IRF), but also the direct activation of dendritic cells. This activation involves the inflammasome, the kinases [spleen tyrosine kinase (Syk), casein kinase 2 (CK2), protein kinase C (PKC)], the production of ROS which leads to the activation of the Janus kinase (JAK) pathway, and the induction of oxidative stress in DCs which is related to the activation of the nuclear factor erythroid-2-related (Nrf-2) transcription factor (Boislève et al., 2004; Sanderson et al., 2007b; Lavergne et al., 2009; Sharma et al., 2013b). Therefore, several allergic molecules may provide danger signals resulting in direct activation of these signaling pathways resulting in modifications of the phenotypes of dendritic cells and secretion of cytokines and chemokines (Boislève et al., 2004, 2005; Sanderson et al., 2007b; Lavergne et al., 2009; Sebastian et al., 2012; Sharma et al., 2013b; X. Zhang et al., 2013b). Several studies have shown that nickel is a direct agonist of TLR4 by binding to the imidazole groups of two histidine residues (His 456 and His 458) present in human TLR4 (Schmidt et al., 2010; Raghavan et al., 2012; Rachmawati et al., 2013; Bechara et al., 2017). Consequently, Nickel and DNCB have been shown to rapidly activate MAPKs in human DCs, which are known to be highly involved in the expression of co-stimulatory molecules and cytokine production (Aiba et al., 2003). Additionally, contact allergens such as TNCB and oxazolone induce the formation of hyaluronic acid (HA) fragments that activate TLR2 and TLR4 (Martin, 2015). NF- κ B has been identified as another transcription factor activated by various chemical sensitizers and implicated in the maturation of DCs (Neves et al., 2011). Studies using a peptide array approach have indicated that Nickel and DNCB activate CK2 in DCs, and inhibition of its activity can alter the phenotype of DCs in response to these chemicals (de Bourayne et al., 2017). CK2 activity has also been shown to regulate the production of pro-

inflammatory cytokines in DCs, leading to polarization towards Th1 cells (de Bourayne et al., 2017). PKC has been shown to be involved in DC migration from the epidermis in response to TNCB, and data suggest that PKC- β is essential for PPD and DNCB-induced CD86 expression in human DCs (Corsini et al., 2014). On the other hand, chemical reactivity and the resulting inflammatory response can lead to cellular stress and death, resulting in the release of intracellular danger signals such as ATP. This ATP is necessary for the activation of the NLRP3 inflammasome through the purinergic receptor P2RX7, leading to the cleavage of pro-interleukins (pro-IL-1 β , pro-IL-18) into their biologically active forms (Weber et al., 2010; Esser et al., 2023).

1.4 Activation of T lymphocytes

The previously described mechanisms lead to DC maturation; however, without a T-cell repertoire, initiating an adaptive immune response is impossible. This section will discuss the cellular and molecular mechanisms of the lymphocyte response.

1.4.1 The structure of the T-cell receptor (TCR)

The TCR is a heterodimer formed by two polypeptide chains, α and β . The TCR specificity depends on the gene rearrangement of these two chains. The TCR locus contains gene segments coding for the V (variable), J (junction), and C (constant) genes as well as the D gene (diversity) only for β chains (Takaba and Takayanagi, 2017). The diversity of the T-cell repertoire is related to arbitrary combinations of individual TCR genes: $V\alpha$, $J\alpha$, and $C\alpha$ for the α chain and $V\beta$, $J\beta$, $D\beta$, and $C\beta$ for the β chain. The human genome and the $\alpha\beta$ rearrangement generate more than 2.5×10^7 different TCRs (Zarnitsyna et al., 2013; de Greef et al., 2020). This unique combination for each TCR results in three hypervariable domains that will allow specific interaction with a given MHC-antigen complex through three complementarity determining regions (CDR) outward loops CDR1, CDR2, and CDR3 (**Figure 19**). More precisely, the CDR1 and CDR2 regions associate with the MHC molecule, while the CDR3 region binds to the

peptide. The TCR includes a CD3 complex which plays an essential role in signal transduction through phosphorylated ITAM motifs under the action of an Lck kinase. CD4 and CD8 molecules stabilize the TCR/pMHC interaction (Malissen and Bongrand, 2015).

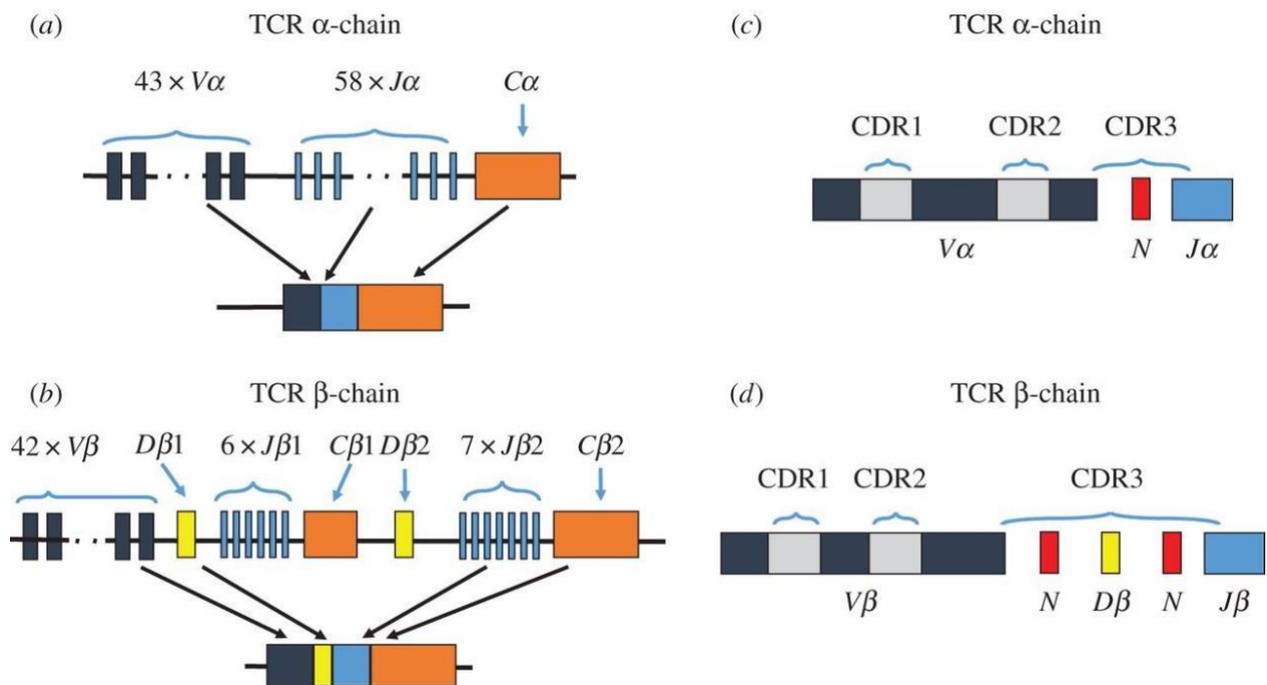


Figure 19. T-cell receptor gene rearrangement adapted from (Laydon et al., 2015)
(a) Variable (V), junction (J), and constant regions (C) of the TCR α -chain. **(b)** V, J, C, and diversity (D) regions the TCR β -chain. Sections from each region are recombined, with additional nucleotides (N), to generate the rearrangement of TCRs. These processes generate substantial T-cell diversity. **(c,d)** Hypervariable complementarity determining regions CDR1-CDR3 of the α and β chains. CDR1 and CDR2 regions are encoded on the V region, while the most variable CDR3 region straddles the VJ and VDJ junctions.

1.4.2 Antigen recognition by the TCR

Most peptides that associate with MHC class I molecules are 8 to 10 amino acids (aa) long. This correlates with the closed structure of the MHC class I molecule, which only holds small peptides into 9 pockets (Wieczorek et al., 2017). The P2 and P9

pockets are MHC anchor positions, and only peptides with preferred aa at these positions, such as Leucine (L), Methionine (M) at P2 or Valine (V) at P9, have optimal binding (Redwood et al., 2018). Furthermore, for peptides presented on MHC class I molecules, the aa directly involved in TCR interaction are located in the center of the peptide sequence between P3 and P7. While aa at other positions (e.g., P1) have been described as potentially influencing TCR affinity, this is likely due to indirect effects on the three-dimensional structure of the epitope (Hunt et al., 1992). All studied TCRs show a similar binding to MHC-peptide complexes at angles ranging from 45° to 80° (Hennecke and Wiley, 2001) **(Figure 20)**.

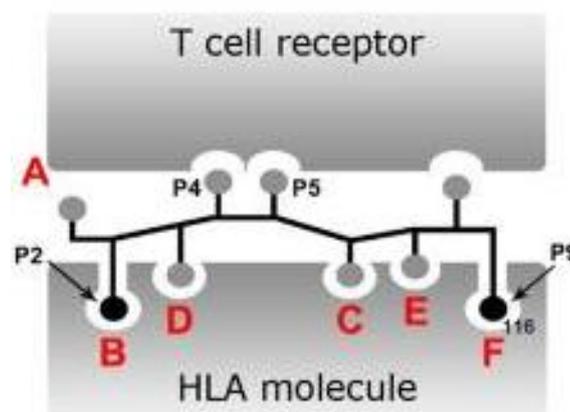


Figure 20. The human leukocyte antigen I (HLA) adapted from (Redwood et al., 2018)

HLA presents peptides to the T cell receptors on the surface of T cells. Peptides bind to class I HLA alleles in the A, B, C, D, E, and F pockets of the peptide binding groove. The peptide's anchor residues P2 and P9 are bound by the B and F pockets, enabling binding specificity to a given HLA molecule.

Unlike peptides associated with class I MHC molecules, those associated with MHC class II molecules are longer (12 to 25 aa), with a preference for 15 residues (Wieczorek et al., 2017). These peptides are composed of a binding sequence of 12 to 13 aa and N- or C-terminal side chains overhanging from the groove due to the open structure of the MHC class II molecule **(Figure 21)**. At the binding site, the peptide adopts an extended conformation where the side chains form angles of approximately 130° (Jardetzky et al., 1996). The formation of the MHC/peptide complex also involves

interactions between certain side chains of the peptide and the pockets of the groove. Nine pockets hold the peptide in the MHC class II binding groove. The side chain of the aa in P1 is buried in the deep hydrophobic pocket located at one end of the groove. The other anchor residues, are in positions P4, P6, P7, and P9. Aa in P2, P5, and P8 point outward and will therefore be recognized by T cells. The P3 residue lodges an intermediate position (Nakayama and Michels, 2019). Each pocket holds certain side chains, which is the origin of the binding specificity of MHC class II molecules. Determining the aa held by each of the 5 pockets (P1, P4, P6, P7, and P9) allows the definition of the MHC class II binding motif (Stern et al., 1994).

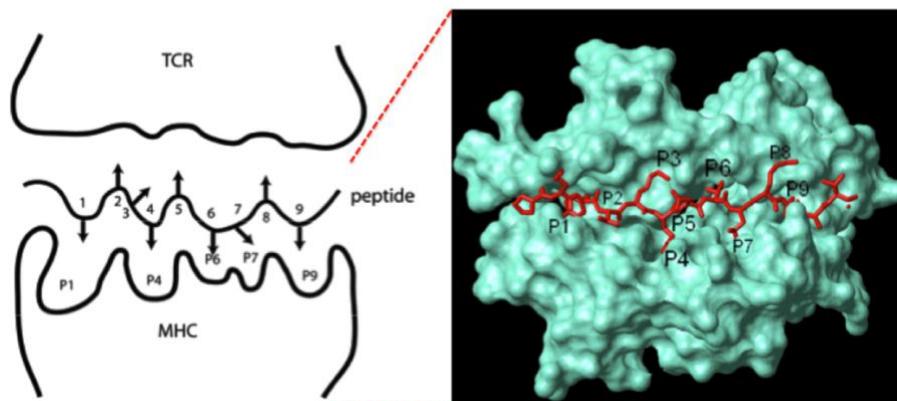


Figure 21. The human leukocyte antigen II (HLA) adapted from the thesis of Marie-Eliane Azoury

1.4.3 The naive T-cell repertoire

A set of antigen-specific naive T cells characterizes the T-cell repertoire of an individual. They are predominant after birth but decline after puberty. Each T-cell is specific for an antigen that is recognized through the TCR. T-cell precursors, also known as thymocytes, develop in the thymus. They undergo a strict selection process where immature double-positive (CD4⁺ CD8⁺) cells are selected based on the reactivity of their TCR to self-peptides (Sprent and Surh, 2011). The negative selection process allows the removal of thymocytes with a high affinity for these peptides. In

contrast, positive selection enables the survival of thymocytes with low affinity of their TCR for self-peptides. This thymic selection eliminates more than 90% of thymocytes (Klein et al., 2014; Takaba and Takayanagi, 2017). The remaining thymocytes survive this double selection and are then differentiated into mature single-positive cells (CD4⁺CD8⁻ and CD4⁻CD8⁺) with a naive phenotype characterized by the expression of CD45RA, CCR7, CD62L, CD127 and CD132 (Eagar and Miller, 2019; Kondo et al., 2019). They are then exported from the thymus to the periphery, forming a peripheral repertoire of naive T cells. Functionally, naive T cells are not capable of mediating effector immune responses. They are considered naive until they encounter their specific antigen (Klein et al., 2014). The survival of these T cells is ensured by an IL-7-rich cytokine cocktail and survival signals induced by the interaction of the TCR with self-peptides presented by APCs (Kondo et al., 2019). During an immune response, naive T cells react with the specific epitopes and become effector cells (Woodland and Kohlmeier, 2009).

After the resolution of the reaction, these cells survive and form memory T cells (Woodland and Kohlmeier, 2009). Two main subpopulations have been defined: the effector memory T cells (T_{EM}) and the central memory T cells (T_{CM}). T_{EMs} play an important role in the immediate response due to their location in the periphery, close to the pathogen entry sites. These cells are CD44^{high} CD62L⁻ CCR7⁻ CD69⁻ CD103⁻ (Lugli et al., 2016). T_{EMs} can rapidly differentiate into effector T cells and secrete large amounts of cytokines (IFN- γ , IL-4, IL-5) after restimulation with the antigen while T_{CMs} require more time (Broere et al., 2011; Golubovskaya and Wu, 2016). T_{CMs} are preferentially localized in lymphoid organs, spleen, bone marrow, and blood, and have low immediate effector capacities but a high expansion capacity. They can produce large amounts of IL-2. In contrast to T_{EMs}, T_{CMs} express CCR7 and CD62L (L-selectin), "homing" receptors allowing them to circulate in the lymph nodes and spleen (Farber et al., 2014; Gehad et al., 2018). A third population of memory T cells called resident memory T cells (T_{RM}) has been identified and persists at the tissue level. These cells are

CD44^{high} CD62L⁻ CCR7⁻ CD11a^{high} CD69⁺ (Mueller et al., 2013; Steinbach et al., 2018; Derksen et al., 2023).

1.4.4 Mechanisms of antigen recognition and T-cell activation

When DCs detect antigens in the presence of danger signals, they are activated and migrate to lymphoid organs to activate naïve T cells. Activation of specific naïve T cells by DCs requires three signals (**Figure 22**) (Bakdash et al., 2013; Tai et al., 2018). This occurs in the immunological synapse, which is a molecular superstructure whose ultimate goal is to optimally rearrange all factors necessary for T-cell activation (Dustin, 2014; Capitani and Baldari, 2022). This structure was initially described as having a discoid shape with a diameter of approximately 5 µm (Monks et al., 1998), but it is much more dynamic. It is organized into three concentric regions: a central-supramolecular activation complex (c-SMAC) region, a peripheral region, and a distal region. The c-SMAC region contains the TCR and co-stimulatory molecules, and is surrounded by a ring where pairs of adhesion molecules are present (Dustin, 2016).

The first activation signal is the recognition of the MHC/peptide complex by the TCR (extensively detailed previously). This results in the reorganization of the expression of surface molecules, facilitating the synapse between the DC and the T cells (Chen and Flies, 2013; Meissner et al., 2017). This reorganization allows the onset of the second activation signal, called the co-stimulatory signal, which is mediated by the co-stimulatory molecules such as CD80 and CD86, particularly expressed on mature DCs. These molecules bind with the CD28 molecule on the surface of T cells, promoting their activation (Finetti and Baldari, 2018; Tittarelli et al., 2020). This binding increases T-cell proliferation and survival, marked by an expanded IL-2 secretion (Bakdash et al., 2013). This co-stimulatory signal is accompanied by the interaction of the CD40 molecule with its ligand CD40L present in T cells. The CD40/CD40L interaction activates the NF-κB pathway, thereby inducing the maintenance of DCs activation and survival (Bakdash et al., 2013). CTLA-4 molecules are expressed in activated T cells and regulatory T cells (Olive et al. 2011). These molecules compete

with CD28 for binding with CD80/CD86 and trigger an inhibitory signal (Chen and Flies, 2013). Indeed, binding CD80/CD86 co-stimulatory molecules with CTLA-4 induces inhibition of T-cell proliferation and IL-2 secretion (Wolchok and Saenger, 2008). In conventional T cells, this mechanism serves as a negative feedback control to avoid excessive lymphocyte activation. The PD-1 receptor is another significant T-cell receptor mainly studied in oncology. Binding to its DC ligand PD-L1 inhibits T-cell proliferation and survival (Olive, 2020). The third activation signal is provided by the secretion of cytokines by DCs such as IL-12, IL-23, IL-10, and TNF- α . These are essential for the survival and polarization of T cells (Blanco et al., 2008). The cytokine cocktail secreted is, therefore, dependent on the antigen. It will trigger the polarization of "helper" CD4 T cells towards the Th1, Th2, Th17, or Th22 responses and "cytotoxic" CD8 T cells towards the Tc1 or Tc2 responses (Deenick et al., 2011; Mittrücker et al., 2014).

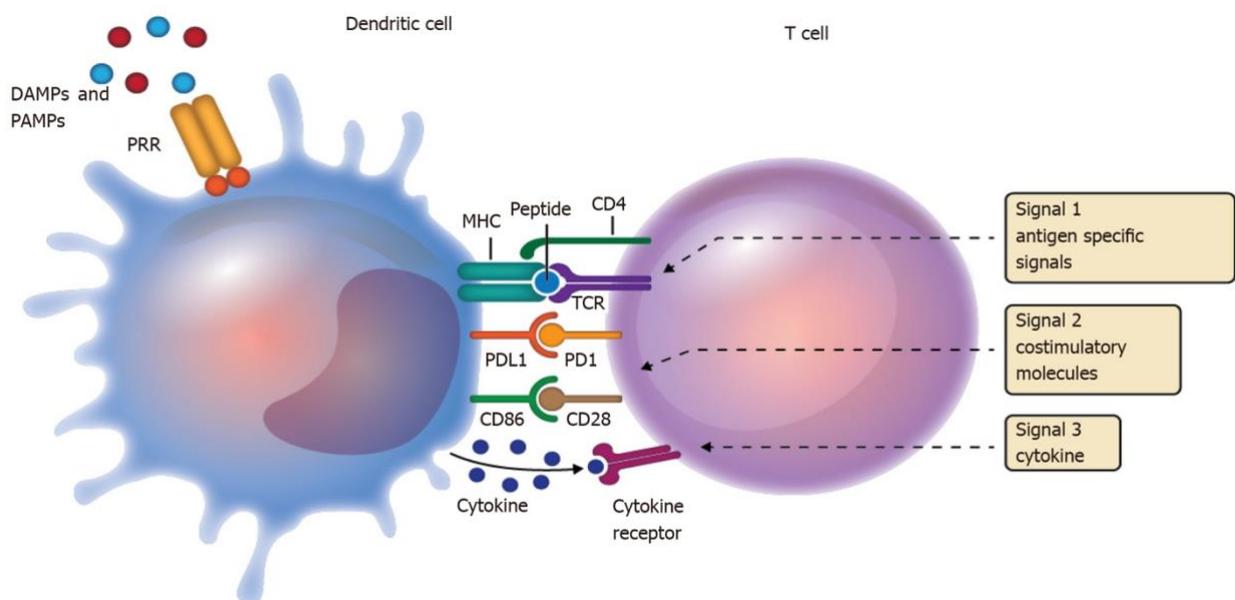


Figure 22. The three-signal model for T-cell activation adapted from (Velazquez-Soto et al., 2022)

1.4.5 Examples of drugs and chemicals

Several *in vitro* studies have shown the ability of strong sensitizers, such as TNBS, DNCB, Bandrowski's Base (BB), and PPD, to induce T-cell proliferation in healthy or allergic donors. (Vocanson et al., 2008; Dietz et al., 2010; Gibson et al., 2015; Faulkner et al., 2016). Indeed, the results of the *in vitro* study by Dietz *et al.*, indicated that human immature DCs modified with DNBS and TNBS, but not with DNCB, elicited primary T-cell responses in healthy donors. The effectiveness of DNBS-modified iDC was determined by measuring the proliferation of dinitrophenyl (DNP)- and trinitrophenyl (TNP)-specific T-cell subsets, as well as IFN- γ production in CD4⁺ and CD8⁺ T-cells (Dietz et al., 2010). Furthermore, it was observed that the HSA protein modified with DNP (HSA-DNP) was also effective in inducing a primary T-cell response when processed by iDC (Dietz et al., 2010). On the other hand PPD-specific and PPD-HSA-specific CD4⁺ and CD8⁺ T-cell clones were generated from three allergic patients (Jenkinson et al., 2010). However, detecting a repertoire of T cells specific to weak sensitizers seems more difficult. This could be due to the low frequency of specific T-cell precursors that require a more sensitive detection method. Efforts in our laboratory previously showed a T-cell repertoire to nickel and cobalt, both considered well described contact sensitizers (Bechara et al., 2019b).

On the other hand, most studies with drugs have been performed mainly on allergic patients and have presented a repertoire of drug-specific T cells or naïve T cells in healthy donors. A few examples are of clavulanic acid (Ariza et al., 2022), sulfasalazine (Hammond et al., 2022), vancomycin (Ogese et al., 2021), dapsone (Alzahrani et al., 2017; Zhao et al., 2019), ticlopidine (Usui et al., 2018), flucloxacillin (Wuillemin et al., 2014; Faulkner et al., 2016), CBZ (Faulkner et al., 2016), and lamotrigine (Naisbitt et al., 2003). Recently, a repertoire of naïve T cells specific for amoxicillin, piperacillin, SMX-NO, and the bioconjugate HSA-benzylpenicillin (HSA-BP) was identified in the blood of healthy donors (Faulkner et al., 2012; Nhim et al., 2013). Furthermore, candidate peptides derived from the HSA-BP bioconjugate, identified by

an *in silico* approach (Scornet et al., 2016), are also recognized by naïve CD4+ T cells from healthy donors: PT-BP, KV-BP, FS-BP, with BP bound to lysines 159, 525 and 212, respectively. T-cell clones specific for BP peptides also recognize the HSA-BP bioconjugate and vice versa, supporting T-cell activation by a hapten-dependent mechanism. Two of these immunodominant peptides (Lys 159 and 525) were also recognized by PBMC of allergic patients confirming their role in patient sensitization (Azoury et al., 2018).

Interestingly, the intensity of the response generated by specific T-cells seems to be correlated with the level of hapteneized proteins (El-Ghaiesh et al., 2012; Meng et al., 2017), and lymphocyte proliferation is abrogated upon inhibition of antigen priming by DCs by binding to glutaraldehyde (Pickard et al., 2007; Jenkinson et al., 2010). In addition to proliferation, the cytokine profile of T-cells has also been investigated in some studies (Pickard et al., 2007; Vocanson et al., 2008; Dietz et al., 2010).

2. Other mechanisms of chemical or drug recognition by T cells

The hapten concept, including internalization priming and presentation of haptenized peptides by APCs, represents only one of the methods of recognition and activation of T cells. Therefore, this section will present other recognition mechanisms and their consequences.

2.1 Pharmacological interaction with an immune receptor or p-i concept

The pi-concept constitutes an alternative mechanism by which a low-molecular-weight molecule can bind reversibly and non-covalently with the MHC or TCR independently of antigen priming by APCs. This bond is considered stable enough to stimulate T cells. The p-i concept has major implications on our understanding of drug interactions with the specific immune system and DHRs. (Schnyder et al., 1997; Zanni et al., 1998b, 1998a; Schnyder et al., 2000; Burkhart et al., 2002; Yang et al., 2007; Pichler et al., 2011; Pichler and Hausmann, 2016). It is based on extensive investigations of T-cell clones reacting with the drugs. It is a highly specific interaction dependent on the expression of a TCR into which the drug can bind with sufficient affinity to cause signaling. Small modification of the drug structure may already annul reactivity. Stimulation of T cells occurs within minutes as revealed by rapid Ca^{2+} influx after drug exposure, thus, before metabolism and processing can occur (Pichler, 2008).

This concept emerged following *in vitro* experimental observations showing that certain drugs such as SMX, allopurinol, CBZ, atabecostat, and lidocaine can directly bind to the MHC and TCR of T cells from allergic patients with sufficient affinity to activate them (Schnyder et al., 2000; Burkhart et al., 2002; Wei et al., 2012; Pan et al., 2019; Thomson et al., 2021). Several studies showed that the inhibition of APC priming by glutaraldehyde exposure did not decrease the activation of drug-specific T cells. Washing APCs loaded with these molecules before co-culture with T cells also prevented activation of the latter (Castrejon et al., 2010). Another argument is the rapid activation of T cells which is incompatible with the cell priming mechanism that

requires at least sixty minutes (Pichler, 2008). As described earlier in this chapter, SMX is a pro-hapten, and its reactive metabolite SMX-NO follows the hapten concept. Although T cells from SMX-allergic patients exclusively recognize SMX-NO (Elsheikh et al., 2011), several *in vitro* studies show that SMX can also be directly presented to T cells and act predominantly not through its reactive metabolites but via the pi-concept (Schnyder et al., 1997, 2000; Elsheikh et al., 2011). Recently the pi-concept has been divided into two subgroups depending on the location of the binding (**Figure 23**). Pi-TCR if the molecule binds primarily to the $\alpha\beta$ -TCR, and pi-HLA if the molecule binds mainly to the HLA (Yun et al., 2016).

For **pi-HLA**, the drug binds directly to HLA molecules, which represents one of the most polymorphic protein systems in humans with more than 14000 HLA alleles (carbamazepine, allopurinol) (Yun et al., 2016). *In vitro* studies have shown that CBZ binds to the allele B*15:02, which is prevalent in Southeast Asia (13%-15%), as well as to A*31:01 in Caucasians. This observation suggests a potential genetic basis for the variable prevalence of CBZ-induced hypersensitivity reactions across different ethnic groups. Additionally, allopurinol, and its metabolite oxypurinol, can trigger direct and immediate activation of specific T cells from HLA-B*58:01 donors with a conceptual p-i mechanism independently of antigen priming (Yun et al., 2013, 2014). Furthermore, it has been suggested that the peptide in the MHC pocket can dissociate from the pocket to create space for allopurinol or oxypurinol. These will then bind to arginine residues of HLA-B*58:01 and alter the conformation of the peptide (Lin et al., 2015).

As an example of **pi-TCR**, the activation of some SMX- or lidocaine-specific T-cell clones is dependent on the unique structure of the specific TCR and does not require the presence of a peptide with a specific amino acid sequence (Zanni et al., 1998a; Burkhart et al., 2002; Pichler et al., 2015). Indeed, T-cell stimulation required the presence of HLA molecules, but the immunogenic peptides bound to HLA were exchangeable. Even the HLA molecules were exchangeable, as some T cells upheld reactivity in the presence of allogeneic APC (Pichler et al., 2015). SMX is considered a

classic example for pi-TCR. Using computer modelling and in vitro experiments, it has been shown that, SMX binds straight into a relatively wide loop in the CDR3 region of the TCRV (TCR "1.3"), which typically interacts with the peptide-HLA complex in an inverted orientation, with its NH2 group pointing to the peptide-binding groove (Yun et al., 2012; Watkins and Pichler, 2013).

Interaction of specific molecules with **both TCR and HLA** may occur (Yun et al. 2016; Zhou et al. 2016). This is the case with CBZ. CBZ-related SJS/TEN was associated with HLA- B*15:02 (Wei et al., 2012) and TCR Vb-11-ISGSY (Ko et al., 2011). This ability of to bind with TCR regions might explain why oligoclonal or even monoclonal T-cells are identified in some of the severe clinical manifestations (SJS/TEN).

