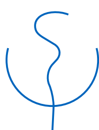

Fakultät für Medizin
Lehrstuhl für molekulare Allergologie

Improved Diagnosis and Therapy of Hymenoptera Venom Allergy by Component-Resolved Approaches

Maximilian Schiener

Vollständiger Abdruck der von der Fakultät für Medizin der Technischen Universität München zur Erlangung des akademischen Grades eines

Doktors der Naturwissenschaften (Dr. rer. nat.)

genehmigten Dissertation.

Vorsitzende: Prof. Dr. Christina Zielinski

Prüfende/-r der Dissertation:

1. Priv.-Doz. Dr. Simon Blank
2. Prof. Dr. Michael Sattler

Die Dissertation wurde am 23.01.2018 bei der Technischen Universität München eingereicht und durch die Fakultät für Medizin am 04.07.2018 angenommen.

Abstract

Patients suffering from allergies due to stings of Hymenoptera like *Apis mellifera*, *Vespula vulgaris* and *Polistes dominula* can experience severe to fatal anaphylactic reactions. Besides emergency treatment, venom immunotherapy (VIT) is the only curative treatment. VIT products are available from different manufactures and treatment efficacy is very high compared to other forms of allergen-specific immunotherapy. Nevertheless, therapeutic failures can be observed and possibly originate from different venom extraction or work-up techniques. To reduce the cost resulting from unnecessary treatment with more than one venom and to avoid the chance of *de novo* sensitization, the identification of the insect causing the systemic reaction has to be unambiguous. Not all patients can correctly identify the causative insect and, moreover, diagnosis by skin testing is not always straightforward. Therefore, serum specific immunoglobulin E (sIgE) levels to crude insect venom can be determined in routine diagnosis. In the past, this was shown to have several disadvantages, as single allergens can be underrepresented in venom extracts due to low biologic availability or stability, thus patients sensitized to those allergens might be missed. Furthermore, patients with clinically irrelevant sIgE reactivity to cross-reactive carbohydrate determinants (CCDs), might be tested (false) positive to the venoms of *Apis mellifera* and *Vespula vulgaris*. However, double-positive sIgE diagnostic test results can also originate from homologous proteins in the venoms of *Apis mellifera*, *Vespula vulgaris* and *Polistes dominula*.

The introduction of single venom allergens for routine diagnosis by recombinant techniques advanced molecular and component-resolved diagnosis of insect venom allergy. Since the beginning of the new millennium, many *Apis mellifera* and *Vespula vulgaris* venom allergens have been identified. Additionally, marker allergens were identified and produced without CCD-reactivity, thus patients showing double-positive diagnostic test results to *Apis mellifera* and *Vespula vulgaris* venom extracts can be diagnosed with high sensitivity and specificity. Discriminating marker allergens are missing for the diagnosis of patients with suspected *Vespula vulgaris* and *Polistes dominula* venom allergy. As both species coexist in the Mediterranean regions of Europe, double-positive tested patients or patients sensitized to both species are frequently observed. As *Polistes dominula* is invading northern regions due to climate changes, venom allergy to stings of this species is most likely to spread in the same manner.

In the first part of this thesis, the available and already identified *Vespula vulgaris* and *Polistes dominula* major venom allergen, antigen 5, was used together with the homologues from other related allergy-relevant Hymenoptera species to demonstrate the present diagnostic challenges. In the next part, the state-of-the-art diagnostic and therapeutic options for Hymenoptera venom allergy were reviewed. Additionally, a previously unknown *Polistes dominula* venom allergen, the dipeptityl peptidase IV, was identified and demonstrated to be a major allergen. Furthermore, the generation of recombinant venom allergens was employed to generate tools to study the composition of available therapeutic venom extracts used for the treatment of *Apis mellifera* venom allergy. Thereby, a possible reason for treatment failures of *Apis mellifera* venom allergic patients was demonstrated by the lack and instability of allergens in some therapeutic venom preparations.

Zusammenfassung

Stiche von Hymenopteren wie *Apis mellifera*, *Vespula vulgaris* oder *Polistes dominula* können schwere bis tödliche anaphylaktische Reaktionen in allergischen Patienten auslösen. Neben der Notfallbehandlung, ist die Immuntherapie mit Insektengift die einzige kurative Therapiemöglichkeit und gereinigte Gifte sind hierfür von verschiedenen Herstellern erhältlich. Trotz der sehr guten Wirksamkeit, im Vergleich zu anderen allergenspezifischen Immuntherapeutika, können Therapieversager beobachtet und möglicherweise durch unterschiedliche Methoden der Giftextraktionen oder der Aufarbeitung erklärt werden. Um die Kosten durch die Behandlung mit mehr als einem Gift zu senken und mögliche *de novo* Sensibilisierungen zu vermeiden, ist eine korrekte Identifizierung des allergieauslösenden Insekts nötig. Da nicht alle Patienten das Insekt identifizieren können und die Diagnose mit Hilfe von Hauttests ebenfalls nicht immer eindeutig ist, kann das spezifische Immunoglobulin E (sIgE) gegen Insektengiftextrakt routinemäßig bestimmt und herangezogen werden. In der Vergangenheit konnte allerdings gezeigt werden, dass eine Messung gegen einen Allergenextrakt mehrere Nachteile hat. Einzelne Allergene können aufgrund geringer biologischer Verfügbarkeit oder Stabilität im Extrakt unterrepräsentiert sein. Patienten mit Sensibilisierungen gegen diese Bestandteile werden eventuell diagnostisch nicht erfasst. Außerdem können klinisch irrelevante sIgE-Werte gegenüber den Giften von *Apis mellifera* und *Vespula vulgaris* aufgrund von kreuzreaktiven Kohlenhydrat-Determinanten (CCDs) die Diagnose beeinträchtigen. Allerdings können doppel-positive sIgE-Werte auch aufgrund von homologen Proteinen in Giften von *Apis mellifera*, *Vespula vulgaris* und *Polistes dominula* beobachtet werden.
Die Einführung von rekombinanten Einzelallergene für die Routinediagnostik hat die Molekulare oder Komponenten-aufgelöste Diagnostik vorangetrieben. Seit Anfang des neuen Millenniums wurden mehrere Allergene aus dem Gift von *Apis mellifera* und *Vespula vulgaris* identifiziert und konnten rekombinant sowie ohne CCD-reaktivität produziert und teilweise als Spezies-spezifisch bzw. Markerallergen bestimmt werden und helfen somit bei der Diagnose von Patienten mit doppelt-positiven sIgE-Ergebnissen mit hoher Sensibilität und Spezifizität. Markerallergene für die Diagnose von Patienten mit vermuteter Allergie gegen das Gift von *Vespula vulgaris* oder *Polistes dominula* sind bisher nicht bekannt. Doppel-positive Testergebnisse oder eine Sensibilisierung gegen beide Spezies wird in mediterranen Gegenden Europas häufig beobachtet, da beide Spezies nebeneinander bestehen. Aufgrund des Klimawandels dringt die *Polistes dominula* in nördliche Gegenden ein und eine Allergie aufgrund von Stichen dieser Spezies könnte sich gleichermaßen ausbreiten.
Im ersten Teil dieser Arbeit wurde das aus dem Gift von *Vespula vulgaris* und *Polistes dominula* bekannte Majorallergen, das Antigen 5, zusammen mit den Homologen aus dem Gift verwandter und allergie-relevanter Spezies genutzt, um diagnostische Tücken aufzuzeigen. Im nächsten Teil wurden der aktuellste Stand der Insektengiftdiagnostik und -therapie in einen Review zusammengefasst. Darüberhinaus