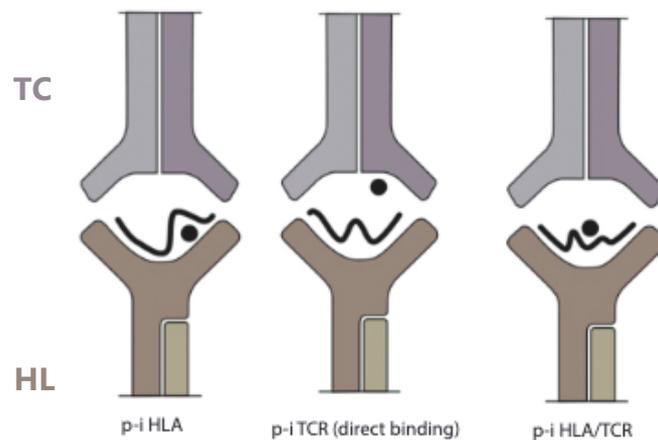


Figure 23 Subtypes of the p-i concept adapted from (Yun et al., 2016)

2.2 Altered self-peptide repertoire

The altered self-repertoire mechanism has been described following the characterization of abacavir-induced hypersensitivity syndrome but also proposed for CBZ. According to this model, the medication can fit into an HLA molecule's binding groove, changing the groove's conformation and the range of self-peptide ligands that APCs can bind to and display. It assumes that a drug can alter the recruitment of peptides to the MHC pocket, allowing an inappropriate repertoire of self-peptides to be present in this pocket and triggering an allogeneic lymphocyte response. A strong association between this syndrome and HLA-B*57:01 has been described with a negative predictive value of 100%, and a positive predictive value of 48% (Mallal et al., 2008).

It has been proposed that abacavir can non-covalently bind to the serine residues of the F-pocket of the HLA-B*57:01 groove and specifically alter the shape and chemistry of the binding pockets resulting in the modification of the repertoire of endogenous peptides presented (**Figure 24**) (Illing et al., 2012a; Jaruthamsophon et al., 2022; Norcross et al., 2012a; Ostrov et al., 2012). ABC favors the presentation of peptides containing small aliphatic residues (valine, alanine, or isoleucine) instead of hydrophobic residues such as tryptophan or phenylalanine that usually bind to the HLA-B*57:01 molecule (Illing et al., 2012b; Norcross et al., 2012b; Illing et al., 2021). In these studies, 20-45% of the peptides eluted from ABC-treated HLA-B*57:01 APCs differed from those eluted from untreated HLA-B*57:01 positive APCs. In this way, abacavir guides the detection of new endogenous peptides by naive T-cells by inducing an alteration of the "immunological self" (Illing et al., 2012b; Norcross et al., 2012b; Illing et al., 2021).

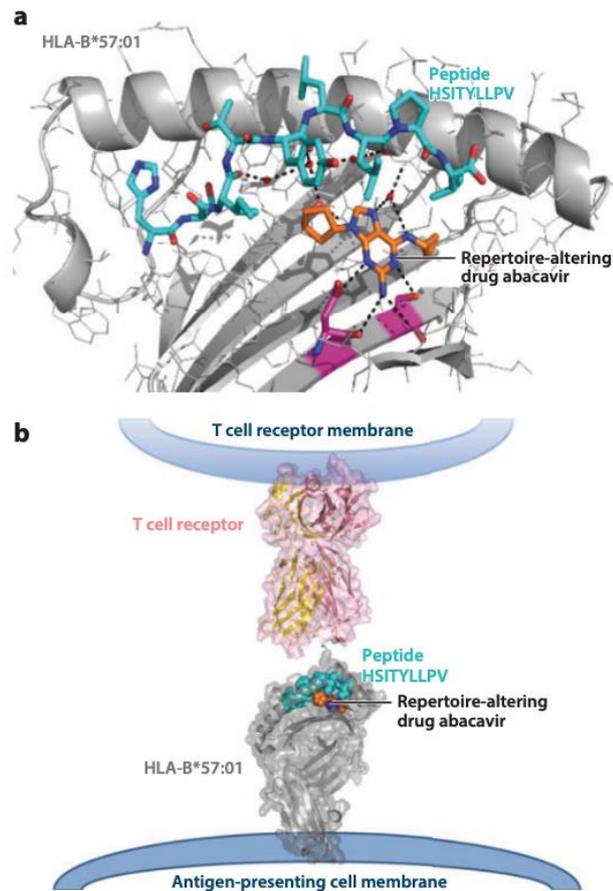


Figure 24. Abacavir binding to the MHC/TCR complex through the altered peptide repertoire from (Pavlos et al., 2015)

(a) The crystal structure of the complex formed between abacavir-peptide-MHC has been elucidated, revealing crucial intermolecular contacts within the antigen-binding cleft of HLA-B*57:01. The HLA-B*57:01 molecule is depicted in gray, while the synthetic peptide is shown in cyan carbons. Abacavir is represented in orange for carbon, blue for nitrogen, and red for oxygen, with hydrogen-bond interactions (black dashes) formed with both the peptide and HLA-B*57:01. Additionally, the residues that differentiate the abacavir-sensitive allele HLA-B*57:01 from the abacavir-insensitive HLA-B*57:03 are depicted in magenta for carbon, blue for nitrogen, and red for oxygen. **(b)** A model of the abacavir-peptide-MHC complex interacting with the T-cell receptor is also presented, where HLA-B*57:01 is depicted in gray, the peptide in cyan carbons, and abacavir as spheres with orange representing carbon and blue representing nitrogen. The T cell receptor is represented in pink.

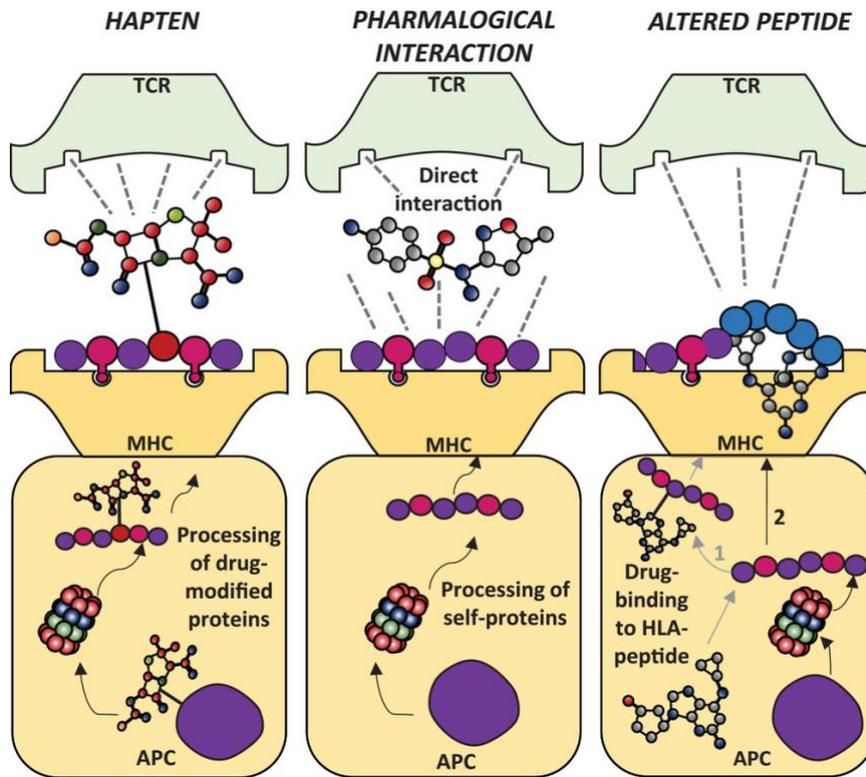


Figure 25. Models of antigen presentation by APCs from (Adair et al., 2021)

PRESENTATION OF THE THESIS PROJECT

PRESENTATION OF THE THESIS PROJECT

Drug and chemical allergies are a significantly growing healthcare concern. Therefore, their assessment and prediction are crucial in ultimately preventing reactions. There is a growing interest in developing tools and models for adequately evaluating and predicting these allergies, as they can help pharmaceuticals, clinicians, and patients. As a result, our work proposes three drug and chemical allergy assessment and prediction methods.

The **first project** involves a decade-long retrospective analysis of DHRs in a Lebanese outpatient population. For the first time, the present study evaluates the characteristics of all reported DHRs in Lebanon over ten years in a national tertiary outpatient clinic. A group of 758 patients met the study criteria. Our study confirmed the findings of previous global research, revealing that among the 72 drugs identified, BLs (53.8%) and NSAIDs (48.9%) were the most commonly implicated drugs. All patients were diagnosed based on a comprehensive clinical history, and an SPT was performed when necessary for confirmation by the physician (22.6%). Additionally, we analyzed the characteristics of 90 patients (11.9%) with a history of ambulatory DHRs and DHRs to one or more molecules used in the perioperative setting.

The **second project** aimed to build a one-of-a-kind weight-of-evidence approach that predicts the immunization risk of new drug candidates based on what is known in the literature on a selection of pro-allergenic therapeutic molecules. This approach analyzes four *in vitro* and *in silico* predictive parameters that mimic an event that may contribute to immunization and their correlation to two clinical parameters. Each parameter was coupled with a coefficient, which allowed us to calculate predictive and clinical scores. Therefore, for each new molecule in the drug development process, the results of *in vitro* and *in silico* experiments allow us to calculate the predictive score and, consequently, the correlation of the clinical score to assess the pro-allergic potential of the molecule.

The **third and final project** was the development of an improved DC/ T-cell autologous co-culture model to detect and analyze the human T lymphocyte response to six chemical sensitizers involved in ACD in healthy donors: 2,4-dinitrobenzenesulfonic acid (DNBS), cinnamaldehyde (CinA), isoeugenol (IsoE), methylisothiazolinone (MIT), nickel sulfate (NiSO₄), and cobalt chloride (CoCl₂). The analysis revealed that specific T cells were detected for all chemicals and in the majority of donors, and their mean frequencies were calculated. CD4⁺ and CD8⁺ T cells were primarily found in CinA and DNBS-responding T cells, respectively. For MIT, both subpopulations were present equally. These results provided insights into the underlying mechanisms of ACD.

Overall, this thesis project seeks to advance our understanding of drug and chemical allergies and to develop new tools and models for predicting and assessing these reactions. The insights and techniques developed in this project could have significant implications for diagnosing and preventing these conditions by shaping clinical decision-making and improving patient outcomes.

RESULTS

RESULTS

ASSESSMENT AND PREDICTION OF DRUG ALLERGIES

1. **Article 1: Drug-induced hypersensitivity reactions in a Lebanese outpatient population: a decade-long retrospective analysis (2012-2021)**

Article in revision in the Journal of Allergy and Clinical Immunology: Global

Drug hypersensitivity reactions (DHRs) constitute a significant health concern worldwide, affecting millions of patients and leading to hospitalizations; therefore, they are considered a substantial burden on the healthcare system and a challenge for physicians. A false allergy diagnosis may limit treatment options and lead to using less effective, more dangerous, or more expensive drugs. Limited epidemiology research on DHRs has been conducted worldwide, specifically in the Middle East. Most of the available literature either studies a particular population or a particular drug class. To our knowledge, there are no published epidemiology research papers on drug allergies in Lebanon, leading us to be the first to evaluate reported DHRs in the Lebanese population.

This retrospective study aimed to evaluate the clinical characteristics and diagnostic workup of reported DHRs to therapeutic molecules used in ambulatory care in Lebanon over a decade (2012-2021) in an allergist practice.

The results are largely confirmatory of other worldwide studies. A group of 758 patients met the study criteria, predominantly females. Most patients presented reactions to only one drug (67.4%), while others had DHRs to multiple molecules. All patients were diagnosed

based on detailed clinical history. For patients presenting an active DHR (aDHR), the physician prescribed an allergy skin prick test (SPT) when necessary for confirmation. A total of 72 suspected drugs were identified. The most frequently implicated drug classes included BL antibiotics (53.8%), followed closely by NSAIDs (48.9%). The characteristics of 90 patients (11.9%) with a history of ambulatory DHRs and concomitant DHRs to one or more molecules used in the perioperative setting were also analyzed.

These results may aid in developing strategies for the accurate diagnosis and management of DHRs in Lebanon and contribute to understanding the genetic and environmental factors influencing DHRs in the region.

1 **Title:** Drug-induced hypersensitivity reactions in a Lebanese outpatient population: a
2 decade-long retrospective analysis (2012-2021)

3 **Short title:** Drug-induced hypersensitivity reactions in a Lebanese outpatient population
4 from 2012 to 2021

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36

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39

40 **Conflict of interest**

41 The authors declare no conflict of interest.

42 **ABSTRACT**

43 **Background:** Drug hypersensitivity reactions (DHRs) are becoming more common
44 due to increasing prevalence and case complexity. Allergists and clinical immunologists
45 worldwide are challenged daily to adequately diagnose and manage these reactions.
46 Data in the literature regarding DHR outpatient consultations is scarce worldwide, limited
47 in the Middle East, and currently unavailable in Lebanon.

48 **Objective:** This retrospective study aims to evaluate the characteristics of all
49 reported DHRs over ten years in a tertiary allergy clinic in Lebanon.

50 **Methods:** We conducted a decade-long (2012-2021) retrospective analysis of the
51 archived medical records of patients with a positive history of DHRs. Demographics,
52 clinical history, diagnostic tools, and characteristics of the DHRs were collected and
53 analyzed.

54 **Results:** 758 patients presented DHRs to therapeutic molecules used for
55 ambulatory care. Our results identified a total of 72 medications. The most frequently
56 implicated drug classes included beta-lactam antibiotics (53.8%), followed closely by
57 non-steroidal anti-inflammatory drugs (48.9%). Of the 758 patients, 32.6% reported DHRs
58 to multiple molecules, and 11.8% reported concomitant DHRs to one or several
59 molecules used in the perioperative setting. Out of those, opioids and neuromuscular
60 blocking agents (NMBAs) were the two most common therapeutic classes recorded.
61 Furthermore, we evaluated the cross-reactivity between molecules of the same class. In
62 NMBAs, rocuronium and cisatracurium were the most commonly cross-reactive, and for
63 opioids, the most common association we recorded was with morphine and pethidine.

64 **Conclusion:** Our findings constitute the first step towards a more comprehensive
65 evaluation of the clinical characteristics of DHRs in Lebanon.

66 **HIGHLIGHT BOX**

67 **What is already known about this topic?** Drug hypersensitivity reactions (DHRs) are
68 becoming more common due to increasing prevalence. Data in the literature regarding
69 outpatient DHRs is currently limited in the Middle East.

70 **What does this article add to our knowledge?** The present study evaluates, for the first
71 time, the characteristics of all reported DHRs in Lebanon over ten years in a national
72 tertiary outpatient clinic.

73 **How does this study impact current management guidelines?** This study evaluates
74 ambulatory outpatient DHRs in Lebanon, concomitant perioperative reactions, and cross-
75 reactivities that potentially impact management.

76

77 **KEYWORDS**

78 Drug hypersensitivity reaction, drug allergy, epidemiology, outpatient clinic, Lebanon,
79 beta-lactams, non-steroidal anti-inflammatory drugs, opioids, neuromuscular blocking
80 agents.

81

82 **ABBREVIATIONS**

83 AAAAI American Academy of Allergy, Asthma and Immunology

84 aDHR Active drug hypersensitivity reaction

85 ADR Adverse drug reaction

86 AMX/CLV Amoxicillin/clavulanic acid

87 BL Beta-lactam

88 COVID Coronavirus disease

89 COX Cyclooxygenase

90	DHR	Drug hypersensitivity reaction
91	ED	Emergency department
92	F	Female
93	FDE	Fixed drug eruptions
94	HDF	Hôtel-Dieu de France
95	Ig	Immunoglobulin
96	M	Male
97	MDM	Minor determinant mix
98	MHC	Major histocompatibility complex
99	MRGPRX2	MAS-related G protein-coupled receptor X2
100	mRNA	Messenger ribonucleic acid
101	NSAID	Non-steroidal anti-inflammatory drug
102	RCM	Radiocontrast media
103	SCAR	Severe cutaneous adverse reaction
104	SJS	Stevens-Johnson syndrome
105	SPT	Skin prick tests
106	TMP/SMX	Trimethoprim/sulfamethoxazole

107 INTRODUCTION

108 Drug hypersensitivity reactions (DHRs) are idiosyncratic, unpredictable dose-
109 independent adverse drug reactions (ADRs) (1–3). They represent 15–20% of ADRs and
110 are potentially life-threatening (4–7). Therefore, DHRs are a frequent reason for
111 consultation in allergy clinics (8,9), as they are responsible for high morbidity and
112 mortality, constituting a significant burden on healthcare resources (10–12).

113 DHRs are classified as immediate or delayed. Immediate reactions can be allergic
114 or non-allergic. Allergic reactions occur when a specific adaptive immune response is
115 involved (9,13). Immediate allergic reactions are typically mediated by specific
116 immunoglobulin E (IgE) and usually arise less than one hour after exposure (14). However,
117 some immediate responses may appear up to six hours after administration, especially if
118 the drug has been administered orally and after food intake, which slows its absorption
119 (2). Delayed-onset reactions can occur more than one hour to a few days after the initial
120 administration of the drug and are mediated by CD4+/CD8+ T lymphocytes (15,16).
121 When mediated by CD8+ T lymphocytes, DHRs are generally severe cutaneous adverse
122 reactions (SCARs) (17–19). However, other organs and tissues may be involved, such as
123 the kidneys, liver, heart, and blood (20). The mechanisms of action of non-allergic
124 reactions (or pseudo-allergies) are numerous and depend on the molecules studied. They
125 are mediated by the release of mediators from basophils and mast cells that are not
126 triggered by IgE (13). One of the mechanisms described, notably with fluoroquinolones
127 and neuromuscular blocking agents (NMBAs), is the direct binding to the MAS-related G
128 protein-coupled receptor X2 (MRGPRX2) on the surface of mast cells, which triggers their
129 degranulation and the release of mediators such as tryptase and histamine that are
130 responsible for the reaction (21,22).

131 Most DHRs appear after the administration of antibiotics, particularly beta-lactams
132 (BLs), non-steroidal anti-inflammatory drugs (NSAIDs), opioids, anticonvulsants, antigout
133 agents, NMBAs, chemotherapy, and radiocontrast media (RCM) (23). The threat of

134 developing DHRs increases in people with risk factors, such as family and personal history
135 or polymorphism of the major histocompatibility complex (MHC) (24).

136 Due to the increase in DHRs, it is a real challenge for physicians to properly
137 diagnose and manage these reactions. A false allergy diagnosis based solely on medical
138 history may limit patients' treatment options and lead to using less effective, more
139 dangerous, or more expensive drugs (25). Additionally, a reported allergy to one drug
140 may falsely suggest that the patient is allergic to all medications in the same therapeutic
141 class (14). The current diagnostic approach for DHRs is based on a complete clinical
142 history, eventually leading to an allergy workup with standardized skin tests, biological
143 and occasionally *in vitro* tests, and finally, drug provocation tests (DPTs), which is the last
144 step for accurate diagnosis of DHRs (26,27).

145 Several epidemiology reports study DHRs. Each differs according to the
146 population studied (hospitalized patients, patients seen in general practice clinics or the
147 emergency departments, children or adults), the methodology used (diagnosis based
148 solely on clinical history, skin testing, or biological testing), and the drugs implicated.
149 Furthermore, epidemiological data on DHRs in non-hospitalized patients is scarce and
150 limited mainly to antibiotics (28–32). There are few publications on drug-allergic
151 outpatients worldwide, and only a few of those previously published were located in the
152 Middle East (33–35). In addition, Lebanon's geographical location and social
153 characteristics have impacted the population's genetic diversity, leading to the
154 identification of numerous distinct genetic particularities (36–39). We conducted this
155 retrospective study in an allergist practice to evaluate the clinical characteristics and
156 diagnostic workup of reported DHRs in Lebanon over ten years.

157 **METHODS**

158 **Study Design**

159 We conducted a decade-long (2012-2021) retrospective review of patients'
160 physical and electronic archived medical records with a positive history of DHR. These
161 patients were referred to the outpatient allergy clinic at the University Medical Center
162 Hôtel-Dieu de France (HDF) hospital in Beirut, which is considered a tertiary allergy clinic
163 in Lebanon. The Lebanese healthcare system is concentrated in the capital, Beirut.
164 Consequently, this clinic welcomes patients from across the Lebanese territory. These
165 patients were transferred from HDF or other hospitals, needing follow-up or referred by
166 other physicians.

167 The clinic's medical records were used as the data source. In 2018, an electronic
168 ISO-certified telemedicine platform, TrakMD, was introduced. All paper medical records
169 from 2012 to 2017 and electronic documents from 2018 to 2021 were studied. Data
170 collection was anonymously performed by assigning an identification number to each
171 patient. All data collected were obtained solely for research reasons. This study was
172 approved by the HDF ethical committee (case number CEHDF 1598).

173 **Demographic information, study variables, and analysis**

174 Our study's primary objective and main inclusion criteria were to identify and
175 evaluate all patients with DHRs to therapeutic molecules used in ambulatory care and,
176 therefore, not hospitalized (758 patients). Moreover, 190 of these patients presented, in
177 addition to their DHRs in ambulatory care, a reaction to one or more molecules used in
178 the perioperative setting. These were described separately. We excluded patients with
179 exclusive reactions to medications used in the perioperative setting.

180 Every patient underwent a physical examination, and a standardized questionnaire
181 was completed. Blood and skin tests were then prescribed when they were applicable.
182 Demographics, clinical history, diagnostic tools, and specific characteristics of the DHRs

183 were collected and analyzed for all individuals. The following data were recorded for each
184 subject: age at the time of consultation, sex, the reason for consultation, urgency of the
185 consultation, date of visit, past medical history, relevant family history, food allergies,
186 respiratory allergies, drugs responsible for the DHRs, types, and symptoms of the
187 reaction, skin tests, and biological diagnostic investigations.

188 Data entry was performed on Microsoft Excel (2019) and exported to GraphPad
189 Prism version 8.0.0 for analysis. The results of the descriptive analysis are presented by
190 the number of patients and percentages of the study population for each variable.

191 **Skin prick test**

192 Skin prick tests (SPTs) were performed according to the American academy of
193 allergy, asthma and immunology (AAAAI) guidelines. For antibiotics of the penicillin
194 family, a diagnostic kit (Diater S.A., Madrid, Spain) was used. This kit contains 0.04mg of
195 benzylpenicilloyl poly-L-lysine (PPL) and a minor determinant mix (MDM) consisting of
196 0.5mg each of benzylpenicillin, benzylpenilloate, and benzylpenicilloate. Pure PPL and
197 MDM prick testing was performed per the manufacturer's recommendation. The
198 investigation of amoxicillin, latex, patent blue, and RCM was performed by SPT using
199 commercial extracts following the manufacturer's guidelines. Other molecules (including
200 drugs used in the perioperative setting) were all provided by the pharmacy or the
201 operating room of HDF hospital. These SPTs were conducted at full concentration using
202 a crushed pill or powder for intravenous solution dissolved extemporaneously before
203 testing in 2ml of purified water. The test was performed via a prick to the skin of the
204 forearm, using Stallerpoint®. The results were read 15 minutes later, with close
205 monitoring. Patients were advised to stop all antihistamine medications five days prior to
206 testing. The SPT results was considered positive when a wheal and flare reaction equal to
207 or greater than 3 mm was observed and compared to histamine and saline used as
208 positive and negative controls, respectively. It is important to note that all tests were
209 conducted by the same operator, enhancing pertinence and reproducibility.

210 **RESULTS**

211 All patient's medical records were retrospectively analyzed, and a group of 758
212 patients met the study criteria. The characteristics of the study population are shown in
213 table I. Patients were predominantly females (72.3%), with a female-to-male ratio of 2.6
214 to 1 and a median age of 39. Patients were included if they had been labeled as allergic
215 by the physician, regardless of whether they presented an active DHR (aDHR) in the clinic
216 as the main reason for their consultation. Other patients had different primary reasons
217 for consultation with a history of DHR. Of these 758 patients, 190 constituting almost
218 25% of cases, were examined by the physician for an aDHR. More than a third (34.6%)
219 had concomitant allergies, including food allergies (52.7%) and respiratory allergies
220 (47.3%). On the other hand, 29.8% had a family history of allergies, including drug
221 allergies (6.2%), and 93.8% had a family record of respiratory allergies. Most patients
222 presented reactions to only one drug (67.4%), 20.9% and 9.9% had DHRs to two or three
223 molecules, respectively, and less than 2% presented reactions to 4 and 5 drugs.

224 All 758 patients were diagnosed based on detailed clinical history. For the 190
225 patients presenting an aDHR, the physician prescribed an allergy SPT 4 to 6 weeks later,
226 when necessary for confirmation. SPTs were performed in 22.6% of cases (figure E1 in the
227 online repository).

228 A total of 72 suspected drugs were identified. The most frequently implicated drug
229 classes included BL antibiotics (53.8%), followed closely by NSAIDs (48.9%). Other
230 antibiotics induced fewer cases of DHRs (Figure 1), followed by RCM, opioids, and
231 acetaminophen.

232 All cases of antibiotic hypersensitivity reactions are represented in table II. The
233 most common drug class responsible for DHRs in our study was BL (53.8%), including
234 penicillins and cephalosporins. The most commonly implicated active substance was
235 amoxicillin associated with clavulanic acid, which was reported in 168 cases. Other
236 prevalent antibiotics were quinolones (7.8%), sulfonamides (5.8%), and nitroimidazole

237 (3.6%). Fewer cases of macrolides, lincomycin, and tetracycline DHRs were observed. Of
238 all the antibiotic cases reported, 94 were confirmed by SPT, most of which were for BLs.
239 Most patients presented immediate DHRs to antibiotics. The symptoms primarily
240 included urticaria, with or without angioedema, and a few cases of anaphylaxis with BLs,
241 metronidazole, and the association of trimethoprim with sulfamethoxazole (TMP/SMX).
242 We also observed delayed DHRs such as skin rash, including erythema multiforme and
243 dermatitis, and a few cases of Stevens-Johnson Syndrome (SJS) and Fixed Drug Eruptions
244 (FDE).

245 Six SJS cases were documented (table E2 in the online repository) following
246 TMP/SMX, metronidazole, or allopurinol administration. All patients were above 60 years
247 old and had no family histories of DHRs, concomitant food, or respiratory allergies.

248 Non-antibiotic DHRs are presented in Table III. Following BLs, the second most
249 commonly found drug class was NSAIDs, responsible for 371 cases of reported DHRs
250 (48.9%). Aspirin was the most frequent in this group (11.9%). Urticaria was again the most
251 frequent symptom, followed by angioedema, anaphylaxis, and skin rash, with mostly
252 immediate reactions (96.2%). Other frequent events included 17 clinically diagnosed RCM
253 cases and 13 opioids for ambulatory use.

254 Within the evaluated time frame, the evolution of aDHR prevalence for NSAIDs,
255 BLs, and other antibiotics was analyzed (Figure 2). Antibiotic hypersensitivity cases were
256 stable between 2012 and 2016. Then a significant drop occurred between 2016 and 2017,
257 followed by a gradual rise until the end of 2021. However, for NSAIDs, the records were
258 constant between 2012 and 2019, and a 50% rise was recorded until 2021. The highest
259 number of DHR visits were recorded between 2020 and 2021 for all drug classes (31 cases
260 for NSAIDs, 38 for BLs, and 24 for other antibiotics).

261 We then detailed the characteristics of 90 patients (11.9%) with a history of
262 ambulatory DHRs and concomitant DHRs to one or more molecules used in the
263 perioperative setting (Table IV). All were immediate reactions, and the predominant

264 symptom observed was anaphylaxis, followed by angioedema, dyspnea, and facial
265 erythema. An SPT workup was systematically performed for all patients using a selection
266 of molecules shown in table V according to the reaction history. Positive reactions to
267 opioids (74 patients), neuromuscular blocking agents (NMBAs) (57 patients), hypnotics
268 (18 patients), and local anesthetics (12 patients) were recorded. Thirty-six patients with
269 latex hypersensitivity and 5 patent blue hypersensitivity cases were also observed.
270 Morphine was found in 52 occurrences, followed by rocuronium (46 cases).

271 Results from SPTs allowed us to study cross-reactivity for NMBAs and opioids. Of
272 the 57 patients with positive SPT to NMBAs, we recorded 39 cases of single-agent
273 sensitization, and 15 showed co-sensitization to two molecules, mostly rocuronium and
274 cisatracurium (n=10). Positive SPTs to three molecules were observed in 3 patients, 2 of
275 which were rocuronium, cisatracurium, and atracurium. Of the 74 patients with a positive
276 SPT to opioids, 49 showed cross-reactivity. The most frequent association of molecules
277 was analyzed. For opioids, 26 were reactive to two molecules, majorly to morphine and
278 pethidine, with 13 cases, 13 to three molecules (n=4 to morphine, pethidine, and
279 fentanyl), 9 to four molecules (n=3 to morphine, pethidine, sufentanil, and remifentanil)
280 and one case of cross-reactivity to morphine, pethidine, sufentanil, remifentanil, and
281 fentanyl. Hypnotics and local anesthetics showed rare cross-reactivities, with one case of
282 cross-reactivity to midazolam and ketamine, one case of midazolam, ketamine, and
283 etomidate, and one case of lidocaine and ropivacaine (table VI).

284 **DISCUSSION**

285 Epidemiological studies of DHRs present multiple outcomes mainly due to
286 divergence in study populations. The populations studied are hospitalized inpatients,
287 outpatients, individuals from national databases, pharmacovigilance databases, or ED
288 attendances, thus resulting in varying outcomes (5,23,40). To our knowledge, these
289 reports are scarce in the Middle East (33,41–44) and have yet to be conducted in Lebanon.
290 The primary aim of our study is to provide a comprehensive retrospective analysis of
291 DHRs in an outpatient population, to provide, for the first time, an overview of national
292 DHRs, and to document specific characteristics.

293 From the 758 patients analyzed, females outnumbered the males, with a female-
294 to-male ratio of 2.6:1. Our results are consistent with those found by others, such as
295 Gamboa *et al.*, who investigated an outpatient Spanish database (30,45,46). It is still
296 unclear as to why women are more prone to DHRs. Some reports suggested that this
297 difference was probably due to the higher drug consumption by women compared to
298 men with more repeated exposure to antibiotics/medications, genetic factors, epigenetic
299 changes, or hormonal influences on immune cells (47–49). The mean age in our study
300 was 39 years. These findings corroborated with evidence published in the literature
301 demonstrating that DHRs in an outpatient population mainly occur in young and middle-
302 aged adults with a mean age of close to 40 years (30,50,51). However, other studies on
303 hospitalized patients showed an older average (32).

304 We showed that BL antibiotics, followed by NSAIDs, were the two most implicated
305 drug classes in the recorded DHRs, with a majority of cases associated with
306 amoxicillin/clavulanic acid (AMX/CLV). Multiple studies showed that BL antibiotics are the
307 most frequent elicitors of DHRs (52–55). Doña *et al.* recorded 25 to 35% of BL DHRs (56).
308 These percentages are lower than those recorded in our study (around 50%), which might
309 be attributed to the high levels of misuse and over-the-counter delivery of antibiotics in
310 Lebanon (57,58). In fact, Cheaito *et al.* reported that 42% of the population in Beirut and

311 its suburbs are self-medicated with antibiotics (57), the majority with AMX/CLV (59).
312 Furthermore, CLV might be an increasing source of adverse reactions because of the
313 possibility of developing specific IgE (60). Another study by Mouhieddine *et al.* showed
314 high level of antibiotic misuse among the Lebanese population. In fact, more than a third
315 of the population does not consult a physician before starting the treatment (61,62). In
316 addition, as recounted by others, NSAIDs were the second most reported DHR in our
317 clinic (10). NSAIDs can cause immune DHRs, or, more frequently, non-immune reactions
318 due to the inhibition of cyclooxygenase-1 (COX-1), leading to a shunt of arachidonic acid
319 metabolism towards the 5-lipoxygenase pathway and increased production of cysteinyl
320 leukotrienes. This shunt results in the depletion of prostaglandin E2 and the release of
321 inflammatory mediators from mast cells and other inflammatory cells (9,63). Aspirin was
322 the most reported NSAID, followed by ibuprofen and diclofenac. Our results were
323 consistent with the presence of the heteroaryl acetic acid group in diclofenac and
324 ibuprofen, which seems to carry a higher risk of anaphylactic reactions than other groups
325 (64). As described by others, we found that quinolones (7.8%) are the third most frequent
326 drug associated with DHRs (65,66).

327 The diagnosis of DHRs in our study was primarily based on the clinical history of
328 the reaction, and in 22.6% of aDHR cases, the clinician performed an SPT for confirmation.
329 Gomes *et al.* also reported the same figures in 2004. They recounted that 22.7% of
330 Portuguese patients underwent skin testing (31). It is noteworthy that neither public nor
331 private insurances cover SPT costs in Lebanon. Additionally, almost half of the Lebanese
332 population lacks sufficient healthcare coverage and turns to out-of-pocket payments. In
333 addition to the economic constraint, these results partially confirmed what is known
334 about Middle eastern patients' hesitation toward drug allergy testing (33).

335 We evaluated the trends of BLs, NSAIDs, and other antibiotic aDHR cases recorded
336 during the ten years. At first, we expected a decrease in cases during 2020 and 2021 since
337 most allergy consultations worldwide were postponed due to the COVID pandemic
338 (67,68). In addition, the nuclear-like Beirut port explosion on August 4, 2020, destroyed

339 vital health infrastructure and left more than half of Beirut's medical facilities non-
340 functional, according to the World Health Organization (69). However, the allergy clinic's
341 diagnostic and treatment activities were not affected. Patients were handled and followed
342 up virtually, and severe cases were granted face-to-face consultations. Another
343 explanation for the rise in the number of cases is the significant increase in DHRs over
344 the last few years (70), which can be due to the perturbation of the human microbiome
345 by environmental impact. (71,72). This increase in DHRs diagnosed in the clinic coincides
346 with the messenger ribonucleic acid (mRNA) COVID-19 vaccination. We believe that
347 patients were concerned by a putative allergic reaction to the COVID-19 vaccine, which
348 might have led them to evaluate their history of DHRs in the clinic before their first
349 vaccine dose (73–76).

350 Our study identified and analyzed six SJS cases (0.8%). Other studies on
351 hospitalized patients recorded more cases, with a percentage of around 2% (32). These
352 disparities can be explained by the severity of the reaction and the need for
353 hospitalization of these patients, thus not recorded in our data. All patients who suffered
354 from SJS were elderly, with a mean age of 67. SJS reactions are known to occur in patients
355 of all ages, but the highest incidence is recorded in adults older than 40 (77). Concerning
356 the molecules causing SJS, we recorded cases of TMP/SMX, allopurinol, and
357 metronidazole, as described by others (78–80).

358 Perioperative DHRs constitute the first-line challenge for anesthesiologists and
359 allergists (81). Our study population recorded 90 cases of DHRs to drugs for ambulatory
360 use and concomitant positive SPT to molecules used in the perioperative setting. We
361 found opioids and NMBAs to be the two most common therapeutic classes involved. It
362 is known that DHRs to NMBAs represent the leading cause of perioperative anaphylactic
363 reactions (about 58% of cases in France) (82–84). However, the percentage of cases
364 reported for opioids is higher than what is typically described (85–87). In our study, the
365 majority of SPT positive results were detected with morphine and pethidine, both of
366 which have potent histamine-releasing properties caused by direct degranulation of mast

367 cells (88). NMBAs are classified as depolarizing (suxamethonium) or nondepolarizing
368 (rocuronium, atracurium, cisatracurium) (89,90). It was previously known that
369 suxamethonium was the most commonly found molecule, yet, its usage is decreasing
370 (91). Recent data suggests a rising incidence of DHRs to atracurium, rocuronium, and
371 cisatracurium (a stereoisomer of atracurium) (82,92), which coincides with our findings.
372 Furthermore, we evaluated the cross-reactivity between molecules of the same
373 therapeutic class. The sensitivity and specificity of SPTs for NMBAs are estimated at 94 to
374 97% and 96 to 98%, respectively (93,94). Therefore, SPTs are useful for diagnosing and
375 evaluating cross-reactivities (93,94). Our SPT results established that rocuronium and
376 cisatracurium were the most commonly cross-reactive. Even though rocuronium has been
377 frequently reported to be implicated in cross-reactivity (more than 90% of cases) (95),
378 current reports showed disparities in cross-reactivity patterns between different
379 geographical regions (96). This might result from the selection bias due to varying rates
380 of NMBA usage, assuming the NMBAs are the sensitizers (86,95–97).

381 The most common opioid association we recorded was morphine and pethidine
382 (also known as meperidine). Based exclusively on chemical structures, natural (morphine,
383 codeine) and semisynthetic opioids (oxycodone) are structurally very similar, and possible
384 cross-reactivity is to be evaluated. Nevertheless, synthetic opioids such as pethidine are
385 structurally different, which suggests no cross-reactivity (98). However, structural
386 similarities between morphine and synthetic opioids are often overlooked. In fact, these
387 two molecules share a phenylpiperidine structure and a phenylpropanolamine group
388 (88), which might explain the cross-reactivity observed.

389 In conclusion, this is the first study evaluating the clinical characteristics of all
390 reported DHRs in Lebanon between 2012 and 2022 in an allergist outpatient clinic. Our
391 retrospective study constitutes the first step toward a more comprehensive knowledge
392 of the reality of DHRs in this region. More outpatient data collection and hospital-based
393 studies must be conducted, biological evaluation such as basophil activation test and a
394 national data-based registry for DHRs must be implemented in Lebanon.

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- 670

671 **Figure legends:**

672 **Figure 1 Percentages of the most common drugs implicated in drug**
673 **hypersensitivity reactions (n=758 patients).** Molecules used in the perioperative
674 setting are presented separately in table IV. The percentages do not add up to 100, as
675 one patient can report multiple drug hypersensitivity reactions. * Other antibiotics include
676 quinolones, sulfonamides, metronidazole, macrolides, clindamycin, and tetracyclines. **
677 Opioids in ambulatory use. *** See other molecules listed in table E1 in the online
678 repository. NSAIDs: non-steroidal anti-inflammatory drugs.

679

680 **Figure 2 Evolution of the number of NSAIDs, beta-lactams, and other antibiotics**
681 **drug hypersensitivity cases from 2012 to 2021.** NSAIDs: non-steroidal anti-
682 inflammatory drugs.

683

684 **Figure E1 Percentage of active drug hypersensitivity patients confirmed by clinical**
685 **history or skin prick test.** The diagnosis of DHRs in our study was majorly based on the
686 clinical history of the reaction; in many cases, a SPT was proposed by the clinician for
687 better confirmation. SPT: skin prick test.

688 **Table I Demographic characteristics of patients with drug hypersensitivity reactions**
 689 **between 2012 and 2021.**

Total number of patients with DHRs included	758	
Sex ratio F:M	2.6:1	
Age range (median)	1-87 years (39 years)	
Reason for consultation	n	(%)
aDHR	190	(24.9)
Pre-anesthesia evaluation	60	(7.9)
Food allergy	26	(3.4)
Respiratory allergy	15	(2.0)
<u>Other symptoms</u>		
Urticaria	147	(19.4)
Rhinitis	86	(11.3)
Angioedema	33	(4.4)
Asthma	31	(4.1)
Dermatitis	29	(3.8)
Chronic cough	25	(3.3)
Rhinosinusitis	14	(1.8)
Pruritis	12	(1.6)
Bronchitis	11	(1.5)
Others*	79	(10.6)
Concomitant history of allergy	262	(34.6)
Respiratory allergy	173	(47.3)
Food allergy	138	(52.7)
Past family history of allergy	226	(29.8)
Respiratory allergy	212	(93.8)
Drug allergy	14	(6.2)
DHR to one or multiple molecules		
One molecule	511	(67.4)
Two molecules	159	(20.9)
Three molecules	75	(9.9)
Four molecules	11	(1.5)
Five molecules	2	(0.3)

690

691 * Others include dyspnea, conjunctivitis, rhinoconjunctivitis, anaphylaxis, alopecia,
 692 shortness of breath, contact dermatitis, nasal congestion, nasal polyposis, vasculitis, otitis,
 693 pneumonia, bullous lichen planus, bronchitis, psoriasis, scalp seborrheic dermatitis.
 694 aDHR: active drug hypersensitivity reaction, DHR: drug hypersensitivity reaction, F:
 695 female, M: male.

696 **Table II Characteristics of antibiotic hypersensitivity reactions.**

697

698

Antibiotic class	Number (% ^a)	Molecules	Clinically diagnosed n (% ^b)	SPT confirmed n (% ^b)	Symptoms (% ^b)	Delayed/Early onset of reaction (% ^b)	
Beta-lactams	408 (53.8)	Penicillins	Amoxicillin/Clavulanic Acid	159 (39.0)	9 (2.2)	Urticaria (79.2) Angioedema (10.8) Gastrointestinal (3.2) Anaphylaxis (3.2) Pruritis (1.4) Others (2.2)	Immediate (95.8) Delayed (4.2)
			Penicillin G	116 (28.4)	38 (9.3)		
			Amoxicillin	21 (5.1)	15 (3.7)		
			Ampicillin	2 (0.5)	-		
		Cephalosporins	Ceftriaxone	9 (2.2)	-		
			Cefixime	7 (1.7)	3 (0.7)		
			Cefuroxime	6 (1.5)	2 (0.5)		
			Cefdinir	3 (0.7)	-		
			Cefpodoxime	3 (0.7)	2 (0.5)		
			Cefadroxil	2 (0.5)	-		
			Cefalexin	1 (0.3)	-		
			Not specified	8 (2.0)	2 (0.5)		
Total	337 (82.6)	71 (17.4)					
Quinolones	59 (7.8)	Levofloxacin	24 (40.6)	1 (1.7)	Urticaria (66.1) Angioedema (13.6) Skin Rash* (10.2) Others (10.1)	Immediate (77.8) Delayed (22.2)	
		Ciprofloxacin	7 (11.9)	9 (15.2)			
		Nadifloxacin	2 (3.4)	-			
		Norfloxacin	2 (3.4)	-			
		Ofloxacin	1 (1.7)	-			
		Not specified	7 (11.9)	6 (10.2)			
		Total	43 (72.9)	16 (27.1)			
Sulfonamides	44 (5.8)	TMP/SMX	44 (100.0)	-	Skin rash* (72.7) Urticaria (13.6) SJS (6.8) FDE (2.3) Angioedema (2.3) Anaphylaxis (2.3)	Immediate (77.3) Delayed (22.7)	

Nitroimidazoles	27 (3.6)	Metronidazole	24 (88.9)	3 (11.1)	Urticaria (74.1) Angioedema (11.1) Anaphylaxis (3.7) Facial erythema (3.7) SJS (3.7) Skin Rash* (3.7)	Immediate (92.6) Delayed (7.4)
Macrolides	10 (1.3)	Clarithromycin	2 (20.0)	1 (10.0)	Urticaria (100.0)	Immediate (100.0)
		Erythromycin	2 (20.0)	-		
		Azithromycin	1 (10.0)	-		
		Spiramycin	1 (10.0)	-		
		Not specified	2 (20.0)	1 (10.0)		
		Total	8 (80.0)	2 (20.0)		
Lincomycin	9 (1.2)	Clindamycin	7 (77.8)	2 (22.2)	Urticaria (55.6) Skin Rash* (22.2) Tachycardia (11.1) FDE (11.1)	Immediate (55.6) Delayed (44.4)
Tetracycline	2 (0.3)	Doxycycline	1 (50.0)	-	Urticaria (50.0) Gastritis (50.0)	Immediate (50.0) Delayed (50.0)
		Not specified	1 (50.0)	-		

699

700 * Skin rash other than urticaria, including erythema multiforme and dermatitis.

701 Clinically, drug hypersensitivity reactions are classified as immediate (typically <1 h following the last intake of the culprit drug) or

702 delayed (typically >1 h to days after the start of treatment with the culprit drug) (14). FDE: fixed drug eruption, SJS: Steven Johnson

703 syndrome, SPT: skin prick test, TMP/SMX: trimethoprim/sulfamethoxazole.

704 ^a percentage of the total number of patients in the study population.

705 ^b percentage of the number of patients in the therapeutic class.

706 **Table III Characteristics of non-antibiotic drug hypersensitivity reactions.**

Molecule class	Number (%^a)	Molecules	Clinically diagnosed n (%^b)	SPT confirmed n (%^b)	Symptoms (%^b)	Delayed/Early onset of reaction (%^b)
NSAIDs	371 (48.9)	Aspirin	44 (11.9)	-	Urticaria (66.8) Angioedema (22.4) Anaphylaxis (2.4) Skin Rash* (1.9) Dyspnea (1.9) Pruritis (1.1) Others (3.5)	Immediate (96.2) Delayed (3.8)
		Ibuprofen	32 (8.6)	-		
		Diclofenac	15 (4.0)	-		
		Ketoprofen	8 (2.2)	-		
		Mefenamic acid	5 (1.3)	-		
		Aceclofenac	4 (1.1)	-		
		Celecoxib	4 (1.1)	-		
		Etoricoxib	4 (1.1)	1 (0.3)		
		Naproxen	3 (0.8)	-		
		Normocoxib	3 (0.8)	-		
		Lysine clonixinate	1 (0.3)	-		
		Not specified	245 (66.0)	2 (0.5)		
<i>Total</i>		<i>368 (99.2)</i>	<i>3 (0.8)</i>			
RCM	17 (2.2)	RCM	17 (100.0)	-	Urticaria (64.7) Anaphylaxis (23.5) Angioedema (11.8)	Immediate (100.0)
Opioids for ambulatory use	13 (1.7)	Tramadol	10 (76.9)	2 (15.4)	Urticaria (61.5) Hypotension (23.1) Angioedema (15.4)	Immediate (76.9) Delayed (23.1)
		Codeine	1 (7.7)	-		
		<i>Total</i>	<i>11 (84.6)</i>	<i>2 (15.4)</i>		
Acetaminophen	9 (1.2)	Acetaminophen	9 (2.4)	-	Urticaria (66.7) Angioedema (33.3)	Immediate (100.0)

707 * Skin rash other than urticaria, including erythema multiforme and dermatitis. Clinically, drug hypersensitivity reactions are classified
 708 as immediate (typically <1 h following the last intake of the culprit drug) or delayed-type (typically >1 h to days after the start of
 709 treatment with the culprit drug) (14). NSAIDs: non-steroidal anti-inflammatory drugs, RCM: radiocontrast media, SPT: skin prick test.

710 ^a percentage of the total number of patients in the study population. ^b percentage of the number of patients in the therapeutic class.

711 **Table IV Characteristics of patients with a history of ambulatory drug**
 712 **hypersensitivity reactions and a positive SPT to molecules used in the**
 713 **perioperative setting.**

	Number (%)	SPT confirmed (%)	Clinically diagnosed	Symptoms (%)	Delayed/Early onset of reaction
Molecules used in the perioperative setting	90 (11.9)	90 (100.0)	–	Anaphylaxis (95.6) Angioedema (2.2) Dyspnea (1.1) Facial erythema (1.1)	Immediate (100.0)

714

715 Clinically, drug hypersensitivity reactions are classified as immediate (typically <1 h
 716 following the last intake of the culprit drug) or delayed-type (typically >1 h to days
 717 after the start of treatment with the culprit drug) (14).

718 **Table V Number of patients with a history of ambulatory drug hypersensitivity**
 719 **reactions with a positive SPT to the different molecules used in the perioperative**
 720 **setting tested.**

Drug class	Number of patients with a positive SPT	Molecule	Number of cases
Opioids	74	Morphine	52
		Pethidine	38
		Sufentanil	26
		Remifentanil	21
		Fentanyl	18
		Oxycodone	2
NMBA	57	Rocuronium	46
		Cisatracurium	19
		Atracurium	10
		Suxamethonium	3
Hypnotics	18	Midazolam	8
		Ketamine	5
		Propofol	4
		Etomidate	3
		Pentobarbital	1
		Sodium thiopental	1
Local anesthesia	12	Lidocaine	7
		Bupivacaine	3
		Ropivacaine	2
		Mepivacaine	1
Others	39	Latex	36
		Patent Blue	5

721

722 NMBA: neuromuscular blocking agent, SPT: skin prick test.

723 **Table VI Most common associations evaluating the possible cross-reactivity to**
 724 **molecules used in the perioperative setting in patients with a history of**
 725 **ambulatory drug hypersensitivity reactions.**

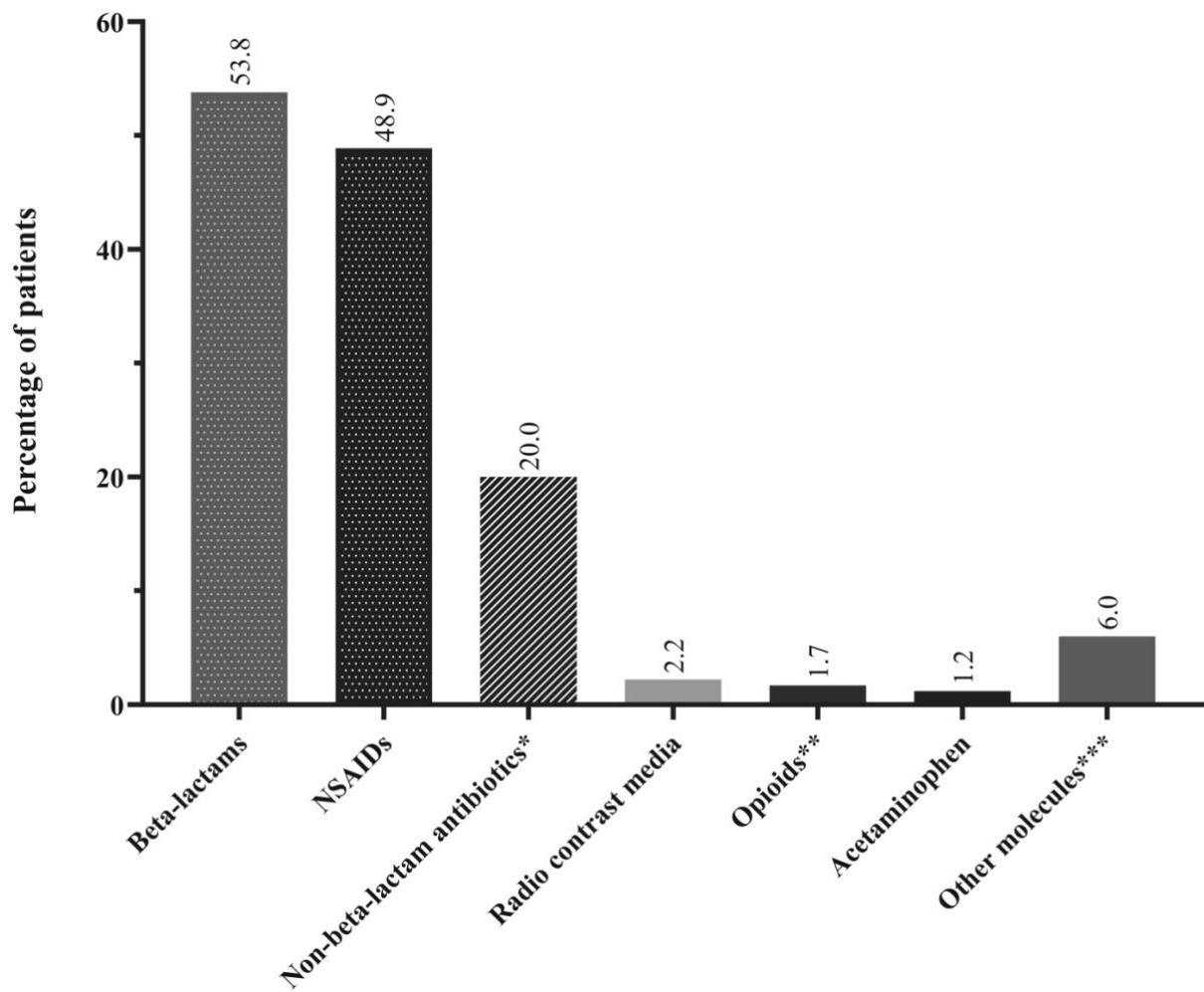
726

Drug class	Number of molecules	n	Most frequent molecules ^a	n
Opioids	One molecule	25	Morphine	11
	Two molecules	26	Morphine, Pethidine	13
	Three molecules	13	Morphine, Pethidine, Fentanyl	4
	Four molecules	9	Morphine, Pethidine, Sufentanil, Remifentanil	3
	Five molecules	1	Morphine, Pethidine, Sufentanil, Remifentanil, Fentanyl	1
NMBA	One molecule	39	Rocuronium	29
	Two molecules	15	Rocuronium, Cisatracurium	10
	Three molecules	3	Rocuronium, Cisatracurium, Atracurium	2
Hypnotics	One molecule	16	Midazolam	6
	Two molecules	1	Midazolam, Ketamine	1
	Three molecules	1	Midazolam, Ketamine, Etomidate	1
Local anesthesia	One molecule	11	Lidocaine	6
	Two molecules	1	Lidocaine, Ropivacaine	1

727

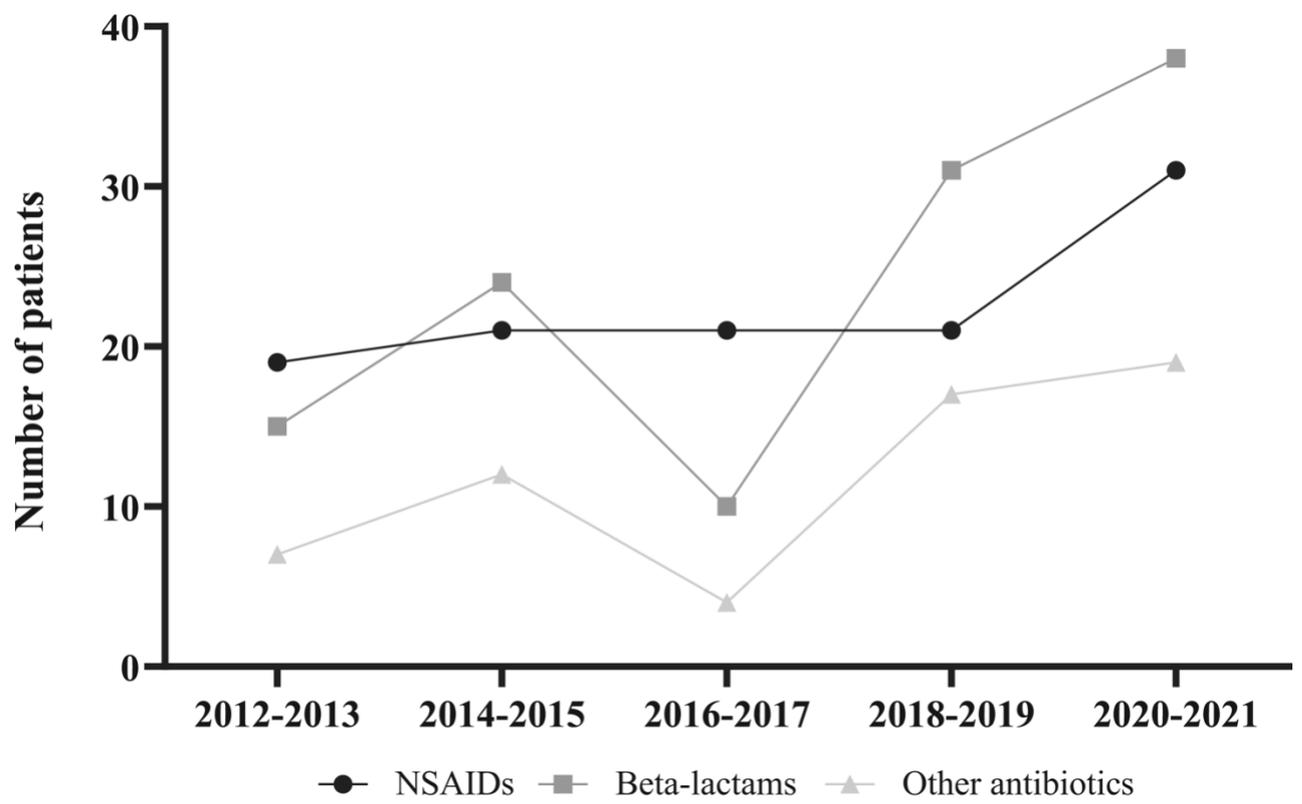
728 ^a For each number of molecules, only the most frequent association of cross-reactivity
 729 is described.

730



731

732 **Figure 1**

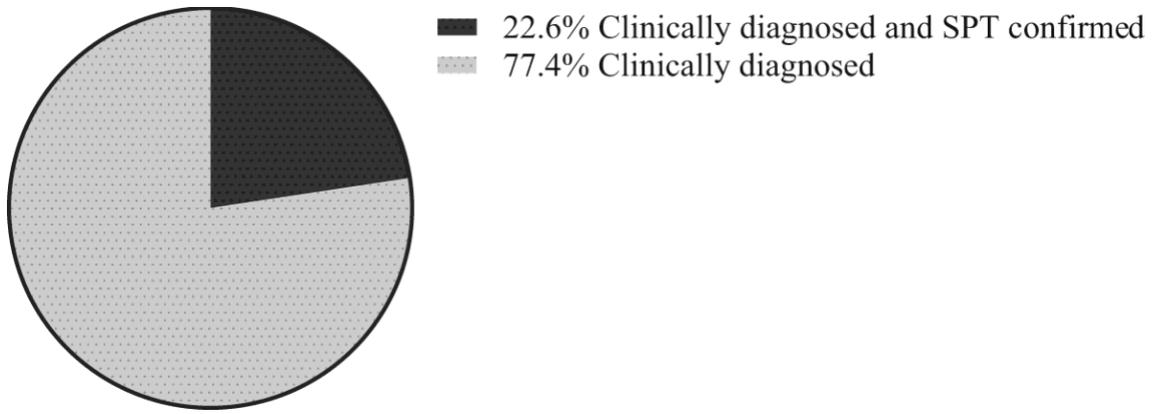


733

734 **Figure 2**

735

736



737

738 **Figure E1**

739

2. The development of an immunization score to predict drug allergies

INTRODUCTION

Drug hypersensitivity reactions are idiosyncratic and can often be independent of the drug's pharmacological effect. As a result, they are rarely detected during the non-clinical and clinical phases of drug development. Therefore, they can lead to late discontinuation of new drug candidates, withdrawal of marketing authorizations, and additional costs related to managing affected patients. With the increasing number of new drugs, there is a growing need in the pharmaceutical industry for a reliable and accurate method to predict the potential for these drugs to induce allergic reactions. Therefore, elaborating on a novel immunization score model to predict the pro-allergenic potential is critical for improving patient safety.

This second part of the thesis project aims to propose a weight-of-evidence (WoE) approach to predict the immunization risk of new drug candidates based on the available literature on a selection of well-known pro-allergenic therapeutic molecules. This approach has two dimensions:

- The **conception** of the score was based on the analysis of four *in vitro* and *in silico* parameters mimicking key immunization events and two clinical parameters that reflected the effect of known pro-allergenic drug molecules based on a thorough literature review. Each parameter was coupled with a coefficient, allowing the calculation of a predictive and a clinical score. These two scores were then correlated. This comprehensive WoE categorization approach may also aid in identifying gaps in the literature and proposing ways to fill them.
- The potential **application** of the score to new molecules was based on the analysis of the same *in vitro* and *in silico* predictive parameters of a drug in

the development process (with no clinical data available) to be able to calculate a predictive score. Researchers have the tools necessary to determine the predictive score. Therefore, based on the correlation previously established, this model will allow the estimation of the clinical score.

METHODS

1. Identification of the parameters

The development of this immunization score requires extensive research and analysis. A critical aspect of this process is the identification of the appropriate parameters. Once these parameters have been identified and approved, they can be used to develop an immunization score that comprehensively assesses the new molecule's potential for immunization.

To identify the predictive parameters, we turned to the four essential steps we believe are involved in the immunization process (**Figure 1**):

1. The **chemical reactivity** of the molecule (or its metabolite). Identifying protein-drug and/or protein-metabolite bioconjugates by western blot or mass spectrometry assays.
2. The **immune danger signals** induced by the molecule (or its metabolite). The measure of dendritic cells surface activation markers (CD83), co-stimulation markers (CD80, CD86, CD40), adhesion molecules (CD54), chemokine receptors (CXCR4, CCR7), and the secretion of pro-inflammatory cytokines (e.g., IL-1 β , IL-6, TNF- α , IL-8).
3. The **interactions** of peptides bound with chemicals (hapten theory) or free molecules (pharmacologic interaction with immune receptors or p-i concept) with major histocompatibility complex molecules (MHC) or T-cell receptors (TCR). This interaction can be evaluated by *in vitro* tests or *in silico* methods such as the immune epitope database (IEDB) or TEPITOPE.

According to the hapten model, it is proposed that low molecular weight (LMW) drugs and their metabolites are not immunogenic in their unbound state. Instead, they form covalent bonds with specific host proteins. The resulting complexes are internalized by antigen-presenting cells (APCs), which then process and present them on major MHC molecules. This presentation has the potential to activate T cells that are specific to the drug (Posadas and Pichler, 2007; Pichler and Hausmann, 2016). Alternatively, the p-i model suggests that drugs can bind reversibly and induce the formation of noncovalent complexes between the drug and MHC molecules or TCRs, which can directly activate T cell responses without requiring a specific peptide ligand (Pichler, 2008).

4. The identification of a **specific T-cell repertoire** recognizing the molecule, the metabolite, or the bioconjugates.

In addition to these predictive parameters, two clinical parameters were also selected to calculate the clinical score. **(Figure 1)**:

1. The **prevalence** of drug allergy in the general population: The prevalence refers to the proportion of individuals in a population who have a drug allergy at a particular time or during a specific period. It represents the total number of allergy cases in the treated population, including new and existing patients.
2. The clinical **symptoms** of drug allergic reactions: understanding the symptoms of drug allergic reactions provides insight into the various mechanisms of action implicated in such reactions. Since multiple symptoms can arise from various mechanisms of action, this might lead to higher chances for these symptoms to manifest in a greater number of patients.

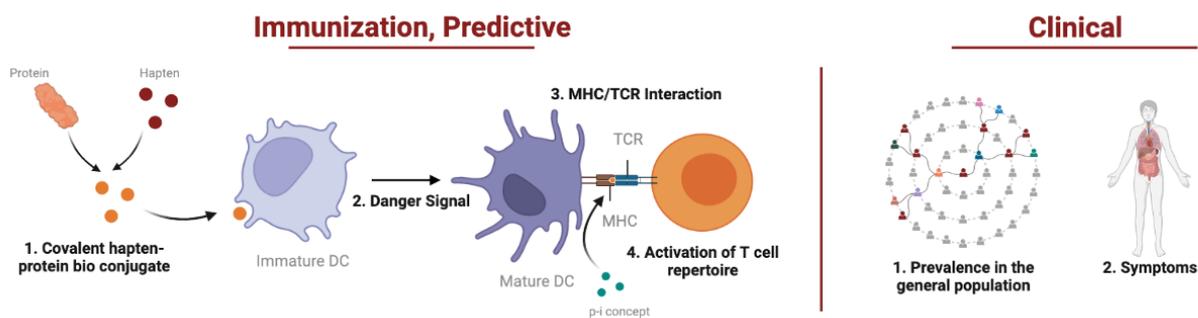


Figure 1. Key events in immunization and clinical events (created in Biorender).

2. Attribution of coefficients and calculation of scores

After identifying the parameters, coefficients were assigned to each parameter to develop a scoring model that could predict the likelihood of an allergic outcome based on the experimental findings and literature review. The coefficients presented in **Table 1** were carefully determined after thorough evaluation.

The predictive score incorporated specific coefficients to assess different aspects. The evaluation of chemical reactivity and molecule binding involved assigning (+1) for binding to a single protein, (+1) for binding to multiple proteins, (+1) for the presence of a covalent bond, and (+1) for binding observed with both the molecule and its metabolite. To evaluate immune danger signals, we consider $(+0.5*x)$, where x represents the number of activation, adhesion, costimulatory markers, and cytokines expressed by the molecule, with an additional $(+0.5*y)$ for metabolite markers. The interaction with the MHC or the TCR is assessed with (+1) for *in silico* interaction with the molecule, (+1) for *in vitro* interaction with the molecule, (+1) for *in silico* interaction with the metabolite, and (+1) for *in vitro* interaction with the metabolite. The identification of the T-cell repertoire is rated as (2) if T-cell activation is demonstrated without subpopulation identification, (2) if demonstrated with CD4+ or CD8+ T-cells, and (4) if demonstrated with both CD4+ and CD8+ T cells.

For the clinical score, specific coefficients are chosen as well. Prevalence ratings range from (1) for a prevalence between 0% and 5%, (2) for a prevalence between 5% and 10%, and (4) for a prevalence exceeding 10%. Regarding clinical symptoms, the number of symptoms is multiplied by 0.5 ($z \cdot 0.5$) to determine their contribution to the overall score.

It is worth noting that in cases where the research for any key event yielded negative results, the coefficient value was set to zero. On the other hand, if the data was unavailable due to a lack of knowledge in the literature, the acronym ND, meaning not defined, was designated in place of the coefficient value. Therefore, if one parameter was ND, the score was not calculated and is then considered ND as the equation is incomplete and no value could be attributed.

Once these parameters were identified, they were used to calculate the two immunization scores using the following formulas:

Predictive score = Coef. [(Chem. reactivity + Danger sig. + Interactions) * T-cell rep].

The sum of coefficients for the initial three parameters was multiplied by the T-cell repertoire value. We chose to give greater importance to the presence of a T-cell repertoire based on the underlying rationale that its absence would nullify the score. This signifies that the absence of a T-cell repertoire indicates the absence of T-cell response, as well as subsequent immunization and allergic reactions. In other words, the presence of a T-cell repertoire is considered a crucial factor in the development of immune reactions and the potential for allergic responses to a given drug. By incorporating this weighting approach, we aim to emphasize the significance of T-cell involvement in the overall assessment of pro-allergic potential

Clinical score = Coef. [Prevalence + Symptoms]

A linear regression analysis was then used to examine the hypothesis concerning the association between the two scores. This helped us establish the connection between the predictive score (considered the independent variable) and the clinical score (considered the dependent variable). We then computed the coefficient of determination R^2 , which determines the accuracy of the regression model in fitting the data. Therefore, for each new molecule in the drug development process, the results of *in vitro* and *in silico* experiments will allow the calculation of the predictive score and, consequently, the correlation of the clinical score to assess the pro-allergic potential of the molecule.

Table 1. Coefficients and calculation of the predictive and clinical scores.

		Predictive parameters						Clinical parameters				
		Chemical reactivity: protein-drug and protein-metabolite bio-conjugates	Identification of danger signals	Interactions with MHC and TCR molecules	Identification of a naïve T-cell repertoire / or drug-specific T cells	Prevalence of allergy	Symptoms					
Methods	Identification of protein-drug and/or protein-metabolite bioconjugates (western blot, mass spectrometry, proteomics)		Measure of mo-DCs surface activation markers, adhesion molecules, chemokine receptors and the secretion of pro-inflammatory cytokines		Interactions of peptides (hapten theory) or free molecules (p-i concept) with MHC or TCR (<i>in vitro</i> tests or <i>in silico</i> methods)		Identification of drug-specific CD4+ and/or CD8+ T cells		Prevalence of allergy in the treated population		Symptoms of the allergic reactions	
	Coefficients	Binding to one protein	+1	Increase of x markers by the molecule	x * 0,5	<i>in silico</i> prediction	+1	T cells (without subpopulation identification)	2	0 - 5%	1	Immediate antibody-mediated reactions
Binding to multiple proteins		+1	Increase of y markers by the metabolite	y * 0,5	<i>in vitro</i> assays	+1	CD4+ or CD8+ T cells	2	5 - 10%	2	Urticaria, maculopapular exanthema, skin rash	+0.5
Covalent binding		+1			Metabolite <i>in silico</i>	+1	CD4+ and CD8+ T cells	4	>10%	4	DILI	+0.5
Binding of the molecule and its metabolite		+1			Metabolite <i>in vitro</i>	+1					SJS/TEN	+0.5
		Negative results = 0; Missing data = ND										
		Missing data = ND										
Score		Coef. [(Chemical reactivity + Danger signal + Interactions) * T-cell repertoire]						Coef. [Prevalence + Symptoms]				

AGEP: acute generalized exanthematous pustulosis; Coef: Coefficient; DIA: drug-induced agranulocytosis; DILI: drug-induced liver injury; DC: dendritic cell; DRESS: drug reaction with eosinophilia and systemic symptoms; IEDB immune epitope database; IL: Interleukin; LABD: Linear IgA bullous disease; MHC: major histocompatibility complex; ND: not defined; SJS/TEN: Stevens-Johnson syndrome/toxic epidermal necrolysis; TCR: T-cell receptor.

3. Literature review and collection of data for the conception of the score

A great deal of expert judgment is required to analyze the available data in the literature. The decision-making process and the data considered are described in this section.

Conducting a comprehensive literature review is essential in developing a new scoring system built on sound scientific knowledge. A thorough review of the existing literature allows the identification of knowledge gaps and an understanding of the current state of research. This knowledge was then applied to guide the development of the new system and ensure its scientific validity.

Information was collected based on a thorough review of all available published information in the literature, including *in vitro* and *in silico* data. We first defined the scope of the research question (the parameters), identified relevant databases (Pubmed, Google Scholar, scientific journals, etc.), and defined keywords. In some instances, other thesis results from our laboratory were used as input data to aid in completing missing information. Once the initial search was conducted, we critically evaluated the studies and determined their relevance. Studies that are deemed relevant are then extracted and analyzed. For the conception of the score, 13 molecules were assigned predictive and clinical scores (penicillin, clavulanic acid, dapsone, allopurinol, carbamazepine, lamotrigine, vancomycin, flucloxacillin, sulfamethoxazole, sulfasalazine, abacavir, ticlopidine, and cisplatin). The literature review also provided a basis for evaluating the data quality collected, focusing on the current state of knowledge in the field.

Results

Understanding the mechanisms of the presentation of antigens to T cells is imperative, as they can significantly impact the efficacy of immunization to different molecules. In this section, we provide a detailed description of the score calculations for each molecule, which are classified based on their mechanism of recognition by T cells. By dividing the immunization scores, we can better understand and optimize using different molecules in our score. Penicillins and clavulanic acid activate T cells exclusively through the hapten mechanism, while allopurinol, carbamazepine, lamotrigine, and vancomycin activate T cells exclusively through the p-i mechanism. Other molecules such as flucloxacillin, sulfamethoxazole, sulfasalazine, and activate T cells by both mechanisms. Abacavir is the only molecule that can alter the conformation of HLA self-peptides in the peptide binding groove (altered self-repertoire). Other molecules were investigated without knowledge of their mechanisms of action, such as ticlopidine and cisplatin. Additionally, we provided a brief background on the drug hypersensitivity reactions associated with each molecule, which will aid in understanding the relevance and clinical significance of the scoring system.

1. Mechanism: Hapten

As briefly described above, the mechanism of antigen presentation is an important factor in T-cell activation that may also have clinical consequences. Multiple drugs such as penicillin, clavulanic acid, and dapsone act exclusively as haptens. They are not immunogenic and need to bind to protein carriers, forming neo-antigens presented to T cells (Posadas and Pichler, 2007; Pichler and Hausmann, 2016).

Penicillin

Penicillins are the most commonly prescribed antibiotics worldwide (Macy, 2014) and the most frequent cause of drug allergy, with a prevalence ranging from 6 to 25% depending on the country (Castells, 2019; Torres et al., 2019). The most common clinical symptoms of penicillin allergy are immediate cutaneous reactions, such as urticaria or maculopapular rash (Gomes and Demoly, 2005; Castells, 2019). Anaphylaxis is rare as it occurs in only 0.02% to 0.04% of penicillin administrations (Patterson and Stankewicz, 2023).

The complete penicillin immunization score was calculated with the following parameters (**Table 2**): predictive score = [4 (covalent binding of the molecule and its metabolite to several proteins) + 0 (no activation of DCs) + 3 (interaction with MHC molecules demonstrated *in vitro* and *in silico* and the metabolite *in vitro*)] * 4 (identification of a repertoire of CD4+ and CD8+ T cells recognizing benzylpenicillin) = 28; clinical score = [4 (prevalence >10%)+ 3 (six symptoms)] = 7.

It is noteworthy that penicillin reported the highest predictive score.

Clavulanic acid

Clavulanic acid, also known as clavulanate, is a beta-lactamase inhibitor. It is commercially available in combination with amoxicillin in various forms, such as tablets, chewable tablets, and suspensions. Despite being initially believed to be non-immunogenic (Edwards et al., 1988), it has been demonstrated that clavulanate can

cause immediate hypersensitivity reactions in some individuals following the hapten model (Sánchez-Morillas et al., 2010; Torres et al., 2010; Ariza et al., 2020, 2022). The prevalence of allergic reactions to clavulanic acid is hard to determine as it is administered exclusively with amoxicillin, and the information is limited to case reports (Sánchez-Morillas et al., 2010).

The incomplete clavulanic acid immunization score was calculated with the following parameters (**Table 3**): score = [3 (Covalent binding of the molecule to multiple proteins) + 2.5 (increase in 5 activation and migration markers) + 2 (binding to MHC *in vitro* and *in silico*)] * 2 (identification of clavulanic acid-specific CD4+ T-cells) = 15; clinical score = [ND (not defined) + 0.5 (one symptom)] = ND.

Table 2. Predictive and clinical scores for penicillin.

Predictive parameters					Clinical parameters	
Methods	Chemical reactivity: protein-drug and protein-metabolite bio-conjugates	Identification of danger signals	Interactions with MHC and/or TCR molecules	Identification of a naïve T-cell repertoire / or drug-specific T cells	Prevalence of allergy	Symptoms
Results	Covalent binding of the molecule and its metabolite to multiple proteins: Albumin, Ig and transferrin	No phenotypic modification of DCs	Immunodominant peptides (PT-BP, KV-BP, and FS-BP) interact with MHC molecules <i>in silico</i> and <i>in vitro</i>	Identification of a CD4 and CD8 T cell repertoire	10-12%	Antibody mediated reactions MPE SJS/TEN DRESS DILI AGEP
References	(Magi et al., 1995; Meng et al., 2011; Ariza et al., 2012; Nhim et al., 2013; Ariza et al., 2014; Azoury et al., 2018)	<i>Thesis results of Cathy Nhim 2013</i>	(Meng et al., 2011; Nhim et al., 2013; Scornet et al., 2016; Azoury et al., 2018)	(Nhim et al., 2013; Azoury et al., 2018; Bechara et al., 2019)	(Joint Task Force on Practice Parameters et al., 2010; Macy and Ho, 2012)	(Joint Task Force on Practice Parameters et al., 2010; Blumenthal et al., 2019)
Coefficients	= 1 + 1 + 1 + 1	= 0	= 1 + 2	= 2 + 2	= 4	= 0.5 * 6
Predictive score = (4 + 0 + 3) * 4 = 28					Clinical score = 4 + 3 = 7	

Table 3. Predictive and clinical scores for clavulanic acid.

Predictive parameters					Clinical parameters	
Methods	Chemical reactivity: protein-drug and protein-metabolite bio-conjugates	Identification of danger signals	Interactions with MHC and/or TCR molecules	Identification of a naïve T-cell repertoire / or drug-specific T cells	Prevalence of allergy	Symptoms
Results	Covalent binding to multiple proteins	Increase of CCR7, CD40, CD80, CD83, CD86	Binding to MHC molecules <i>in vitro</i> and <i>in silico</i>	CD4 T cells	- <i>Allergic reports are uncommon</i> <i>Mainly case reports</i>	Immediate antibody-mediated reactions
References	(Ariza et al., 2012; Torres et al., 2016; Barbero et al., 2019)	(Fernandez-Santamaria et al., 2022)	(Ariza et al., 2022)	(Ariza et al., 2022)	-	(Blanca-Lopez et al., 2015; Pineda et al., 2015; Stover et al., 2019)
Coefficients	= 1 + 1 + 1	= 0.5 * 5	= 1 + 1	= 2	= ND	= 0.5
Predictive score = (3 + 2.5 + 2) * 2 = 15					Clinical score = ND + 0.5 = ND	

2. The pi concept

The hapten theory was developed to explain the inability of the immune system to recognize and respond to low-molecular-weight compounds by suggesting that chemical reactivity and subsequent binding to a protein carrier is essential for antigenicity. However, another model has been proposed to supplement the hapten/prohapten concept, challenging this assumption (Schnyder et al., 2000; Burkhart et al., 2002; Wei et al., 2012; Pan et al., 2019; Thomson et al., 2021). This model, known as the p-i concept (which stands for "direct pharmacological interaction of drugs with immune receptors"), suggests that certain drugs can bind specifically and reversibly to HLA or TCR molecules without covalently modifying the MHC-peptide complex (Pichler, 2008). We will present the characteristics of allopurinol, carbamazepine, lamotrigine, and vancomycin.

Allopurinol (ALP), (Oxypurinol OXY)

Allopurinol is primarily used as a hypouricemic for the treatment of gout and certain types of kidney stones. It exerts its therapeutic effect by inhibiting xanthine oxidase involved in the production of uric acid (Pacher et al., 2006). Despite its efficacy, allergic reactions to allopurinol can occur in some individuals, which can be severe and life-threatening. The allopurinol hypersensitivity syndrome occurs in approximately 0.4% of patients treated (Lee et al., 2008). Studies have shown that HLA-B*58:01 allele carriers are at higher risk for developing a hypersensitivity reaction to allopurinol (Yun et al., 2013; Chung et al., 2015b). The hypersensitivity reactions are thought to be related to the production of a reactive metabolite, oxypurinol (OXY). ALP and OXY can bind to HLA molecules to stimulate an immune response (Yun et al., 2013).

The complete ALP immunization score was calculated with the following parameters: predictive score = [0 (no binding to proteins) + 0 (no activation of DCs) + 3 (binding of ALP to HLA *in vitro* and *in silico*, binding of OXY to HLA *in vitro*) * 2 (identification of an OXY-specific CD8+T-cells)= 6; clinical score = [1 (prevalence 2%) + 2 (four symptoms)] = 3.5.

Carbamazepine (CBZ)

Carbamazepine is an anticonvulsant drug used to treat seizures, neuropathic pain, and bipolar disorder associated with other molecules. Approximately 5% of individuals receiving carbamazepine treatment may develop a hypersensitivity reaction. The hypersensitivity reaction is characterized by cutaneous and hepatic symptoms such as SJS/TEN or DRESS and can involve multiple organs (Mockenhaupt et al., 2005; Fricke-Galindo et al., 2018).

This reaction is associated with the presence of the HLA-B*15:02 allele in some ethnic populations, particularly in individuals of Asian descent. To mitigate the risk of developing a hypersensitivity reaction to carbamazepine, testing for the HLA-B*1502 allele before initiating treatment is recommended in individuals of Asian descent (Illing et al., 2012; Faulkner et al., 2016).

The complete CBZ immunization score was calculated with the following parameters: predictive score = [3 (binding of the molecule and its metabolite to multiple proteins) + 0 (no activation of DCs) + 3 (binding of CBZ to HLA *in vitro* and *in silico*, binding of its metabolite to HLA *in vitro*) * 2 (identification of a CBZ-specific naïve T-cells)= 6; clinical score = [1 (prevalence 5%) + 3 (six symptoms)] = 4.

Lamotrigine (LTG)

LTG is a widely used anticonvulsant drug that belongs to the class of phenyltriazines. It is primarily used for treating epilepsy, bipolar disorder and as an adjunctive therapy for partial seizures in patients with Lennox-Gastaut syndrome (Brigo et al., 2019, 2021).

Despite its efficacy, LTG is associated with various severe cutaneous adverse reactions (SCARs). These cutaneous eruptions are often part of a generalized syndrome known as DRESS but can also be maculopapular exanthema (MPE) or SJS/TEN. These reactions are comparable to those observed with the aromatic anticonvulsants phenobarbital, phenytoin, and carbamazepine (Huang et al., 2002; Zaccara et al., 2007; Cheung et al., 2013).

The incomplete LTG immunization score was calculated with the following parameters: predictive score = [2 (binding of the molecule to multiple proteins) + ND + ND * 4 (identification of an LTG-specific CD4+ and CD8+ T-cells) = ND; clinical score = [2 (prevalence between 5 and 10%) + 1.5 (three symptoms)] = 3.5.

Vancomycin

Vancomycin is a glycopeptide antibiotic acting as a cell wall synthesis inhibitor. It is effective against many gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Streptococcus pneumoniae* (De Luca et al., 2020).

True allergic reactions due to vancomycin administration are immediate antibody-mediated or delayed, particularly Linear IgA bullous dermatosis. On the other hand, vancomycin can also cause an IgE-independent mast cell degranulation by the activation of MRGPRX2 resulting in a histamine-mediated reaction. This reaction is called the red man syndrome, which causes flushing and pruritis of the face, neck, and upper body (Sivagnanam and Deleu, 2003; Azimi et al., 2017; Huang et al., 2020; Alvarez-Arango et al., 2021).

The incomplete vancomycin immunization score was calculated with the following parameters: score = [2 (binding of the molecule to multiple proteins) + ND (not defined) + 1 (pi concept)] * 2 (identification of a naïve T-cell repertoire recognizing vancomycin) = ND; clinical score = [1 (prevalence <5%)+ 2.5 (five symptoms)] = 3.5.

Table 4. Predictive and clinical scores for allopurinol.

Predictive parameters					Clinical parameters	
Methods	Chemical reactivity: protein-drug and protein-metabolite bio-conjugates	Identification of danger signals	Interactions with MHC and/or TCR molecules	Identification of a naïve T-cell repertoire / or drug-specific T cells	Prevalence of allergy	Symptoms
Results	No protein binding for ALP and OXY	No phenotypic modification of DCs	ALP and OXY interact with MHC molecules <i>in vitro</i> . ALP <i>in silico</i>	ALP and OXY-specific T cells in allergic patients Naïve OXY-specific T cells in HLA-B*58 :01 donors	0.4%	Skin rash SJS/TEN DRESS DIA
References	(Yun et al., 2014)	(Gonçalo et al., 2015)	(Yun et al., 2014)	(Yun et al., 2013; Chung et al., 2015a; Faulkner et al., 2016)	(Lee et al., 2008; Jung et al., 2015)	(Jung et al., 2015; Stamp et al., 2016)
Coefficients	= 0	= 0	= 2 + 1	=2	= 1	= 0.5 * 4
Predictive score = (0 + 0 + 3) * 2 = 6					Clinical score = 1 + 2 = 3	

Table 5. Predictive and clinical scores for carbamazepine.

Predictive parameters					Clinical parameters	
Methods	Chemical reactivity: protein-drug and protein-metabolite bio-conjugates	Identification of danger signals	Interactions with MHC and/or TCR molecules	Identification of a naïve T-cell repertoire / or drug-specific T cells	Prevalence of allergy	Symptoms
Results	CBZ and CBZ epoxide bind to plasma proteins	No phenotypic modification of DCs	CBZ interact with MHC molecules <i>in vitro</i> and <i>in silico</i> Stable metabolites (carbamazepine 10,11 epoxide, 10-hydroxy carbamazepine interact with MHC molecules <i>in vitro</i>)	Identification of CBZ naïve T cells in 2 HLA-B*15 :02 donors	2-5%	Skin rash MPE DRESS SJS/TEN AGEP DILI
References	(Patsalos et al., 2017)	(Gonçalo et al., 2015)	(Wu et al., 2006; Ko et al., 2011; Wei et al., 2012; Illing et al., 2012)	(Faulkner et al., 2016)	(Roujeau and Stern, 1994; Beghi and Shorvon, 2011; McCormack et al., 2012)	(Mockenhaupt et al., 2005; Gidal, 2011; Fricke-Galindo et al., 2018)
Coefficients	= 1 + 1 + 1	=0	=3	=2	=1	= 0.5 * 6
Predictive score = (3 + 0 + 3) * 2 = 12					Clinical score = 1 + 3 = 4	

Table 6. Predictive and clinical scores for lamotrigine.

Predictive parameters					Clinical parameters	
Methods	Chemical reactivity: protein-drug and protein-metabolite bio-conjugates	Identification of danger signals	Interactions with MHC and/or TCR molecules	Identification of a naïve T-cell repertoire / or drug-specific T cells	Prevalence of allergy	Symptoms
Results	LTG binds to plasma proteins	-	-	Identification of lamotrigine specific CD4+ and CD8+ T cells	5.1-10% Incidence of cutaneous symptoms	MPE SJS/TEN DRESS
References	(Patsalos et al., 2017)	-	-	(Naisbitt et al., 2003)	(Guberman et al., 1999; Huang et al., 2002; Kverno et al., 2018)	(Huang et al., 2002; P-Codrea (Tigaran) et al., 2005; Zaccara et al., 2007; Cheung et al., 2013; Kim et al., 2017; Kverno et al., 2018)
Coefficient	= 1 + 1	= ND	= ND	= 4	= 2	= 0.5 * 3 = 1.5
Predictive score = (2 + ND + ND) * 4 = ND					Clinical score = 2 + 1.5 = 3.5	

Table 7. Predictive and clinical scores for vancomycin.

Predictive parameters					Clinical parameters	
Methods	Chemical reactivity: protein-drug and protein-metabolite bio-conjugates	Identification of danger signals	Interactions with MHC and/or TCR molecules	Identification of a naïve T-cell repertoire / or drug-specific T cells	Prevalence of allergy	Symptoms
Results	Vancomycin binds to multiple proteins (HSA IgA)	-	Interaction with HLA	Repertoire of naive T cells	0.3% - 2.3% Hard to determine since they all include Red Man Syndrome	Ac (anaphylaxis) AGEP SJS/TEN Linear IgA bullous dermatosis (LABD) DRESS
References	(Sun et al., 1993)	-	(Ogese et al., 2021) (Pichler, 2019)	(Ogese et al., 2021)	(Alvarez-Arango et al., 2021; Liu et al., 2022)	(Rocha et al., 2002; Lin et al., 2014; De Luca et al., 2020; Huang et al., 2020; Alvarez-Arango et al., 2021)
Coefficients	= 1 + 1	ND	=1	= 2	= 1	= 0.5 * 5 = 2.5
Predictive score = (2 + ND + 1) * 2 = ND					Clinical score = 1 + 2.5 = 3.5	

3. Mechanism: Hapten + p-i (sulfamethoxazole, sulfasalazine, flucloxacillin)

Flucloxacillin (FLU)

Flucloxacillin is a beta-lactamase-resistant isoxazolyl penicillin with a narrow spectrum of activity. Like other antibiotics in this class, flucloxacillin can elicit both types I and IV immune-mediated hypersensitivity reactions (specifically drug-induced liver injury or DILI) (Lindh et al., 2018), which can be directed towards both the shared beta-lactam ring and the specific side chains of the molecule (Kennard et al., 2019).

The activation of T cells in patients with flucloxacillin-induced DILI showed a specific association with HLA-B*57:01 molecules (Monshi et al., 2013). Hapten and p-i can occur together and result in different clinical symptoms; the hapten reactions which appear in HLA-B*57:01- individuals may be responsible for other non-hepatic symptoms (Jenkins et al., 2009; Monshi et al., 2013), while the p-i stimulations HLA-B*57:01+ individuals may be involved in the HLA-B*57:01 linked DILI (Wuillemin et al., 2013).

The complete FLU immunization score was calculated with the following parameters: predictive score = [3 (covalent binding of the molecule and its metabolites to albumin) + 0 (no activation of DCs) + 1 (binding of FLU to HLA *in vitro*) * 4 (identification of a FLU-specific naïve CD4+ and CD8+ T-cells)] = 16; clinical score = [1 (prevalence 0.2%) + 2.5 (five symptoms)] = 3.5.

Sulfamethoxazole

Sulfamethoxazole is a sulfonamide antibiotic commonly used to treat urinary tract infections, respiratory tract infections, and pneumocystis pneumonia. The exact mechanism of sulfamethoxazole hypersensitivity reactions has yet to be understood entirely. However, it is believed that these reactions may be triggered by the metabolism of sulfamethoxazole into its hapten-reactive metabolite, leading to the activation of the immune system and subsequent hypersensitivity reactions (Sanderson et al., 2007). Indeed, SMX is the most described pro-hapten and is metabolized in the liver by CYP2C9, into SMX-hydroxylamine (SMX-NHOH). The latter undergo spontaneous oxidation into nitroso-SMX (SMX-NO), which is the toxic metabolite that binds to soluble or cellular proteins (Naisbitt et al., 1999; Ogese et al., 2015). However, SMX can also undergo a N-acetylation to form a non-toxic metabolite. Therefore, a decrease in the rate of SMX acetylation would be expected to increase the formation of the oxidized metabolite(s) with subsequent onset of toxicity (Yang et al., 2014). However, SMX can also activate the immune system by directly binding to TCR independently of antigen processing (Burkhart et al., 2002).

The complete SMX immunization score was calculated with the following parameters: score = [2 (conjugation with several proteins in a covalent manner) + 1 (activation of DCs demonstrated on a marker with SMX and its metabolite) + 1 (interaction with MHC molecules demonstrated in vitro)] * 4 (identification of a repertoire of naïve CD4+ and CD8+ T cells recognizing SMX) = 24; clinical score = [2 (prevalence >5%)+ 3 (six symptoms)] = 5.

Sulfasalazine

Sulfasalazine is a prodrug metabolized by colonic bacteria into its active components, the antibiotic sulfapyridine and the anti-inflammatory 5-aminosalicylic acid (5-ASA). The 5-ASA component of sulfasalazine is thought to be responsible for its anti-inflammatory effects, which makes it a useful treatment option for

inflammatory bowel disease (IBD), such as ulcerative colitis and Crohn's disease (Choi and Fenando, 2023). Sulfasalazine hypersensitivity reactions are caused by the direct binding of sulfapyridine to TCRs (pi-TCR) but can also follow the hapten model with the hydroxylamine metabolite of sulfapyridine (Schmid et al., 2006; Pichler, 2013; Hammond et al., 2022).

The complete sulfasalazine immunization score was calculated with the following parameters: score = [3 (Covalent binding of the molecule to albumin) + 0 (no activation of DCs) + 1 (interaction of the metabolite with MHC molecules demonstrated *in vitro*)] * 2 (identification of a specific CD4+ T cells recognizing sulfasalazine) = 8; clinical score = [2 (prevalence >5%)+ 2 (four symptoms)] = 4.

Dapsone

Dapsone is a sulfone medication used for its antibiotic and anti-inflammatory properties to manage leprosy and *Pneumocystis jirovecii* pneumonia in patients with HIV and various dermatoses such as dermatitis herpetiformis (Liu et al., 2019). Dapsone is known to cause a severe and unique adverse reaction called dapsone hypersensitivity syndrome which is a type of drug reaction with eosinophilia and systemic symptoms (DRESS), but also Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) (Liu et al., 2019). Since 2013, multiple reports have discovered HLA-B*13:01 as a genetic determinant of dapsone hypersensitivity syndrome in the Chinese population (Zhang et al., 2013).

The incomplete dapsone immunization score was calculated with the following parameters (**Table 4**): predictive score = [4 (covalent binding of the molecule and its metabolite to multiple proteins) + ND (not defined) + 4 (molecule and its metabolite bind to MHC molecules *in vitro* and *in silico*)] * 2 (identification of dapsone-specific T cells) = ND; clinical score = [1 (prevalence <5%)+ 1 (two symptoms)] = 2.

Table 8. Predictive and clinical scores for flucloxacillin.

Predictive parameters					Clinical parameters	
Methods	Chemical reactivity: protein-drug and protein-metabolite bio-conjugates	Identification of danger signals	Interactions with MHC and/or TCR molecules	Identification of a naïve T-cell repertoire / or drug-specific T cells	Prevalence of allergy	Symptoms
Results	Covalent binding of FLU and its metabolite to human serum albumin	No phenotypic modification of DCs	Interaction of FLU with MHC molecules <i>in vitro</i>	Identification of a naïve FLU-specific T-cell repertoire CD8+ > CD4+	0.1-0.2%	Antibody-mediated reactions DILI AGEP SJS/TEN DRESS
References	(Batchelor et al., 1965; Jenkins et al., 2009; Monshi et al., 2013; Yaseen et al., 2015; Tailor et al., 2016)	(Ogese et al., 2017)	(Daly et al., 2009)	(Wuillemin et al., 2013; Faulkner et al., 2016)	(Walker et al., 2014)	(van Hattem et al., 2014; Fan et al., 2017; Kennard et al., 2019)
Coefficients	=1 + 1 + 1	=0	=1	=4	=1	= 0.5 * 5
Predictive score = (3 + 0 + 1) * 4 = 16					Clinical score = 1 + 2.5 = 3.5	

Table 9. Predictive and clinical scores for sulfamethoxazole.

Predictive parameters					Clinical parameters	
Methods	Chemical reactivity: protein-drug and protein-metabolite bio-conjugates	Identification of danger signals	Interactions with MHC and/or TCR molecules	Identification of a naïve T-cell repertoire / or drug-specific T cells	Prevalence of allergy	Symptoms
Results	The metabolite SMX-NO binds covalently to HSA, transferrin and glutathion S-transferase	SMX and SMX-NO increase the expression of CD40 SMX increases the expression of CD86 and IL-8	SMX binds to TCR molecules <i>in vitro</i>	Identification of a CD4+ and CD8+ SMX-specific naïve T cell repertoire	5-8% 30-80% HIV	Antibody-mediated reactions Skin rash DIA SJS/TEN DRESS DILI
References	(Callan et al., 2009; Lavergne et al., 2009; Taylor et al., 2016)	(Sanderson et al., 2007)	(Schnyder et al., 1997; Naisbitt et al., 2002; Hammond et al., 2022)	(Schnyder et al., 1997, 2000; Burkhart et al., 2001; Ogese et al., 2015)	(Giles et al., 2019; Khan et al., 2019)	(Kardaun et al., 2013; Khan et al., 2019)
Coefficients	= 1 + 1 + 1	= 0,5 * 3 + 0,5	= 1	= 2 + 2	= 2	= 0.5 * 6
Predictive score = (3 + 2 + 1) * 4 = 24					Clinical score = 2 + 3 = 5	

Table 10. Predictive and clinical scores for sulfasalazine.

Predictive parameters					Clinical parameters	
Methods	Chemical reactivity: protein-drug and protein-metabolite bio-conjugates	Identification of danger signals	Interactions with MHC and/or TCR molecules	Identification of a naïve T-cell repertoire / or drug-specific T cells	Prevalence of allergy	Symptoms
Results	Covalent binding to albumin of the drug and its metabolite	No phenotypic modification of DCs	The metabolite sulfapyridine binding to TCR <i>in vitro</i>	Identification of specific CD4 T cells	3% Sulfa allergy 7% DRESS	Immunologic neutrophilic leukopenia leading to agranulocytosis DRESS
References	(Równicka-Zubik et al., 2009)	(Matasić et al., 2001)	(Hammond et al., 2022)	(Hammond et al., 2022)	(Tilles, 2001; Kardaun et al., 2013; Khan et al., 2019)	(Wadelius et al., 2000; Mennicke et al., 2009)
Coefficients	= 1 + 1 + 1	= 0	= 1	= 2	= 2	= 0.5 * 4
Predictive score = (3 + 0 + 1) * 2 = 8					Clinical score = 2 + 2 = 4	

Table 11. Predictive and clinical scores for dapsons.

Predictive parameters					Clinical parameters	
Methods	Chemical reactivity: protein-drug and protein-metabolite bio-conjugates	Identification of danger signal	Interactions with MHC and/or TCR molecules	Identification of a naïve T-cell repertoire / or drug-specific T cells	Prevalence of allergy	Symptoms
Results	Dapsone and nitroso dapsone covalently bind to multiple proteins	-	Dapsone and nitroso dapsone bind to MHC molecules <i>in vitro</i> and <i>in silico</i>	Identification of dapsone-specific T cells	0.5-3.6%	Dapsone hypersensitivity syndrome (DRESS) SJS/TEN
References	(Vyas et al., 2006; Roychowdhury et al., 2007)	-	(Zhang et al., 2013; Alzahrani et al., 2017; Zhao et al., 2021)	(Alzahrani et al., 2017; Zhao et al., 2021)	(Zhu and Stiller, 2001; Koseifi et al., 2006; Liu et al., 2019)	(Koseifi et al., 2006; Gibson et al., 2023)
Coefficients	= 1 + 1 + 1 + 1	ND	= 1 + 1 + 1 + 1	= 2	= 1	= 0.5 * 2
Predictive score = (4 + ND + 4) * 2 = ND					Clinical score = 1 + 1 = 2	

4. Mechanism: Altered self-repertoire

Abacavir (ABC)

Abacavir is a nucleoside reverse transcriptase inhibitor (NRTI) used in the treatment of human immunodeficiency virus (HIV). It acts by inhibiting the reverse transcriptase enzyme necessary for the virus replication (Cruciani et al., 2018).

Abacavir treatment may lead to a specific abacavir hypersensitivity reaction in some individuals. The presence of the HLA-B*57:01 allele has been associated with the onset of the reaction. Abacavir can fit into the binding groove of the HLA molecule and alter the recruitment of peptides to the MHC pocket (Adam et al., 2014). This alteration leads to the presentation of modified self-peptides in the pocket and triggers an allogeneic lymphocyte response. A strong association between this syndrome and HLA-B57:01 has been established, with a negative predictive value of 100%. Therefore, it is recommended that individuals be tested for the HLA-B*57:01 allele before starting Abacavir treatment to identify those at risk for developing the hypersensitivity reaction (Mallal et al., 2008).

The complete ABC immunization score was calculated with the following parameters: predictive score = [1 (binding to one protein) + 0 (no activation of DCs) + 2 (binding to HLA in vitro and in silico) * 1 (identification of an ABC-specific CD8+T-cells, HLA restriction)= 3; clinical score = [2 (prevalence between 5 and 8%) + 0.5 (one symptom)] = 2.5

Table 12. Predictive and clinical scores for abacavir.

Predictive parameters					Clinical parameters	
Methods	Chemical reactivity: protein-drug and protein-metabolite bio-conjugates	Identification of danger signals	Interactions with MHC and/or TCR molecules	Identification of a naïve T-cell repertoire / or drug-specific T cells	Prevalence of allergy	Symptoms
Results	Binding to one protein: Hemoglobin	No phenotypic modification of DCs	ABC modifies the repertoire of peptides HLA-B*57 :01 <i>in vitro</i> and <i>in silico</i>	Identification of naive CD8+ T cells in HLA-B*57 :01 donors	5–8% of patients in Europe 6–7% frequency of HLA-B*57 :01 in Europe	Abacavir-associated hypersensitivity syndrome
References	(Grilo et al., 2013)	(Martin et al., 2007; Adam et al., 2014; Cardone et al., 2018)	(Yang et al., 2009; Illing et al., 2012; Norcross et al., 2012; Ostrov et al., 2012; Adam et al., 2014; Illing et al., 2021)	(Adam et al., 2014; Lucas et al., 2015)	(Hetherington et al., 2001; Clay, 2002; Orkin et al., 2010; Böhm et al., 2018)	(Clay, 2002; Böhm et al., 2018)
Coefficient _s	= 1	= 0	= 2	= 2 - 1 (HLA restriction)	= 2	= 0.5
Predictive score = (1 + 0 + 2) * 1 = 3					Clinical score = 2 + 0.5 = 2.5	

Mechanism: Unknown

Ticlopidine and Cisplatin

Ticlopidine is an antiplatelet drug, and cisplatin is a chemotherapy agent used for the treatment of solid tumors ("Ticlopidine," 2012; Dasari and Tchounwou, 2014)

For ticlopidine, recent work was able to isolate specific CD8+ T cells in HLA-A*33:03 positive individuals (Usui et al., 2018). However, further efforts must be employed to determine the true prevalence of the allergic reactions. Three out of the six total parameters are missing. As for cisplatin, no specific-T cells were identified. Additionally, the mechanisms of action of both molecules are still being determined, resulting in missing data in the literature translated into incomplete scores in our model.

The incomplete ticlopidine immunization score was calculated with the following parameters: score = [3 (Covalent binding of the molecule to multiple proteins) + ND (not defined) + ND (not defined)] * 2 (identification of ticlopidine-specific CD8 T-cells) = ND; clinical score = [ND (not defined) + 1 (two symptoms)] = ND.

The incomplete cisplatin immunization score was calculated with the following parameters: score = [2 (Binding of the molecule to multiple proteins) + 0 (no activation of DCs) + ND (not defined)] * ND (not defined) = ND; clinical score = [4 (>10%) + 0.5 (one symptom)] = 4.5

Table 13. Coefficients and calculation of the predictive and clinical scores for ticlopidine.

Predictive parameters					Clinical parameters	
Methods	Chemical reactivity: protein-drug and protein-metabolite bio-conjugates	Identification of danger signals	Interactions with MHC and/or TCR molecules	Identification of a naïve T-cell repertoire / or drug-specific T cells	Prevalence of allergy	Symptoms
Results	Covalent bond to multiple proteins: Albumin glycoprotein (GP) IIb/IIIa complex	-	-	CD8+ T cells in HLA-A*33:03 donors	- <i>Liver function abnormalities 4.4% in the large-scale clinical study "CATS" of ticlopidine in North America</i> <i>Hepatic disorders comprised approximately 10% by WHO</i>	Thrombotic thrombocytopenic purpura (TTP) DILI
References	(Afrin et al., 2019)	-	-	(Usui et al., 2018)	-	(Mizushima et al., 2005)
Coefficients	= 1 + 1 + 1	ND	ND	= 2	ND	= 0.5 * 2
Predictive score = (3 + ND + ND) * 2 = ND					Clinical score = ND + 1 = ND	

Table 14. Coefficients and calculation of the predictive and clinical scores for cisplatin.

Predictive parameters					Clinical parameters	
Methods	Chemical reactivity: protein-drug and protein-metabolite bio-conjugates	Identification of danger signals	Interactions with MHC and/or TCR molecules	Identification of a naïve T-cell repertoire / or drug-specific T cells	Prevalence of allergy	Symptoms
Results	Binding to multiple proteins	Cisplatin impaired the phenotypic maturation of LPS-activated DCs by down-regulating the expression of CD80, CD86, MHC-I and II in a dose-dependent manner.	-	-	Directly related to the number of exposures. Most frequently in the second line of treatment 5 to 20 %	Immediate antibody-mediated reactions
References	(Möltgen et al., 2020; Wang et al., 2021; Ariza et al., 2022)	(Kim et al., 2016)	-	-	(Koren et al., 2002; Makrilia et al., 2010; Boulanger et al., 2014; Otani et al., 2017; Tsao et al., 2022)	(Koren et al., 2002; Otani et al., 2017; Tsao et al., 2022)
Coefficients	= 1 + 1	= 0	ND	ND	= 4	= 0.5
Predictive score = (2 + 0 + ND) * ND = ND					Clinical score = 4 + 0.5 = 4.5	

CORRELATION

Table 15. Coefficients and calculation of the predictive and clinical scores

	Molecules	Mechanism of recognition by T-cells			Predictive score	Clinical score
		Hapten	P-i	Altered rep		
Complete scores	Abacavir			x	3	2.5
	Allopurinol		x		6	3
	Sulfasalazine	x	x		8	4
	Carbamazepine		x		12	4
	Flucloxacillin	x	x		16	3.5
	Sulfamethoxazole	x	x		24	5
	Penicillin	x			28	7
Incomplete scores	Vancomycin		x		ND	3.5
	Clavulanic acid	x			15	ND
	Dapsone	x	x		ND	2
	Lamotrigine		x		ND	3.5
	Cisplatin				ND	4.5
	Ticlopidine				ND	ND

Of the 13 molecules that were assigned predictive and clinical scores, 7 had complete scores, meaning no data was missing: abacavir, allopurinol, sulfasalazine, carbamazepine, flucloxacillin, sulfamethoxazole, and penicillin (**Table 15**). Abacavir is found to have the lowest predictive and clinical scores. This finding is consistent with the fact that the presentation of Abacavir is restricted to the altered peptide repertoire mechanism and only in patients who carry the HLA-B*57:01 allele. This limited use of the drug is likely to result in a lower incidence of allergic reactions, and thus a lower score. In contrast, penicillins are found to have the highest values of both predictive and clinical scores. This is in line with the fact that penicillins are known to be one of the most common causes of drug allergies. The high scores indicate that penicillins have a high risk of causing allergic reactions, and this risk is reflected in the high prevalence of penicillin allergy in the general population. For the 5 other molecules,

we hypothesize that if the molecule acts like a hapten, it is more likely to have a higher predictive score. This observation is explained by the choice of our different immunization parameters that are mainly based on the haptentation of small molecules. If the molecule acts as a hapten it subsequently score higher. Additionally, we also hypothesized that, in majority, if the molecules acts both as a hapten and p-i, it is more likely to have a higher predictive score. For example, the predictive scores of flucloxacillin (16) and sulfamethoxazole (24) are much higher than what was reported with allopurinol (6). This is consistent with the fact that molecules with diverse mechanisms of presentation to T-cells have a higher likelihood of being recognized by different individuals. It is important to note that these scores are specific to the approach and parameters used to calculate them. Other scoring systems may yield different scores for the same drugs based on different parameters or approaches. Therefore, the scores presented here should be interpreted in the context of the specific scoring system used in this study.

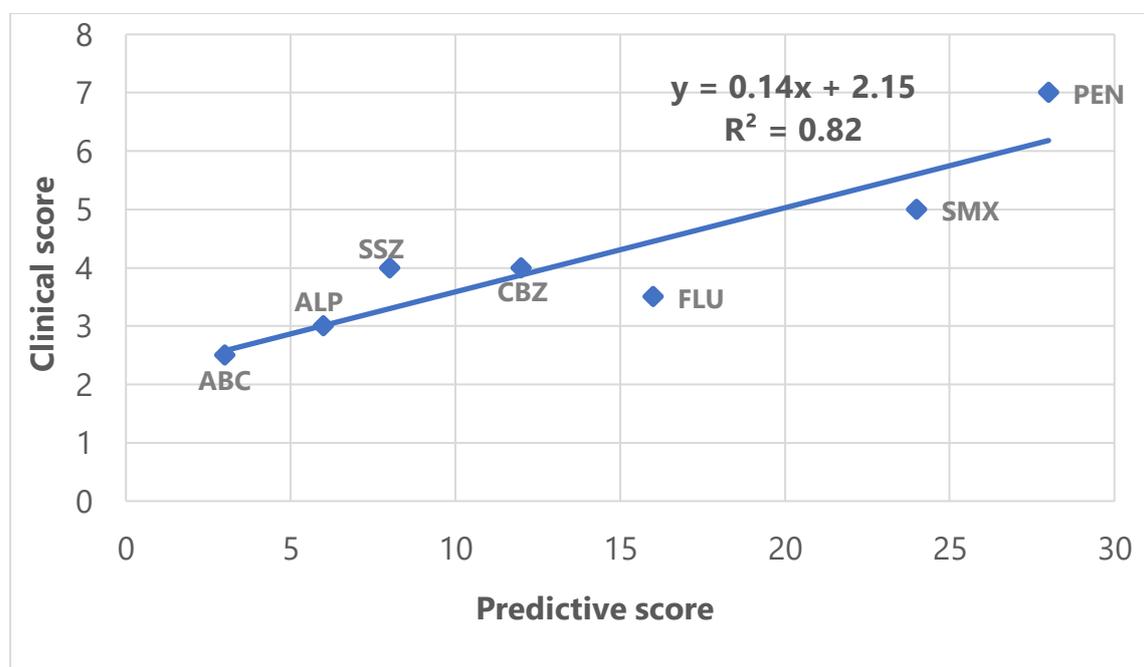


Figure 2. Correlation between the clinical and predictive scores. ABC: abacavir; ALP: allopurinol; CBZ: carbamazepine; FLU: flucloxacillin; PEN: penicillin; SMX: sulfamethoxazole; SSZ: sulfasalazine.

For abacavir, allopurinol, sulfasalazine, carbamazepine, flucloxacillin, sulfamethoxazole, and penicillin, a good positive correlation has been demonstrated between the predictive and clinical scores ($R^2=0.82$) (**Figure 2**), suggesting that the predictive score can accurately capture the potential for a drug to induce an immune response, and the clinical score reflects the actual immune response that occurs in patients. Molecules with missing data have been excluded from the analysis as further efforts must be employed to complete the lack of knowledge to enhance the correlation. Furthermore, our model could potentially be used to identify drugs with a high potential for inducing hypersensitivity reactions before they are introduced into clinical use. This could lead to more efficient drug development, as potential safety concerns could be identified earlier in the drug development process. Moreover, this predictive model should be refined and improved with additional data, leading to more accurate predictions of drug hypersensitivity reactions.

ASSESSMENT AND PREDICTION OF CHEMICAL ALLERGIES

Article 2: A new model to detect and analyze the human T lymphocyte response to chemical sensitizers

Article in preparation

Allergic contact dermatitis (ACD), caused by exposure to contact sensitizers, is a delayed type IV hypersensitivity reaction mediated by CD4 and CD8 T cells. These T cells work together and are responsible for the inflammatory response observed in allergic patients. In this third and final part of the project, we set up a highly sensitive autologous DC/T-cell co-culture model to detect human T cells responding to six well-known chemical sensitizers of different potencies in healthy donors and semi-quantified their frequency. The following chemicals were tested 2,4-dinitrobenzenesulfonic acid (DNBS), cinnamaldehyde (CinA), isoeugenol (IsoE), methylisothiazolinone (MIT), nickel sulfate (NiSO₄), and cobalt chloride (CoCl₂). Furthermore, we identified the subpopulations of these chemical-specific T cells (CD4 and/or CD8) for DNBS, CinA, and MIT. Our month-long assay is based on the weekly repeated stimulation of T cells with chemical-loaded autologous DCs. This allows the expansion of the specific T cells, usually present at low frequencies, and facilitates their detection.

Our results identified responding T cell lines for all the chemicals and in the majority of the donors tested. Using the Poisson distribution law, we found mean frequencies ranging from 0.30 to 2.00 chemical-recognizing T cells per million circulating T cells. We further tested the positive T-cell lines responding to DNBS, CinA, and MIT in a secondary ELISpot assay using antibodies directed to MHC class I or II molecules to determine the subpopulations of these responding T-cell lines. The results confirmed their positivity and showed that the response primarily depended on MHC class I molecules for DNBS, indicating the implication of CD8⁺ T cells. However, CD4⁺ T cells were mainly observed for CinA. For MIT, both subpopulations were

equally present. Lastly, we identified that DNBS binds to intra and extracellular proteins, suggesting a presentation by MHC II molecules to CD4+ T cells and MHC I molecules to CD8+ T cells.

The results of this study contribute to a better understanding of the mechanism of immunization to organic and inorganic chemical sensitizers and propose a sensitive and improved T-cell assay to identify responding T cells.

Title: A new model to detect and analyze the human T lymphocyte response to chemical sensitizers

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Conflict of interest

The authors declare no conflict of interest.

ABSTRACT

Allergic contact dermatitis (ACD), caused by contact sensitizers, is a delayed type IV hypersensitivity reaction. This study aims to set up a highly sensitive model to detect human T lymphocytes responding to chemical sensitizers and to semi-quantify their frequency.

We developed an *in vitro* human monocyte-derived dendritic cells (mo-DC) /T-cell autologous co-culture assay, allowing us to evaluate the activation of T lymphocytes and their subpopulations (CD4+ or CD8+) using peripheral blood mononuclear cells (PBMCs) from healthy donors. Purified T cells were seeded in multiple wells and stimulated weekly by autologous mo-DCs loaded overnight with the chemical sensitizer in media supplemented with 10% human AB serum. Detection of chemical-specific T cells was performed after three rounds of stimulation using an IFN- γ ELISpot assay. A secondary ELISpot was performed on positive cell lines using antibodies directed to major histocompatibility complex (MHC) class I or II molecules.

We were able to detect isoeugenol (IsoE), cobalt chloride (CoCl₂), 2,4-dinitrobenzenesulfonic acid (DNBS), cinnamaldehyde (CinA), methylisothiazolinone (MIT), and nickel sulfate (NiSO₄) responding T cells in 3/3, 2/3, 4/4, 3/4, 3/3 and 2/3 donors, respectively. We calculated a mean frequency of 0.30 for IsoE, 0.58 for CoCl₂, 0.90 for DNBS, 1.22 for CinA, 1.37 for MIT, and 2.00 for NiSO₄ per million circulating T cells using the Poisson distribution law. We then tested 24 T-cell lines specific to DNBS, 17 specific cell lines to CinA, and 15 responding T cells to MIT in a secondary ELISpot assay with MHC-blocking antibodies. The results showed that the response was primarily dependent on MHC class I molecules for DNBS, indicating the implication of CD8+ T-cells, and MHC II molecules for CinA, suggesting the implication of CD4+ T-cells. However, CD4+ and CD8+ T-cells were equally observed for MIT. Western blot assays further demonstrated that DNBS covalently binds to many intracellular proteins and probably to the albumin component of human serum, suggesting an intracellular

and extracellular haptentation leading to the activation of CD8+ and CD4+ T cells, respectively.

Our results contribute to a better understanding of the immunization mechanism to chemical sensitizers. We proposed a T-cell assay to identify T lymphocytes responding to chemical sensitizers responsible for ACD in the general population.

KEYWORDS

Allergic contact dermatitis, Chemical sensitizers, Dendritic cells, T lymphocytes.

ABBREVIATIONS

ACD: Allergic contact dermatitis

AOP: Adverse Outcome Pathway

APC: antigen-presenting cell

BSA: bovine serum albumin

CinA: cinnamaldehyde

CoCl₂: cobalt chloride

DC: dendritic cell

DNBS: 2,4-dinitrobenzenesulfonic acid

DNCB: dinitrochlorobenzene

DNP: dinitrophenol

DPRA: Direct peptide reactivity assay

EFS: Etablissement Français du Sang

ELISpot: enzyme-linked immunosorbent spot

FCS: fetal calf serum

GM-CSF: granulocyte-macrophage colony-stimulating factor

h-CLAT: human cell line activation test

IFN- γ : interferon-gamma

IL: interleukin

IMDM: Iscove's Modified Dulbecco's Medium

IsoE: Isoeugenol

KE: Key events

KLH: keyhole limpet hemocyanin

LMW: Low molecular weight

MHC: major histocompatibility complex

MIT: methylisothiazolinone

Mo-DC: monocyte-derived dendritic cells

NiSO₄: Nickel sulfate

NK: natural killer

NS: not significant

OECD: organization for economic co-operation and development

PBMC: peripheral blood mononuclear cell

PHA: phytohemagglutinin

SEM: standard error of the mean

Th: T helper cells

INTRODUCTION

Allergic contact dermatitis (ACD), caused by the repeated contact of low-molecular-weight (LMW) chemicals with the skin, is a widespread delayed type IV hypersensitivity reaction mediated by T lymphocytes (Kimber et al., 2002a; Martin, 2015; Esser and Martin, 2017; Kostner et al., 2017). It affects around 20% of the general population and is responsible for up to 95% of occupational skin diseases (Thyssen et al., 2007; Diepgen et al., 2016; Alinaghi et al., 2019).

ACD begins with the contact of the allergen with immune cells in the skin leading to an inflammatory immune response after repeated exposure (Rustemeyer et al., 2011; Nassau and Fonacier, 2020). It is mainly composed of two phases: sensitization and elicitation. In the sensitization phase, which is clinically silent, chemicals bind to self-proteins to form a complex processed by antigen-presenting cells (APCs), such as dendritic cells (DCs) (Eisenbarth, 2019). DCs are considered the main culprit in initiating adaptive immune response acting as one of the essential pillars of the immune system (Bonilla and Oettgen, 2010). The resulting neo-antigens are presented through the major histocompatibility complex (MHC) class I or II molecules to T cells in the draining lymph node (Théry and Amigorena, 2001; Hilligan and Ronchese, 2020). Re-exposure to the same molecule initiates the elicitation phase characterized by a rapid recruitment and activation of chemical-responding T cells, leading to clinical symptoms (Christensen and Haase, 2012; Martin, 2012). Tissue damage caused by inflammation is primarily induced by cytokines produced by CD4+ Th1 cells and cell lysis caused by cytotoxic CD8+ T cells, leading to local erythema and epidermal spongiosis (Wang et al., 2000; Pichler, 2007; Vocanson et al., 2009; Scheinman et al., 2021). These chemicals are primarily organic molecules such as dinitrochlorobenzene (DNCB), cinnamaldehyde (CinA), methylisothiazolinone (MIT), and isoeugenol (IsoE), or inorganic molecules such as cobalt chloride (CoCl₂), and nickel sulfate (NiSO₄) (Karlberg et al., 2008).

Animal models such as the murine local lymph node assay (LLNA) have been used to assess the skin sensitization potential of sensitizers (Kimber et al., 2002b; Gerberick et al., 2007; OECD, 2010). However, the growing ethical pressure to move away from animal experimentation has led to the development of alternative *in vitro* or *in silico* methods. In order to develop these methods, it was important to identify the key events (KE) leading to skin sensitization. The organization for economic co-operation and development (OECD) has provided a comprehensive understanding of the biological mechanism underlying skin sensitization by organizing them into an Adverse Outcome Pathway (AOP) (Vandebriel and van Loveren, 2010). This pathway highlights the four KE involved in the pathomechanism of ACD: covalent binding to skin proteins (KE1), keratinocyte responses (KE2), activation of DCs (KE3), and T-cell proliferation (KE4) (OECD 2012). To date, several *in vitro* methods accepted by the OECD can assess the first three KE, and several others are still in various stages of development (Bialas et al., 2023). The five most common tests used are the direct peptide reactivity assay (DPRA), which addresses KE1, the KeratinoSens, addressing KE2, the human cell line activation test (h-CLAT), the U937 cell line activation test (U-SENS), and the IL-8Luc assay addressing KE3 (Gerberick et al., 2004; Ashikaga et al., 2006; Emter et al., 2010; Kimura et al., 2015; Alépée et al., 2015; OECD, 2022a, 2022b).

However, despite significant efforts, there are no current standardized alternative test methods to address KE4 or T-cell proliferation (van Vliet et al., 2018). Nevertheless, multiple T-cell assays have been proposed for chemical or drug sensitizations, such as the human T cell priming assay (hTCPA) (Dietz et al., 2010; Richter et al., 2013), T-cell repertoire identification (Nhim et al., 2013; Azoury et al., 2015; Scornet et al., 2016; Bechara et al., 2019a, 2019b), T-cell assays to assess the immunogenic potential of drugs (Faulkner et al., 2012), the human artificial lymph node model (HuALN) (Giese and Marx, 2014), or other *in silico* methods (Wang et al., 2008). Their lack of sensitivity, high donor-to-donor variability and low value to predict sensitizer potency require further investment.

In this study, we propose a highly sensitive *in vitro* monocyte-derived DC (mo-DC) and autologous T-cell co-culture model to identify and characterize the CD4+ and CD8+ human T lymphocytes responding to six organic and inorganic chemical sensitizers in healthy donors. The chemical-specific T-cells were amplified by repeated stimulations with antigen-loaded DCs. We then semi-quantified their frequency and determined responding T-cell subpopulations (CD4+ or CD8+) using neutralizing anti-MHC class I or II antibodies. We demonstrated the presence of DNBS, CinA, IsoE, MIT, NiSO₄, and CoCl₂ responding T lymphocytes in the majority of tested donors. DNBS, CinA, and MIT-responding T-cell lines also showed a dependence on MHC I or II processing by the DCs. Our outcomes contribute to a better understanding of the pathomechanism of ACD by identifying a repertoire of chemical responding CD4+ and CD8+ T cells in healthy donors.

MATERIALS AND METHODS

Blood collection and isolation of peripheral blood mononuclear cells

Whole blood from anonymous healthy donors was provided by the Etablissement Français du Sang (EFS, Rungis, France) after informed consent and following EFS guidelines. Peripheral blood mononuclear cells (PBMCs) were then isolated using the Ficoll-Paque density gradient centrifugation method (GE Healthcare Biosciences AB, Sweden).

Autologous co-culture assay

Monocytes were isolated from PBMCs by positive antibody anti-CD14 magnetic sorting (Miltenyi Biotec, Bergisch Gladbach, Germany) and differentiated into DCs at day 4 in the presence of 550 U/ml granulocyte-macrophage colony-stimulating factor (GM-CSF, Miltenyi biotec, Bergisch Gladbach, Germany) and 550 U/ml interleukin 4 (IL-4, Miltenyi biotec, Bergisch Gladbach, Germany). The cells were incubated at 37°C in humidified air containing 5% CO₂ in RPMI 1640 cell culture medium (Gibco, Invitrogen, Villebon sur Yvette, France) supplemented with 10 % heat-inactivated fetal calf serum (FCS, Gibco, Invitrogen, Saint Aubin, France), 1% sodium pyruvate (Gibco, Invitrogen, Saint Aubin, France) and 1% penicillin/streptomycin (Gibco, Invitrogen, Saint Aubin, France). Percentages of monocyte purity (CD14^{high}), DC differentiation (DC-SIGN^{high}, CD1a^{high}), and activation (CD83^{low}, CD86^{low}) markers were evaluated by flow cytometry (Attune™ NxT Flow Cytometer, Invitrogen by Thermo Fischer). CD4⁺ and CD8⁺ T cells were then retrieved from CD14⁻ cells of the same donor by magnetic sorting using CD4⁺ and CD8⁺ microbeads simultaneously (Miltenyi Biotec, Bergisch Gladbach, Germany). T cells were co-cultured with autologous DCs previously loaded overnight (18h) with the chemical sensitizer at the following concentrations: 5 mM DNBS, 100 μM CinA, 600 μM IsoE, 150 μM MIT, 500 μM NiSO₄, and 500 μM CoCl₂ (all from Sigma-Aldrich, St. Louis, USA). The concentrations used for each chemical were carefully determined based on the literature findings, cytotoxicity assays (propidium iodide

staining with flow cytometry readout or trypan blue exclusion staining with cell count readout, with a 70% minimum viability), and toxicity of solvents (<0.1% DMSO). Lipopolysaccharide (LPS, Sigma-Aldrich, St. Louis, USA, L6529-1MG) was added to the culture 2 hours after loading of DCs with the chemicals. The co-culture was conducted in 96 well plates at DC to Tf cells ratio of 1:10 in Iscove's modified Dulbecco's medium (IMDM, Lonza, Basel, Switzerland) supplemented with 10% human AB serum (SAB, Sigma-Aldrich, St. Louis, USA), a mix of amino acids, 1000 U/ml IL-6 (Biotechne R&D), 10 ng/ml IL-12 (Biotechne R&D), and 100µg/ml primocine (Invivogen). Purified T cells were restimulated weekly for a total of three rounds of stimulation on days 7, 14, and 21 with autologous chemical-loaded DCs supplemented with 5 ng/ml IL-7 (Biotechne R&D), 20 IU/ml IL-2 (Biotechne R&D), and primocine. For each assay, 10 keyhole limpet hemocyanin (KLH; Thermo fisher scientific) T-cell lines were used as a co-culture positive control in the same culture conditions.

IFN- γ ELISpot assay

On day 28, an enzyme-linked immunosorbent spot (ELISpot) assay was performed to detect interferon- γ (IFN- γ) released by chemical-responsive T-cells. The assay was performed following the manufacturer's guidelines. Briefly, T cells were cultured with autologous chemical-loaded or unloaded DCs 18h prior to the assay readout on MultiScreenHTS-HA filter plates (Merck, Germany) precoated with capture antibody (Mabtech, Sweden). Positive and negative ELISpot controls were also performed: culture media, unloaded DCs, T cells, and T cells stimulated with 5 µg/ml phytohemagglutinin (PHA). Spots were counted using the AID iSpot ELISpot reader (Autoimmune Diagnostika GmbH). The specificity of the response was confirmed if the spot count was at least two-fold higher in the presence of the chemical compared to unloaded DCs, and a minimum of 30 spots per well was required for the validity of the analysis.

MHC class I and II molecules blocking: secondary ELISpot assay

At day 30, anti-human MHC class I (10 µg/ml; anti-HLA-A, B, C; BD biosciences, France) and II (anti-HLA DP, DQ, DR; 10 µg/ml; BD biosciences, France) blocking antibodies were used to determine the role of MHC molecules in chemical-T-cell responses found in the first ELISpot assay. DCs were pre-treated with the different MHC class I or class II antibodies directly before starting the ELISpot assay with responding cell lines from the first ELISpot assay. Inhibition with MHC class I or II antibodies was considered specific if the percentage of the different wells was above the calculated cut-off compared to the loaded wells (see statistical analysis).

Anti-DNP Western blot for the identification of intra and extracellular DNBS haptenized proteins

DCs were treated for 6h with DNBS 5 mM in IMDM medium with and without 10% SAB. Thirty micrograms of protein from cell lysates and supernatants were loaded on 10% SDS-PAGE polyacrylamide gels (TGX Stain-Free FastCast; Bio-Rad Laboratories, Hercules, CA) and transferred to PVDF membrane (Bio-Rad Laboratories). Membranes were then incubated with a goat anti-dinitrophenyl (anti-DNP) polyclonal antibody (Bethyl BE-A150-117A, Thermo fisher scientific) to detect the formation of the DNBS-haptenized proteins. Immunoreactive bands were detected by enhanced chemiluminescence (ChemiDoc XRS+, Bio-Rad Laboratories, CA). Bovine serum albumin, 2,4-dinitrophenylated (BSA-DNP) (1 mg/ml, Invitrogen A23018), and unloaded DCs were used as positive and negative controls, respectively.

Statistical analysis

Statistical analysis was performed using GraphPad Prism® software (version 8). The Wilcoxon test was used to compare two groups, and the Friedman test was used to compare more than two groups. Data are expressed as means ± standard error of the mean (SEM) and considered statistically different if the p-value was < 0.05.

The frequency of human T cells responding to DNBS, CinA, IsoE, MIT, NiSO₄, and CoCl₂ was estimated for each donor using the Poisson distribution law, which is a standard probabilistic model for describing random occurrences. The mean frequency was then calculated for each molecule.

$$\text{Frequency} = \frac{-\ln(\text{number of negative T cell lines} / \text{Total number of T cell lines tested})}{\text{Number of T cells per well}}$$

The percentage of inhibition obtained with MHC class I or II blocking antibodies was calculated for each identified chemical-specific T-cell line using the following formulas.

$$\text{MHC I inhibition percentage} = \frac{\text{Loaded DCs} - \text{Loaded DCs with } \alpha \text{ MHC I}}{\text{Loaded DCs}}$$

$$\text{MHC II inhibition percentage} = \frac{\text{Loaded DCs} - \text{Loaded DCs with } \alpha \text{ MHC II}}{\text{Loaded DCs}}$$

We separately calculated MHC class I or II inhibition cut-offs by subtracting the standard error from the mean inhibition percentages. Each cut-off allowed us to evaluate the specificity of the inhibition for each well.

$$\text{Cut off} = \underbrace{\frac{\text{Loaded DCs} - \text{Loaded DCs with } \alpha \text{ MHC I or II}}{\text{Loaded DCs}}}_{\text{Mean inhibition percentage}} - \underbrace{\frac{\delta}{\sqrt{n}}}_{\text{Standard Error}}$$

RESULTS

Detection of blood circulating T cells recognizing DNBS

A total of 20 healthy donors were tested using a DC/T-lymphocyte co-culture model to detect chemical-specific CD4⁺ and CD8⁺ T cells isolated from PBMCs (**Figure 1**). Each donor's individual T-cell lines (T cells in a single well) were evaluated for their specificity to the chemical using an IFN- γ ELISpot assay. For donor P21.76, used as a representative donor, 21 positive T-cell lines out of 100 tested wells were found positive and produced a significant amount of IFN- γ (**Figure 2A, 2B**). These results showed that we were able to identify T-cells responding to DNBS after repeated stimulations. The mean number of spots of the pooled DNBS-responding T cells from all donors tested (P21.72 P21.73 P21.76 P21.77) was significantly higher with DNBS-loaded DCs versus unloaded DCs (**Figure 2C**).

Detection and quantification of the frequency of blood circulating T cells recognizing contact sensitizers

We tested a total of 6 contact sensitizers. Responding T cells were detected in 3/3, 2/3, 4/4, 3/4, 3/3, and 2/3 healthy donors, for IsoE, CoCl₂, DNBS, CinA, MIT, and NiSO₄, respectively. We found that only three donors were non-responders (P22.05 for CoCl₂, P22.03 for CinA, and P22.54 for NiSO₄). Using the number of positive T-cell lines versus the total number of wells tested, we calculated the frequency of chemical-responding T cells for each donor using the Poisson distribution law (see formula above). For each chemical sensitizer, the antigen-specific mean T-cell frequency was calculated. The results were the following: 0.30, 0.58, 0.90, 1.22, 1.37, and 2.00 chemical-recognizing T cells per million circulating T cells for IsoE, CoCl₂, DNBS, CinA, MIT, and NiSO₄, respectively (**Figure 3**).

Role of MHC class I and II molecules in T-cell activation and identification of T-cell subsets

To determine if MHC molecules mediated the T cell response to chemical sensitizers, we evaluated the ability of MHC class I (A, B, and C) and II (DP, DQ, and DR) blocking antibodies to inhibit the activation of DNBS, CinA, and MIT-specific T-cell lines. Using a second IFN- γ ELISpot performed on responding T-cell lines identified in the first assay, we assessed the inhibition by adding DCs treated with anti-MHC I or II antibodies to T-lymphocytes (**Figure 4A**). We observed a reduction of IFN- γ production with both anti-MHC I and II antibodies for all 3 chemicals tested. For DNBS, the activation of all responding T cell lines from all donors obtained in the first assay significantly depended on MHC class I ($P < 0.05$) and not on MHC class II (**Figure 4B**). On the other hand, the activation of CinA-responding T cells depended on MHC II inhibition ($P < 0.01$) (**Figure 4C**). However, for MIT, both MHC I ($P < 0.001$) and MHC II ($P < 0.0001$) were implicated in the activation of T cells (**Figure 4D**). Furthermore, it is well established that MHC class II molecules generally present peptides to CD4⁺ T cells, and MHC class I molecules present peptides to CD8⁺ T cells. Inhibition of either MHC molecule results in the identification of the predominant subpopulation of T cells, i.e. CD4⁺ or CD8⁺ T lymphocytes.

However, due to the variability observed between the different T cell lines in each donor, we sought to calculate the inhibition percentages for both antibodies in each well individually to assess the specificity of the inhibition. The inhibition percentages allowed us to calculate each chemical's MHC I and II cut-offs based on the inhibitory effects of the antibodies, as previously described in the material and methods. Each inhibition percentage above the corresponding cut-off was considered specific (**Figure 5**). Out of the 24 DNBS-responding T-cell lines found in three donors, we identified 13 significant MHC I inhibitions (inhibition percentages above the 42.3 cut-off), compared to only 3 significant MHC II inhibitions (inhibition percentages above the 30.7 cut-off) (**Figure 5A**). Therefore, DNBS seems to primarily induce a CD8-

mediated T-cell response through MHC class I. However, CinA mainly causes an MHC class II reaction mediated by CD4+ T cells, with 10 significant MHC II inhibitions versus only 5 significant MHC I inhibition (**Figure 5B**). For MIT, the exact number of T-cell lines (n=9) showed a significant MHC I and MHC II inhibition leading to the implication of both CD4+ and CD8+ T cells equally (**Figure 5C**).

2,4-Dinitrobenzenesulfonic acid binds to intracellular and extracellular proteins

MHC class I and II molecules are known to present peptides derived from intracellular and extracellular proteins, respectively. To determine the localization of DNBS protein haptization, DCs isolated from three donors were incubated with DNBS in media with or without SAB for 6 hours. The cellular proteins and supernatants were then extracted and analyzed by Western blot assay (**Figure 6**). Anti-DNP monoclonal antibody was used to detect the formation of DNBS-haptized proteins. It revealed multiple labeled bands suggesting that the DNBS covalently binds to a large number of intracellular proteins. Additionally, it revealed that DNBS probably binds to the albumin component of human serum. BSA coupled with DNP was used as a positive control.

DISCUSSION

Chemical-specific T-cell activation in ACD generally requires the binding of the chemical to skin or cellular proteins and their processing into antigenic hapten-modified MHC-binding peptides (McFadden et al., 2013; Pallardy and Bechara, 2017). Most approaches characterizing the T-cell responses of chemical allergens are *in vivo* animal models, such as the gold standard local lymph node assay (LLNA) (Kimber et al., 1994). To address this issue, other human qualitative or quantitative *in vitro* specific T-cell assays (with different readouts) have been implemented with a limited ability to detect T-cells due to low sensitivity and specificity (van Vliet et al., 2018). These assays are collectively aiming to be validated for alternative KE4 assays in the AOP framework of skin sensitization.

In this work, we propose a new and improved *in vitro* human DC/T-cell co-culture assay to detect and semi-quantify the frequency of strong and moderate contact sensitizer-specific T cells with the identification of CD4⁺ and CD8⁺ subpopulations in healthy donors. The originality of our model lies in the repeated stimulation of T-cells with autologous chemical-loaded DCs for a total of four weekly restimulation rounds which allows the expansion of the T-cell culture over one month. Several briefer T-cell priming assays involving co-culturing DCs and T-cells and subsequent restimulations have been used to investigate the response of T-cells to different therapeutic antibodies, chemicals, and drugs (Dietz et al., 2010; Martin et al., 2010; Faulkner et al., 2012; Richter et al., 2013; Vocanson et al., 2014; Usui et al., 2018). However, the three-fold restimulation has shown better sensitivity and specificity (Delluc et al., 2011; Nhim et al., 2013; Bechara et al., 2019a, 2019b), especially in our current work with moderate sensitizers. Additionally, the culture conditions have been adjusted in our assay to promote a CD4⁺ Th1 and CD8⁺ T-cell profile, characterized by the production of IFN- γ which is a surrogate marker for detection of antigen-specific T cells (Kasahara et al., 1983; Bhat et al., 2017). The addition of IL-6 and IL-12 enhanced T-cell stimulation and the specificity of the ELISpot IFN- γ readout. Unlike other readout methods, the ELISpot assay is considered a powerful tool for measuring T-cell

activation due to its high sensitivity, specificity, and reproducibility (Leehan and Koelsch, 2015), making it the method of choice in our model.

The proposed repeated *in vitro* stimulation model successfully identified, for the first time, specific T-cells for moderate and strong contact sensitizers in a single study and for the majority of healthy donors. The mean T-cell frequency for IsoE, CoCl₂, DNBS, CinA, MIT, and NiSO₄ ranged from 0.30 to 2.00 per million circulating T-cells, depending on the chemical tested. As anticipated, these low frequencies of antigen-specific T-cells are consistent with the range of T-cell repertoires reported by our laboratory and others for benzylpenicillin (Nhim et al., 2013; Azoury et al., 2015; Bechara et al., 2019a), proteins (Delluc et al., 2010), therapeutic antibodies (Delluc et al., 2011), and nickel (Bechara et al., 2019b). For example, a recent evaluation of the naïve T-cell repertoire for nickel yielded mean frequencies of 0.49 for CD4⁺ and 0.37 for CD8⁺ naïve T-cells per million circulating T-cells (Bechara et al., 2019b), consistent with our findings. In comparison, for therapeutic antibodies the responding T-cell frequency ranges between 0.01 and 0.35 per million naïve CD4⁺ T cells (Delluc et al., 2011). As for KLH, which is considered a primary antigen, the responding T-cell frequency ranges between 5 and 30 per million naïve CD4⁺ T cells (Delluc et al., 2011).

The complex involvement of different T-cell lineages has been discussed in various research studies regarding ACD. Both CD4⁺ and CD8⁺ T cells have been shown to be essential mediators of the immune response to contact sensitizers in murine models (Gocinski and Tigelaar, 1990; Kondo et al., 1996; Xu et al., 1996; Wang et al., 2000; Vocanson et al., 2005), and with a limited set of molecules in humans (Moulon et al., 1995; Albanesi et al., 1999; Sieben et al., 2001; Moed et al., 2004; Coulter et al., 2008). CD8⁺ T cells play an inflammatory role, whereas CD4⁺ T cells seem to play both an inflammatory and a regulatory role (Xu et al., 1996; Wang et al., 2000; He et al., 2009). Nevertheless, our *in vitro* model could identify the subpopulations of chemical-specific T cells in healthy donors for the three strong and moderate contact sensitizers tested: DNBS, CinA, and MIT, by using an MHC blocking experiment. Our

results confirmed that both subpopulations are involved in sensitization for all three molecules.

The findings of this assay showed a predominance of CD8⁺ T cells with DNBS, which is the soluble form of the strong contact sensitizer DNCB. Previous research involving mouse models of skin sensitization has demonstrated that strong skin sensitizers elicit an oligoclonal T-cell response characterized by a high frequency of CD8⁺ effector T cells (Martin, 2004; Martin et al., 2004; Vocanson et al., 2009). Analysis of the composition of T cell subsets in the draining lymph nodes of rats also revealed a decrease in the quantity of CD4⁺ T cells but an increase in the amount of CD8⁺ cells following treatment with DNCB (Popov Aleksandrov et al., 2015). Moreover, other studies demonstrated that the inflammatory response mediated by 1-Fluoro-2,4-dinitrobenzene (DNFB), which is a homologue of DNCB, involves specific CD4⁺ and CD8⁺ T cells. However, CD8⁺ T cells may serve as the primary effector cells in mice, as DNFB-specific CD8⁺ effector T cells could develop in the absence of CD4⁺ T-cells, but the opposite was not observed (Bour et al., 1995; Bouloc et al., 1998; Natsuaki et al., 2014). These results were also suggested in humans (Kimber and Dearman, 2002). However, Vocanson *et al.* explained that although immunologic studies in ACD reveal the activity of cytotoxic T cells following DNCB exposure, CD4⁺ Th cell subpopulations also play a role by releasing a particular cytokine pattern (Vocanson et al., 2010; Peiser et al., 2012).

Moreover, MHC class I and II molecules are renowned for presenting peptides derived from intracellular and extracellular proteins to CD8⁺ and CD4⁺ T cells, respectively. To complete our findings, we also evaluated the capacity of DNBS to bind to intracellular and extracellular proteins following MHC blocking assay. We showed that DNBS binds to multiple intracellular proteins and to a protein migrating at the same level of the albumin component of human serum in DCs. The binding of DNBS to serum albumin has already been established by Eisen *et al.* since 1953 (Eisen et al., 1953). In addition, in the study conducted by Pickard *et al.*, DNCB was found to have

the ability to elicit Th1 and Tc1 responses in humans by forming numerous haptens with intracellular and extracellular proteins (Pickard et al., 2007). This latter finding was also shown in our research as haptenized DNBS proteins present the DNP group, used to identify haptenization by the Western blot assay.

For MIT, also considered as a strong sensitizer, our study recorded both T-cell subpopulations equally. Little data is available for MIT, but one study observed that the CD4+/CD8+ ratio was notably decreased for extremely MIT allergic individuals (Wicks et al., 2019). Nevertheless, it is generally accepted that both CD4+ and CD8+ cells producing IFN- γ are involved in sensitization to chemical sensitizers (Zhao et al., 2009; Gibson et al., 2015).

On the other hand, moderate or weak sensitizers are not well documented in T cell assays. CinA, considered as a moderate sensitizer found mainly in fragrances (Clouet et al., 2019), seems to predominantly induce an MHC class II reaction mediated by CD4+ T cells in our study. To our knowledge, no studies looked into the characterization of the subpopulations for CinA responding-T cells. However, murine studies have demonstrated a significant distinction between ACD elicited by potent versus weak sensitizers (Vocanson et al., 2006).

In conclusion, our study highlights the ability of a repeated stimulation model to identify and semi-quantify the frequency of antigen-specific T-cells in healthy donors for several moderate and strong chemical sensitizers. Furthermore, our results indicate that different sensitizers can induce different patterns of MHC-mediated reactions by CD4+ and CD8+ T-cells. We, therefore, hypothesized that stronger sensitizers recruit higher numbers of CD8+ cytotoxic T cells while moderate sensitizers recruit higher numbers of CD4+ Th1 cells. Overall, the development of this *in vitro* model could propose a new tool in the field of skin sensitization testing, as it provides a first step into a new and improved assay for the assessment of the sensitization potential of chemical sensitizers, and the KE4 step of the skin sensitization AOP.

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Figure 1 Co-culture experiment protocol. Monocytes were isolated from the PBMCs of healthy donors by positive anti-CD14 magnetic sorting and differentiated into dendritic cells (DCs) in the presence of GM-CSF and IL-4 for 4 days. CD4⁺ and CD8⁺ T cells were retrieved from CD14⁻ cells of the same donor by magnetic sorting using CD4⁺ and CD8⁺ microbeads simultaneously. T cells were co-cultured with autologous DCs previously loaded overnight with the chemical sensitizer and restimulated weekly for a total of three rounds of stimulation on days 7, 14, and 21 with autologous chemical-loaded DCs for a total of 4 stimulations. On day 28, an ELISpot was performed to detect IFN- γ released by chemical-responsive T-cells. A secondary ELISpot assay was done with anti-human MHC class I and II blocking antibodies to determine the role of MHC molecules in chemical T-cell responses. α -MHC: anti-major histocompatibility complex antibodies; ELISpot: enzyme-linked immunosorbent spot; GM-CSF: granulocyte-macrophage colony-stimulating factor; IFN: Interferon; IL: interleukin; LPS: lipopolysaccharide; Mo-DC: monocyte-derived dendritic cell; NK: natural killer; PBMC: peripheral blood mononuclear cells.

Figure 2 Identification of human T-cells responding to 2,4-dinitrobenzenesulfonic acid. **(A)** ELISpot results of all 21 T cell lines recognizing 2,4-dinitrobenzenesulfonic acid (DNBS) from donor P21.76. Unloaded DCs, DNBS-loaded DCs, and T cells served as negative controls. Phytohemagglutinin (PHA) was used as a positive control for T cell proliferation. **(B)** IFN- γ spot count for DNBS responding T cells in donor P21.76. The dotted line represents the minimum required spot count (30) for the analysis to be valid. **(C)** Comparison of the mean number of spots for DNBS responding T cells to unloaded DCs versus DNBS loaded DCs in all donors (P21.72 P21.73 P21.76 P21.77). DC: dendritic cell, DNBS: 2,4-dinitrobenzenesulfonic acid; IFN: interferon; PHA: phytohemagglutinin. **** P < 0.0001 Wilcoxon.

Figure 3 Frequency of human T-cells recognizing chemical sensitizers. The frequency was calculated for isoeugenol, cobalt, 2,4-dinitrobenzenesulfonic acid, cinnamaldehyde, methylisothiazolinone and nickel-recognizing T cells per million total

T cells for each donor using the Poisson distribution law. Above each frequency, the number of positive T-cell lines (wells) over the total number of T-cell lines tested is indicated in brackets. A dashed line on the graphs identifies the mean frequency for each molecule. Donor numbers are presented on the x-axis.

Figure 4 Implication of MHC I or II molecules in T-cell response to chemical sensitizers. **(A)** The IFN- γ spot count for 2,4-dinitrobenzenesulfonic, cinnamaldehyde, and methylisothiazolinone-specific T-cell lines in the presence of unloaded-DCs, chemical-loaded DCs, and chemical-loaded DCs with anti-MHC I or II antibodies were recorded for all donors. The inhibition of the spot count for **(B)** 2,4-dinitrobenzenesulfonic, **(C)** cinnamaldehyde, and **(D)** methylisothiazolinone. α -MHC: anti-major histocompatibility complex antibodies; CinA: cinnamaldehyde; DC: dendritic cell; DNBS: 2,4-dinitrobenzenesulfonic acid; IFN: interferon; MIT: methylisothiazolinone; NS: not significant. * $P < 0.05$, ** $P < 0.01$ *** $P < 0.001$, **** $P < 0.0001$ Friedman.

Figure 5 Role of MHC I and MHC II in the T-cell response for each chemical sensitizer among the different classes. The calculated MHC I or II inhibition percentages for each **(A)** 2,4-dinitrobenzenesulfonic, **(B)** cinnamaldehyde, and **(C)** methylisothiazolinone-responding T-cell line allowed us to determine their respective cut-offs (see formulas above). CinA: cinnamaldehyde, DNBS: 2,4-dinitrobenzenesulfonic acid, MHC: major histocompatibility complex, MIT: methylisothiazolinone, NS: not significant.

Figure 6 2,4-dinitrobenzenesulfonic acid (DNBS) binds to multiple intracellular proteins and the albumin component of human serum in dendritic cells. The membranes were incubated with a goat anti-dinitrophenyl monoclonal antibody (anti-DNP) to detect the formation of DNBS-protein adducts. Bovine serum albumin coupled with DNP (BSA-DNP) was used as a positive control. Representative experiment out of four. BSA-DNP: bovine serum albumin 2,4-dinitrophenylated, DNBS: 2,4-dinitrobenzenesulfonic acid, kD: kilodalton, MW: molecular weight, SAB: human AB serum.

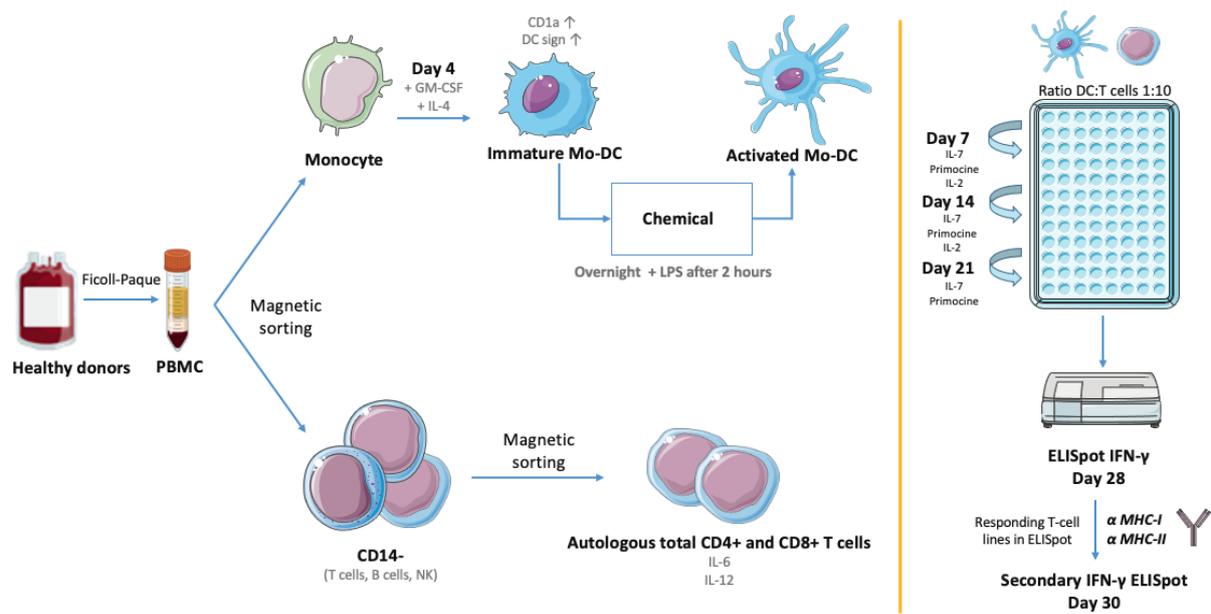


Figure 1

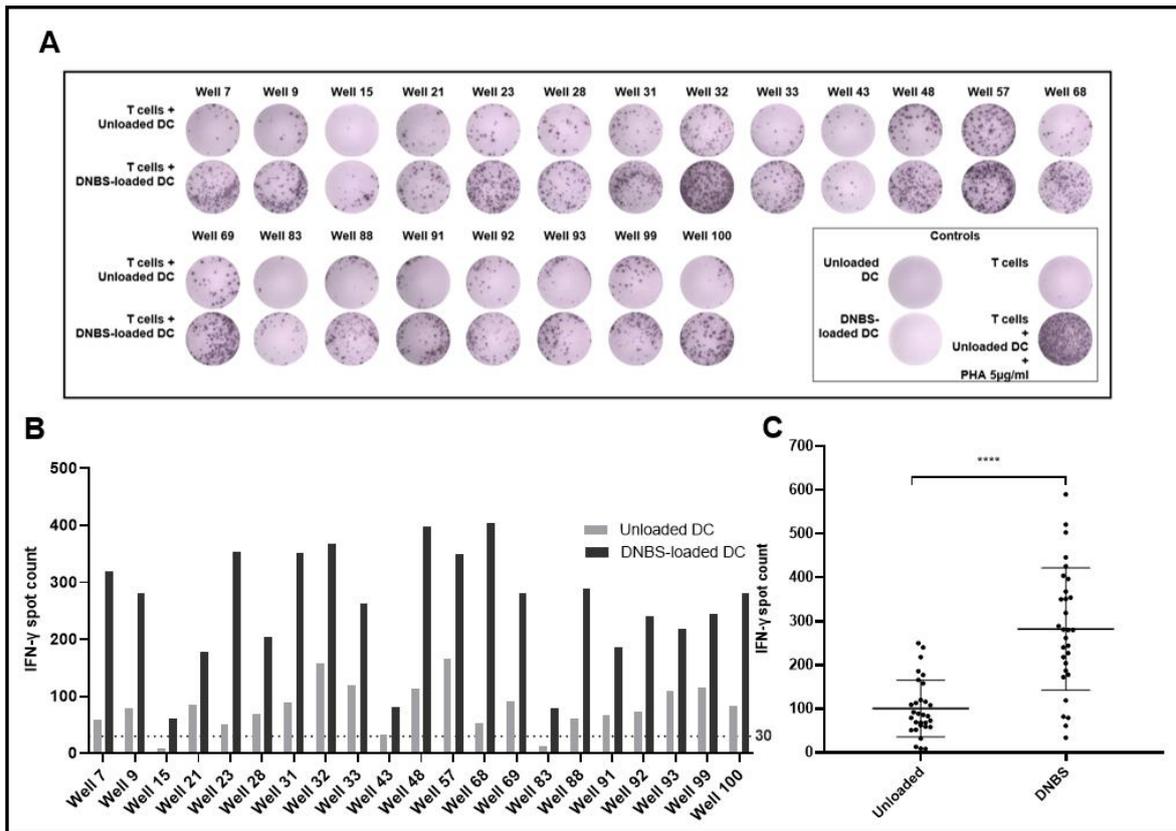


Figure 2

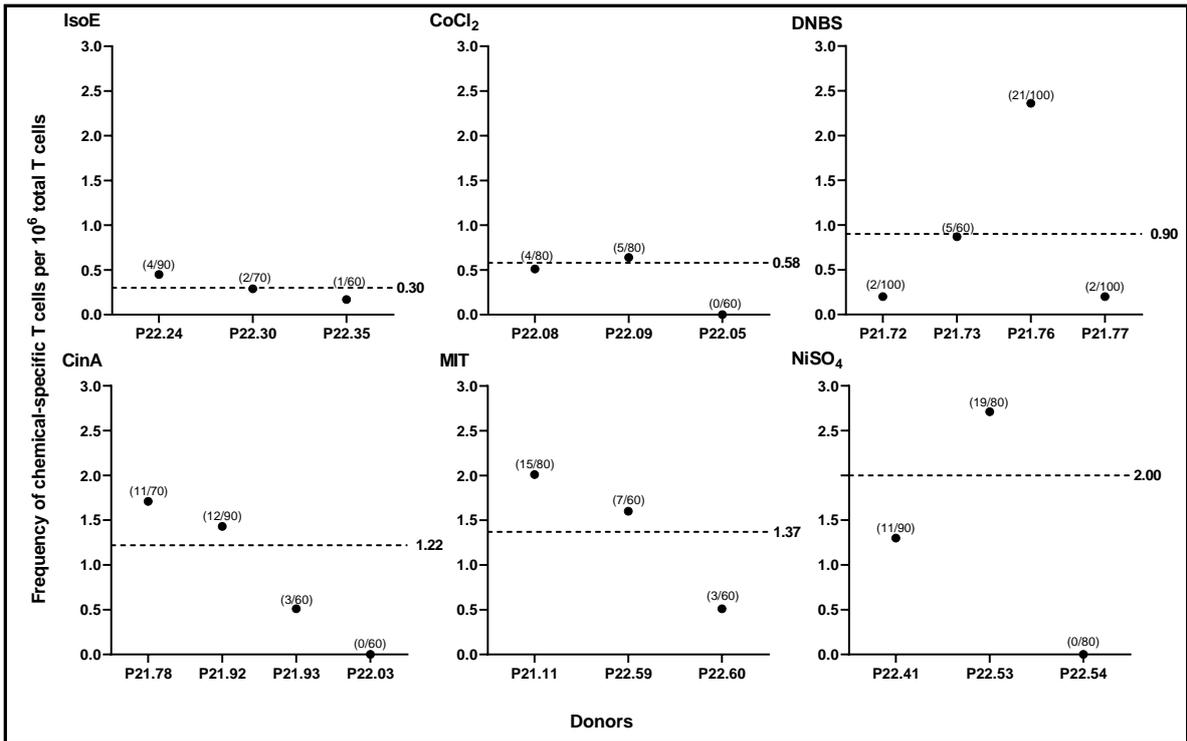


Figure 3

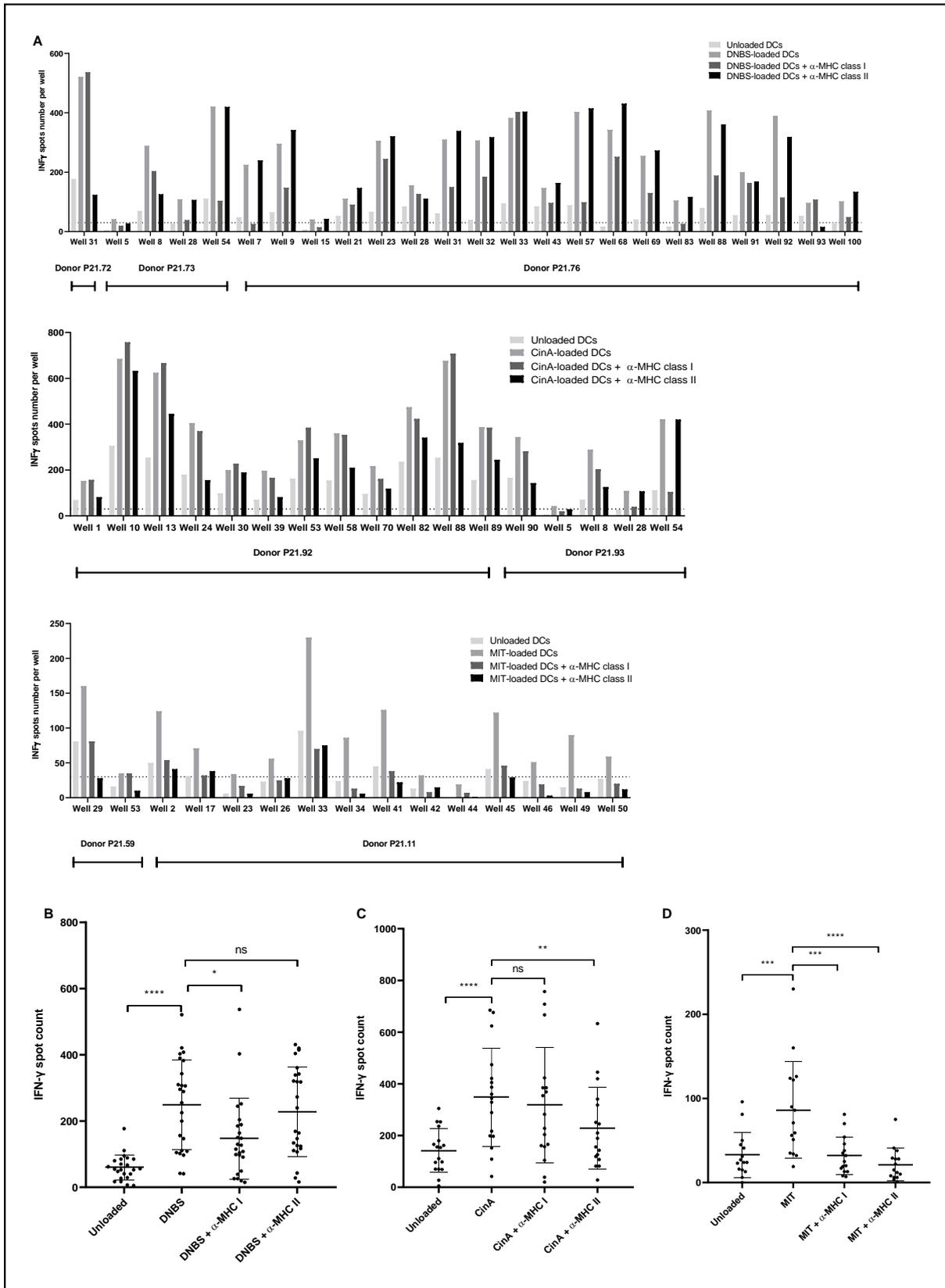


Figure 4

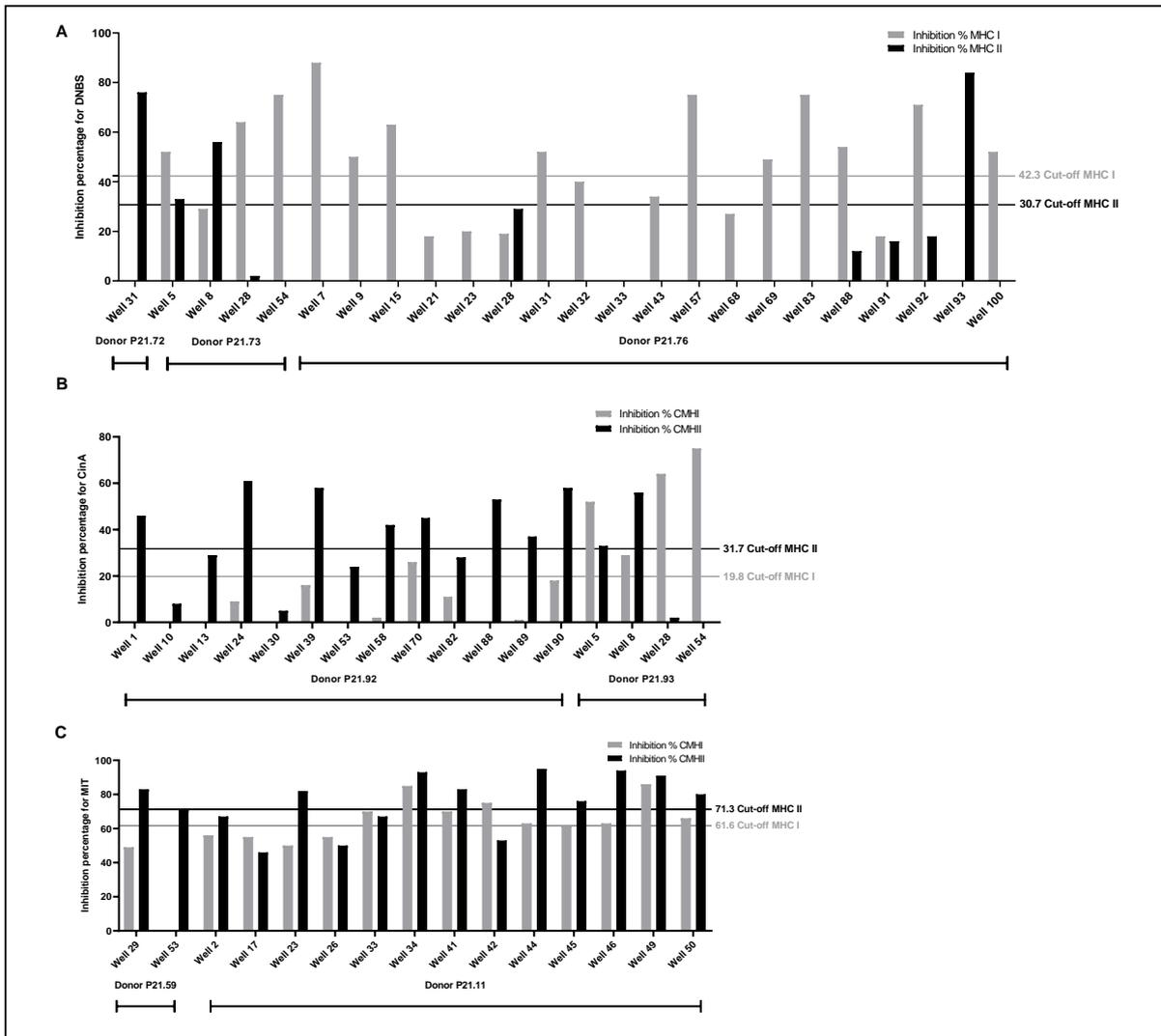
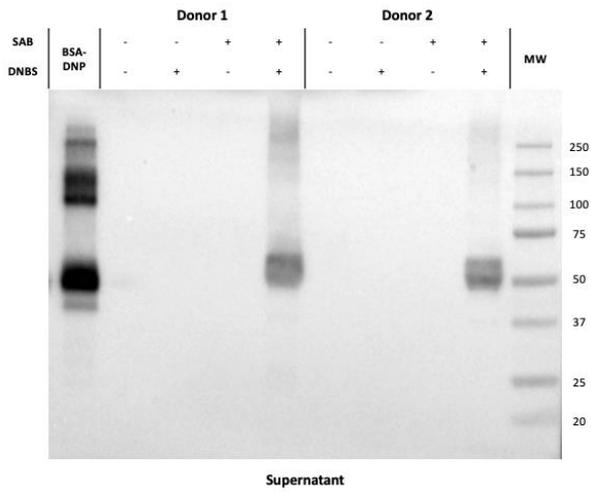


Figure 5



Intracellular haptentation
(cell lysate) in progress

Figure 6

GENERAL DISCUSSION

GENERAL DISCUSSION

Drug and chemical allergies are a significant health concern worldwide. Our thesis project focuses on assessing and predicting drug and chemical allergies that are critical for early diagnosis, appropriate management, and prevention. These two concepts will constitute the basis of our discussion. Assessment commonly focuses on data evaluation, while prediction is about generating insights for future decision-making based on current or past data. Therefore our **epidemiology study** falls into the assessment category as it involves the analysis of existing data on drug allergies. At the same time, the new model for detecting the **T-cell response to chemical sensitizers** engages both the evaluation of the pathomechanism of allergic contact dermatitis but also the prediction of the immunization potential of certain chemicals. Lastly, our **immunization score** falls into the prediction category as it suggests a model for predicting the likelihood of an immune response to certain drugs.

Similarly, our discussion is divided into three parts:

- I. Characteristics of drug hypersensitivity reactions in a Lebanese outpatient population
- II. Identification of chemical-specific T cells in healthy donors
- III. Development of a novel immunization score to predict drug allergies

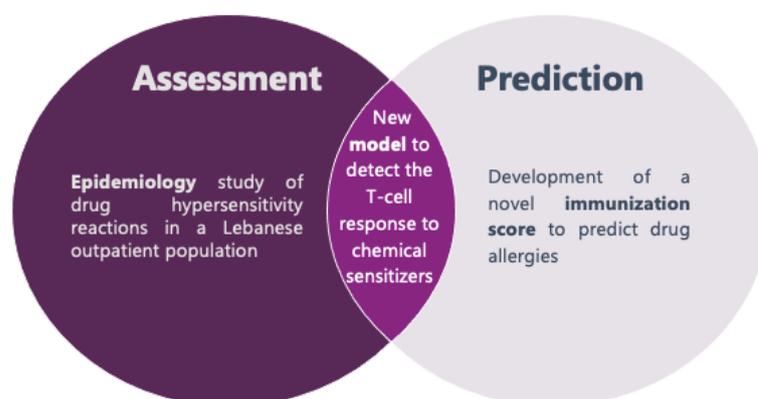


Figure 26. General overview of the thesis project.

i. Characteristics of drug hypersensitivity reactions in a Lebanese outpatient population

To accurately predict drug allergies, it is crucial to evaluate their characteristics, including epidemiological features and associated risk factors. This evaluation may involve various methods, such as surveys, clinical trials, and retrospective analysis of medical records. However, reports of drug allergies in the Middle East region are limited in number and have yet to be conducted in Lebanon (Al-Ahmad et al., 2021; Albin and Agarwal, 2014; Dietrich et al., 2008). Therefore, our study aims to provide a comprehensive overview of DHRs nationwide and document specific characteristics that have not yet been explored in the Lebanese population. By doing so, we aspire to bridge the gaps in knowledge regarding DHRs in Lebanon.

Our ten-year retrospective study reviewed electronic and paper patient charts who visited a tertiary care allergy center in Beirut between 2012 and 2021 and identified all patients with allergic reactions to drugs used in ambulatory care. This center is highly frequented and serves as a primary allergy clinic in the capital, attracting patients from all over the country. Therefore, our 758 patients sample is considered representative of Lebanon's population in this period. A previous study on self-reported food allergies in Lebanon has been conducted (Irani and Maalouly, 2015). However, no other research focused on drug allergies in particular. Additionally, it was difficult to compare our findings to other studies conducted in other countries due to the scarcity of research assessing DHRs in outpatient populations. However, as previously mentioned, the characteristics of DHRs vary greatly depending on the selected population.

From the 758 patients analyzed, females outnumbered the males, with a female-to-male ratio of 2.6:1. Our results are consistent with those found by others, such as Gamboa *et al.*, who investigated an outpatient Spanish database (Caballero Martínez, 2009; Gamboa, 2009; Thong and Tan, 2011). Our study found that the mean age was

39 years, which is consistent with previous literature showing that DHRs are primarily seen in young and middle-aged adults with an average age of around 40 years (Gamboa, 2009; Pagani et al., 2022; Warrington et al., 2018). The findings of our study showed a similar pattern of DHRs as reported in the literature, with BL antibiotics being the most common culprit drugs, followed by NSAIDs (Gomes and Demoly, 2005). Additionally, multiple studies conducted in Europe and the USA showed that BL antibiotics were the most frequent elicitors of DHRs (Iuliano et al., 2022; Macy, 2014; Stone Jr et al., 2020; Torres and Blanca, 2010). However, the high levels of antibiotic allergies we reported exceeded what was found in the literature, which might be attributed to the high levels of misuse and over-the-counter delivery of antibiotics in Lebanon, as previously described (Cheaito et al., 2014; Lahoud et al., 2021). This may be a call to implement educational campaigns about the appropriate use of antibiotics and their threats, whether these risks are pharmacological or toxicological. Additionally, requiring prescriptions for all antibiotics, reducing over-the-counter availability, and limiting the number of antibiotics dispensed can help lower the chances of allergic reactions by avoiding unnecessary administration.

Furthermore, we evaluated the trends of BLs, NSAIDs, and other antibiotic DHRs during the ten-year period. We hypothesize that the number of cases detected would decrease between 2020 and 2021 following the socio-economic state of the country (COVID, the nuclear-like Beirut port explosion on August 4, 2020, and the economic crisis), which had an important impact on the healthcare system (Shaker et al., 2020; World Health Organization, 2020; Alvaro-Lozano et al., 2022). However, our results highlighted that the allergy clinic's diagnostic and treatment activities were unaffected. Patients were handled and followed up virtually, and severe cases were granted face-to-face consultations.

Although the results are promising, we identified some limitations in our study. The first is the need for more DHR diagnosis based on SPT testing in most patients. The diagnosis was primarily based on the clinical history of the reaction. Multiple other

studies conducted in other parts of the world showed similar results, such as the Portuguese study on self-reported drug allergies that recounted around 22.7% of the population underwent skin testing (Gomes et al., 2004). Our findings are probably associated with Lebanon's lack of private or public insurance coverage for SPTs.

Another limitation is the investigation of only a few cases of DHRs to drugs used in the perioperative setting, such as NMBAs, hypnotics, or intravenous opioids, even though perioperative DHRs constitute the first-line challenge for anesthesiologists and allergists (Kosciuczuk and Knapp, 2021). This was due to constraints on time and resources. We focused on DHRs to molecules used in ambulatory care; therefore, the findings of our study concerning anesthesia agents may only be generalizable to some of the population. However, our results are comparable to others as it is known that DHRs to NMBAs are responsible for most perioperative anaphylactic reactions (about 58% of cases in France) (Mertes et al., 2011; Pouessel et al., 2017; Guerci et al., 2020). Our study identified opioids as the primary cause of DHRs in the perioperative setting, which has not been reported in previous literature. This discrepancy may be attributed to our method of distinguishing between opioids used in the perioperative setting and those used in an ambulatory setting, which could account for the high number of cases observed.

A complementary study explicitly focusing on perioperative DHRs in the same clinic is currently underway to address this limitation. This would provide a more comprehensive understanding of the prevalence and patterns of DHRs in this specific setting. A larger sample size could also increase our findings' statistical power and generalizability.

Drug allergies and genetics are intimately linked as associations between HLA alleles and allergic reactions have been frequently detected and linked to particular ethnicities (Ghattaoraya et al., 2017). Lebanon's unique geographical location and social characteristics have contributed to the population's genetic diversity,

discovering several distinct genetic peculiarities (Jabara et al., 1998; Mansour et al., 2001; Mustapha et al., 2001; Khneisser et al., 2006). It would be interesting to investigate the potential genetic risk factors and their association with allergic reactions to identify the particularities in the Lebanese population.

This study provides valuable insights into the patterns of DHRs in the Lebanese population for the first time. Our findings are consistent with previous studies, but additional complementary data is needed to provide a more comprehensive understanding of the prevalence and underlying mechanisms of DHRs, especially in the perioperative setting. This information could be used to develop more effective strategies for the prevention, diagnosis, and treatment of DHRs in the future.

ii. Identification of chemical-specific T cells in the general population

Skin sensitization is an important issue in chemical safety assessment. The AOP is a conceptual framework developed by the OECD that describes the biological sequence of events linking exposure to a chemical to an adverse outcome. In the case of skin sensitization, the AOP describes the four key events (KE) involved in the pathomechanism of allergic contact dermatitis (ACD): covalent binding to skin proteins (KE1), keratinocyte responses (KE2), activation of dendritic cells (KE3), and T-cell proliferation (KE4). To date, five standardized *in vitro* methods can assess the first three KEs. However, despite extensive efforts, no standardized alternative *in vitro* test methods are currently available to address KE4 or T-cell proliferation (van Vliet et al., 2018). However, numerous assays have been proposed for chemical or drug sensitization evaluation, such as the human T cell priming assay (hTCPA), T-cell repertoire identification, T-cell assays to assess the immunogenic potential of drugs, the human artificial lymph node model (HuALN), or other *in silico* methods (Wang et al., 2008; Dietz et al., 2010; Faulkner et al., 2012; Richter et al., 2013; Giese and Marx, 2014; Scornet et al., 2016). These methods still require further investment due to their lack of sensitivity, high donor-to-donor variability, and low ability to predict sensitizer

potency. Our work aimed to apply and refine the *in vitro* assay, previously developed for fundamental work to evaluate its potential for identifying chemical sensitizers (Delluc et al., 2010, 2011; Nhim et al., 2013; Azoury et al., 2015; Bechara et al., 2019a). Thus, we will begin by commenting our study's model.

Our work's originality lies in using an autologous co-culture model initially developed to predict the immunogenicity of therapeutic proteins (Delluc et al., 2011) in which DCs and CD4+ and CD8+ T cells are derived from the same healthy donor. Dendritic cells (DCs) are pivotal in recognizing chemicals or drugs, followed by their presentation to T cells, leading to their subsequent activation (Hilligan and Ronchese, 2020). Therefore, the DC and T cells co-culture model provides an ideal system for studying the mechanism of T cell activation and the role of MHC molecules in presenting the chemical sensitizers to T cells. In the autologous co-culture model, the two autologous cell types do not induce a basal immune reaction, unlike the allogeneic co-culture model, in which DCs and T cells are derived from two different donors. The autologous model gives us the possibility to study an external antigen.

The use of total CD4+ and CD8+ T cells mimics *in vivo* conditions, providing a more physiologically relevant model for studying immune responses to small molecules. Moreover, the simultaneous isolation of naive CD4+ and CD8+ T cells was impossible as it is highly complex and time-consuming for test development.

The choice of molecules was based on a thorough literature review, and the desired outcome of the assay. We selected sensitizers well-characterized in the literature, showing consistent and reliable responses in previous studies. Additionally, we selected strong and moderate sensitizers (based on EC3 results of LLNA) to test the model. The concentrations used for each chemical sensitizer were carefully determined based on the literature findings, cytotoxicity assays, and toxicity of solvents: 5 mM DNBS, 100 μ M CinA, 600 μ M IsoE, 150 μ M MIT, 500 μ M NiSO₄, and 500 μ M CoCl₂ were finally selected. The cytotoxicity was assessed by propidium iodide (PI) staining with

flow cytometry readout or trypan blue exclusion staining with cell count readout. A cytotoxicity threshold of 30% was chosen as set by previous experiences in our laboratory, which ensures the maintenance of the functionality of the DCs. This percentage is similar to the hCLAT skin sensitization test threshold of 25% on the THP-1 cell line (organization for economic cooperation and development OECD 2022). Additionally, we considered the maximum concentration of the solvents in the final cell culture; for instance, we used less than 0.1% of DMSO. Given the cytotoxicity of the chemicals and the short lifespan of human DCs *in vitro*, incubation times must be carefully chosen. In our study, we exposed DCs to the chemicals for 18 hours, which was sufficient for observing the desired effects while remaining below an acceptable cytotoxicity threshold.

Our *in vitro* repeated stimulation T-cell assay has previously identified Nickel and BP T-cell repertoires and their potential role in various immunological processes (Nhim et al., 2013; Azoury et al., 2015; Bechara et al., 2019a, 2019b). The high sensitivity of our DC/T-cell co-culture model due to three-fold repeated stimulation has allowed the expansion of the T-cell culture leading to their identification and quantification, which may otherwise be missed using other methods.

The frequency of the specific T cells was calculated for all molecules and donors. We calculated mean frequencies of 0.30, 0.58, 0.90, 1.22, 1.37, and 2.00 chemical-recognizing T cells per million circulating T cells for IsoE, CoCl₂, DNBS, CinA, MIT, and NiSO₄, respectively. The results vary depending on the antigen and are thought to be correlated with the strength of the T-cell response, thus to the intensity of the allergic reactions in the clinical setting. However, this hypothesis has yet to be fully confirmed, as other factors can also play a role in the clinical manifestations of chemical allergic reactions (Kimber et al., 2012; Moon et al., 2007).

Additionally, the results of this assay suggest that it provides an efficient and reliable system for evaluating the potency of different sensitizers and their ability to

induce T-cell responses. However, despite our ability to determine the frequency of chemical-specific T cells, several additional steps must be followed to consider this assay a regulated alternative method. It begins with the analytical and biological validation studies to demonstrate the assay's accuracy, precision, and reliability. These studies will typically include testing with known standards in multiple centers before submitting the assay for data validation by the regulatory body. Therefore, further efforts must be employed to optimize this method.

The second part of our model successfully identified the different subtypes of T cells involved in ACD caused by three contact sensitizers, DNBS, CinA, and MIT, using an MHC blocking assay. The three tested molecules showed different patterns of T-cell subtypes. DNBS primarily induces an MHC I mediated-reaction activating CD8+ T cells. However, CinA mainly causes an MHC class II mediated-reaction activating CD4+ T cells. For MIT, an equally significant MHC I and MHC II inhibition leading to the implication of CD4+ and CD8+ T cells was revealed. Over the past two decades, murine experimental investigations have shown that CD8+ T cells are responsible for the effector response in strong hapten-induced contact hypersensitivity. In contrast, CD4+ T cells have been found to possess downregulatory functions, which may confirm its involvement at a higher rate in moderate contact sensitizers (Vocanson et al., 2005). To our knowledge, these findings have yet to be confirmed in human healthy donors. Our study constitutes the first insights into the implication of these two subpopulations in ACD in humans, however, complementary experiments must be conducted to confirm these findings.

As previously mentioned, the evaluation of skin sensitizers plays a crucial role in ensuring the safety of chemicals and products. The introduction of this assay in the evaluation of skin sensitizers (KE4 in the AOP) can provide several advantages. Firstly, it offers a more human-relevant approach, reducing the need for animal testing. This aligns with the principles of the 3Rs (Replacement, Reduction, and Refinement) in animal experimentation. Secondly, the use of this assay becomes particularly valuable

for molecules with uncertain results in KE1, KE2, and KE3. These molecules may exhibit ambiguous or inconsistent behavior in traditional assays, making their hazard assessment challenging. However, the validation process of the new *in vitro* assay should follow established guidelines and regulatory requirements. It involves assessing its reliability, and reproducibility. Comparative studies with existing assays and correlation with known skin sensitizers are essential to demonstrate its accuracy and predictivity.

iii. The development of an immunization score to predict drug allergies

Drug development is a complex and multifaceted process that involves numerous stages and considerations (Horien and Yuan, 2017). At each step of the process, several key questions must be addressed to ensure the safety and effectiveness of the drug. One of the main questions in drug development is whether the drug is pharmacologically active and has the desired effect on the body. In addition to efficacy, drug development also considers other factors such as formulation, delivery, and manufacturing. These factors are essential in ensuring the drug is stable, easy to administer, and can be manufactured at a large scale. Another key element is considering the economic viability of the drug and if the needs of the patients and healthcare providers are met. This involves evaluating the potential market for the drug and the cost of manufacturing and distribution. Finally, drug safety is a crucial question in pre-clinical and clinical trials. These trials aim to identify potential adverse effects and evaluate the drug's overall safety. Despite the rigorous development process of pharmaceuticals, rare adverse effects, such as drug allergies, may still go undetected (Whitebread et al. 2005), which results in the late-stage termination of drug development or the withdrawal of marketing authorizations, leading to significant human and financial costs. A few different approaches have been used to predict the potential for a drug to cause allergic reactions. One common approach is the structure-

activity relationship (SAR) model to identify features of the chemical structure of a drug that are associated with an increased risk of allergy. Other approaches have used machine learning algorithms or bioinformatics tools to analyze large drug and allergy-related datasets. However, there is a growing interest within the pharmaceutical industry to develop early prediction immunization models for drug allergies to improve safety assessment during drug development.

This research project aims to combine all the key events in immunization to develop a predictive score of the overall allergic risk of a drug molecule in development. To achieve this model, we developed the predictive immunization score, which was calculated based on coefficients obtained from **four** key events: **(1)** the chemical reactivity of the molecule (or its metabolite) leading to the formation of a stable protein-drug or protein-metabolite bioconjugates, **(2)** the danger signal effect of the drug (or its metabolite) leading to the activation of DCs, **(3)** the presence of interactions between the drug or metabolite peptides (hapten theory) and/or the free drug or its metabolite (p-i concept) with MHC molecules or the TCR, and **(4)** the presence of a T-cell repertoire recognizing the drug (or its metabolite). Each key event was attributed a coefficient used to calculate the immunization score according to the following formula: predictive immunization score = (Coefficient 1 + Coefficient 2 + Coefficient 3) * Coefficient 4. A greater weight was assigned to Coefficient 4, which constitutes an important event for initiating immunization and allergic reactions. We applied this formula to calculate the scores of 13 allergenic molecules: penicillin, clavulanic acid, dapsone, allopurinol, carbamazepine, lamotrigine, vancomycin, flucloxacillin, sulfamethoxazole, sulfasalazine, abacavir, ticlopidine, and cisplatin, which induce allergic reactions by multiple mechanisms.

After completing the predictive immunization score, it was evident that the model was built on theoretical predictions, which may not necessarily reflect the actual incidence of allergic reactions observed in real life. This is primarily because immunization to a drug does not always result in an allergic reaction. While

immunization and allergic responses involve the activation of the immune system, the latter occurs when the immune tolerance mechanisms fail to regulate the immune response, leading to an exaggerated and harmful reaction (Zhang et al., 2014). Hence, there was a need to compare and correlate the predictive immunization score with clinical data. The clinical data would provide an accurate representation of the **(1)** prevalence and **(2)** symptoms of allergic reactions observed in the population: clinical score = (Coefficient 1 + Coefficient 2). The development of a clinical score was deemed necessary to assess the predictive immunization score's real-life applicability and ability to accurately predict the likelihood of developing an allergic reaction to a drug.

To test the hypothesis about the relationship between the two variables, we performed a linear regression analysis. This allowed us to determine the relationship between the clinical score (the dependent variable) and the predictive score (the independent variable). We then obtained the coefficient of determination R^2 , which measures how well the regression model fits the data. For the molecules without missing data (abacavir, allopurinol, sulfasalazine, carbamazepine, flucloxacillin, sulfamethoxazole, and penicillin), the coefficient of determination was $R^2=0.82$, which yields a good positive correlation. However, the proposed model developed in this study is still in its early stages, and more efforts should be employed before its clinical use.

Missing data is a common issue in scientific research, and the predictive immunization score is no exception. It poses a significant challenge as some of the key events might not have any data or insufficient data to determine a coefficient accurately. This may occur because the experiment was not conducted or was inconclusive or because the data was only available from one research publication. Further research should be undertaken to obtain the missing information in case the data is missing. Notably, this approach also allowed us to determine the information missing in the literature and propose multiple tools to fill these gaps.

However, the data quality is also crucial for the model's accuracy. Biases in the literature review and differences in experimental protocols used to measure the key events can introduce significant coefficient variation and ultimately affect the score's predictive power. Therefore, ensuring that the data used to develop the score is of high quality is essential. To achieve this, standardized tests can be designed to homogenize the experiments and ensure that the data is comparable and reproducible across different studies. Standardizing the experimental protocols will allow researchers to compare and validate the results obtained by various groups, reducing variability in the data and increasing the overall quality of the data used to develop the score. Additionally, researchers are advised to look for a T-cell repertoire first, as its absence leads to the annulment of the predictive score.

To conclude, developing an immunization score represents a promising tool for predicting the risk of drug-induced allergies. By combining knowledge of key events in the immune response with *in vitro* models and bioinformatics tools, we have calculated the immunization score for known and unknown molecules. This score has been correlated to a clinical score. However, further studies are needed to validate the accuracy and reliability of this score by adding more molecules. If successfully validated, the immunization score could provide a valuable tool for improving the safety and efficacy of drugs in development, ultimately benefiting patients and healthcare providers alike.

CONCLUSION AND PERSPECTIVES

CONCLUSION AND PERSPECTIVES

Evaluating and predicting drug and chemical allergies are critical for early diagnosis, appropriate treatment, and prevention. The three projects discussed in this thesis provide valuable information on the epidemiology of drug allergies in Lebanon, the development of an immunization score to predict the likelihood of an individual's immune response to a drug, and the detection and semi-quantification of contact allergen-specific T cells.

In our work, the immunization score takes the central focus as the epidemiology study, and the *in vitro* model can feed information into the clinical and predictive scores, respectively (**Figure 27**).

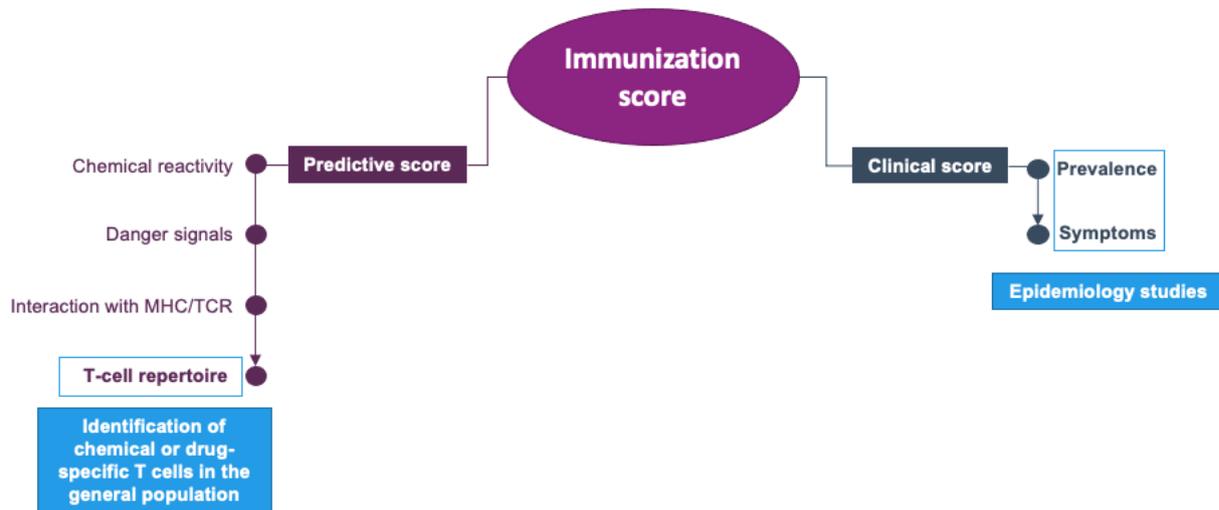


Figure 27. Interconnected themes: the epidemiology study and identifying chemical or drug-specific T cells inform the immunization score.

Through epidemiological studies, the assessment of drug allergies can provide valuable insights into the clinical aspects of these reactions. These studies can help determine the prevalence of allergic reactions in the treated population and identify the associated symptoms. By calculating the clinical score, evaluating the severity of these reactions and their impact on the treated population is possible.

On the other hand, besides being a promising KE4 assay in the AOP of skin sensitization, our repeated stimulation model can also be useful in identifying and semi-quantifying the T-cell response to drugs. This would be especially useful for calculating the T-cell repertoire in our predictive score. However, further research is necessary to validate these findings. With ongoing advances in technology and immunology, there is great potential for this method to lead to new insights and approaches to managing chemical and drug allergies in the future.

Lastly, developing the immunization score represents an innovative approach to predicting and preventing drug allergies. By identifying the key events involved in drug sensitization, this approach can provide a more accurate assessment of the risk of developing drug allergies.

The results of these three projects can help improve the diagnosis, treatment, and prevention of drug and chemical allergies, ultimately improving the health and well-being of patients. Further research in this area is necessary to continue advancing our understanding and developing effective strategies for their assessment and prediction.

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ANNEXE I. SCIENTIFIC CONTRIBUTIONS

Publications

Submitted article

Joelle Dagher, Diane Antonios, Sylvie Chollet-Martin, Luc de Chaisemartin, Marc Pallardy, Hayat Azouri, Carla Irani.

Drug-induced hypersensitivity reactions in a Lebanese outpatient population: a decade-long retrospective analysis (2012-2021).

Journal of Allergy and Clinical Immunology: Global (JACI: Global).

Article in preparation

Joelle Dagher, Natacha Szely, Estefania Tumbaco Valarezo, Diane Antonios, Hayat Azouri, Marc Pallardy.

A new model to detect and analyze the human T lymphocyte response to chemical sensitizers.

Abstract

Irani, C., Dagher, J., Antonios-Gholam, D., Pallardy, M., Azouri-Tannous, H.

An enhanced technique exploring the role of T cells in drug allergies.

Journal of Allergy and Clinical Immunology. 147, AB10, 2021.

<https://doi.org/10.1016/j.jaci.2020.12.081>.

Oral communications

Joelle Dagher, Natacha Szely, Estefania Tumbaco Valarezo, Diane Antonios, Hayat Azouri, Marc Pallardy.

A new model to detect and analyze the human T lymphocyte response to chemical sensitizers.

Immunotoxicology and Chemical Allergy Specialty Section – European Research Group on Experimental Contact Dermatitis (ITCASS-ERGECD) 2022 meeting, Amsterdam, Netherlands.

Joelle Dagher, Natacha Szely, Estefania Tumbaco Valarezo, Diane Antonios, Hayat Azouri, Marc Pallardy.

A new model to detect and analyze the human T lymphocyte response to chemical sensitizers.

13^{ème} journée de la recherche USJ 2022, Beirut, Lebanon.

Poster presentations

Joelle Dagher, Natacha Szely, Estefania Tumbaco Valarezo, Diane Antonios, Hayat Azouri, Marc Pallardy.

A new model to detect and analyze the human T lymphocyte response to chemical sensitizers.

International congress of toxicology (ICT), Federation of European Toxicologists and European Societies of Toxicology (EUROTOX), XVIth International Congress of Toxicology 2022, Maastricht, Netherlands.

AstraZeneca Student Innovation Award 2022

Joelle Dagher, Natacha Szely, Estefania Tumbaco Valarezo, Diane Antonios, Hayat Azouri, Marc Pallardy.

A new model to detect and analyze the human T lymphocyte response to chemical sensitizers.

Society of Toxicology (SOT) 62nd annual meeting 2023, Nashville, Tennessee.

Joelle Dagher, Natacha Szely, Estefania Tumbaco Valarezo, Diane Antonios, Hayat Azouri, Marc Pallardy.

A new model to detect and analyze the human T lymphocyte response to chemical sensitizers.

Journée de l'école doctorale, Innovation Thérapeutique du Fondamental à l'Appliqué, Université Paris Saclay, Châtenay Malabry, France.

Joelle Dagher, Natacha Szely, Estefania Tumbaco Valarezo, Diane Antonios, Hayat Azouri, Marc Pallardy.

A new model to detect and analyze the human T lymphocyte response to chemical sensitizers.

13^{ème} journée de la recherche USJ 2022, Beyrouth, Liban.

ANNEXE II. RESUME DE THESE EN FRANÇAIS

Les allergies aux molécules chimiques et médicamenteuses sont considérées un problème majeur de santé publique, affectant des millions de personnes dans le monde. Elles sont souvent imprévisibles, ont un impact sur la qualité de vie des patients et leur prise en charge pose des défis aux cliniciens. Ce travail de thèse vise à présenter trois modèles d'évaluation et de prévision des allergies aux molécules chimiques et médicamenteuses, chacun abordant un aspect différent du problème.

I. Évaluation et prévision des allergies médicamenteuses

- i. Étude rétrospective sur dix ans (2012-2021) des allergies médicamenteuses dans une population Libanaise

La première partie du projet de thèse consiste en une analyse rétrospective sur dix ans des réactions d'hypersensibilité médicamenteuses (HSM) aux molécules médicamenteuses utilisées en ambulatoire dans la population libanaise. Les études épidémiologiques sur les HSM sont limitées, notamment au Moyen-Orient. La plupart des études disponibles portent sur une population particulière ou une classe de médicaments spécifique. À notre connaissance, aucun travail de recherche épidémiologique portant sur les HSM a été précédemment publié au Liban, d'où l'utilité de notre travail. Notre étude rétrospective vise à évaluer les caractéristiques cliniques et le diagnostic des réactions d'HSM aux molécules utilisées en soins ambulatoires au Liban sur une période de dix ans (2012-2021) dans une clinique externe d'allergologie à Beyrouth.

Nos résultats ont largement confirmé ceux d'autres études menées dans d'autres pays. Un groupe de 758 patients ont été inclus, majoritairement des femmes. La plupart des patients ont présenté des réactions à une seule molécules (67,4 %), tandis

que d'autres ont présenté des HSM à plusieurs molécules. Tous les patients ont été diagnostiqués suite à l'historique clinique détaillé. Pour les patients présentant une HSM au moment de la consultation, un prick test cutané (SPT) d'allergie a été prescrit lorsque cela était nécessaire pour la confirmation du diagnostic. Un total de 72 médicaments ont été identifiés. Les classes de médicaments les plus fréquemment impliquées étaient les antibiotiques de la classe des bêtalactamines (53,8 %), suivis par les anti-inflammatoires non stéroïdiens (AINS) (48,9 %). Les caractéristiques de 90 patients (11,9 %) ayant des antécédents de HSM ambulatoires et de réactions concomitantes à une ou plusieurs molécules utilisées en peropératoire ont également été analysées.

ii. Développement d'un score d'immunisation capable de prédire les allergies médicamenteuses

Les réactions d'hypersensibilité médicamenteuse sont rarement détectées pendant les phases cliniques et pré-cliniques. Elles peuvent entraîner une interruption tardive du développement du médicament, le retrait des autorisations de commercialisation et des coûts supplémentaires liés à la prise en charge des patients concernés. La deuxième partie du projet visait à construire une approche unique fondée sur une analyse bibliographique des mécanismes d'immunisations aux molécules médicamenteuses afin de prévoir le risque d'immunisation des nouveaux candidats médicaments.

Cette approche analyse quatre paramètres prédictifs *in vitro* et *in silico* qui contribuent à l'immunisation des patients et leur corrélation avec deux paramètres cliniques liés à l'allergie. Chaque paramètre a été couplé à un coefficient, ce qui nous permet de calculer les deux scores. Pour le score prédictif, les quatre paramètres ont été sélectionnés en fonction de leur implication dans le processus d'immunisation : la

réactivité chimique de la molécule (ou de son métabolite), les signaux de danger immunologique induits par la molécule (ou son métabolite), les interactions de la molécule (ou de son métabolite) avec les molécules du complexe majeur d'histocompatibilité (CMH) ou les récepteurs des lymphocytes T (TCR pour T-cell receptor), et l'identification de lymphocytes T (LT) spécifiques reconnaissant la molécule, le métabolite ou le bioconjugué. Deux paramètres ont également été sélectionnés pour calculer le score clinique: la prévalence de l'allergie liée à la molécule étudiée et les symptômes cliniques des réactions allergiques. Pour la conception du score, 13 molécules ou famille de molécules ont été étudiées : bêta-lactams, acide clavulanique, dapsons, allopurinol, carbamazépine, lamotrigine, vancomycine, flucloxacilline, sulfaméthoxazole, sulfasalazine, abacavir, ticlopidine et cisplatine.

Une analyse de régression linéaire a ensuite été utilisée pour valider l'hypothèse concernant l'association entre les deux scores. Cela nous a permis d'établir le lien entre le score prédictif (la variable indépendante) et le score clinique (la variable dépendante). Par conséquent, pour toute nouvelle molécule en développement, les résultats d'expériences *in vitro* et *in silico* permettront le calcul du score prédictif et, par conséquent, la corrélation avec le score clinique pour évaluer le potentiel pro-allergique de la molécule.

II. Évaluation et prévision des allergies aux molécules chimiques

i. Développement d'un nouveau modèle pour la détection et l'analyse de la réponse des lymphocytes T aux allergisants de contact

La dermatite allergique de contact (DAC), causée par une exposition aux molécules appliquées sur la peau, est une réaction d'hypersensibilité retardée de type IV, médiée par les LT CD4+ et CD8+. Dans cette troisième et dernière partie du projet, nous avons mis en place un modèle de co-culture de cellules dendritiques et de LT autologues humains pour détecter les LT répondant à six molécules chimiques

connues pour provoquer une DAC. Ce modèle permet de quantifier la fréquence des LT répondant à ces molécules. L'originalité de notre protocole est la stimulation répétée des LT avec des cellules dendritiques autologues chargées avec les molécules chimiques. Cela permet l'expansion des LT spécifiques, normalement présents à une très faible fréquence, ce qui permet leur détection. Les molécules choisies sont les suivants: l'acide 2,4-dinitrobenzènesulfonique (DNBS), le cinnamaldéhyde (CinA), l'isoeugénol (IsoE), la méthylisothiazolinone (MIT), le sulfate de nickel (NiSO₄) et le chlorure de cobalt (CoCl₂). Nos résultats ont identifié des lignées de LT répondant à toutes les molécules chimiques chez la majorité des donneurs testés. En utilisant la loi de distribution de Poisson, nous avons calculé des fréquences moyennes allant de 0,30 à 2,00 LT spécifique par million de LT circulants. De plus, nous avons identifié les sous-populations des LT (CD4+ et/ou CD8+) répondants aux DNBS, CinA et MIT dans un second ELISpot en utilisant des anticorps dirigés contre les molécules du complexe majeur d'histocompatibilité de classe (CMH) I ou II. Les résultats ont confirmé la positivité des lignées lymphocytaires et ont montré que la réponse dépendait principalement des molécules du CMH I pour le DNBS, indiquant l'implication des LT CD8+. Pour le CinA nous avons retrouvés une implication principale des LT CD4+ et pour la MIT une réponse mixte CD4+ et CD8+. Les résultats de cette étude contribuent à une meilleure compréhension du mécanisme d'immunisation aux allergisants de contact organiques et inorganiques, et proposent un nouveau protocole sensible pour la détection des LT.

Dans l'ensemble, ce travail de thèse a permis de faire progresser notre compréhension sur les allergies aux molécules chimiques et médicamenteuses et à développer de nouveaux outils et modèles pour prévoir et détecter ces réactions. Les connaissances et les techniques développées dans le cadre de ce projet pourraient avoir des répercussions sur le diagnostic et la prévention des allergies aux produits chimiques et médicamenteuses.

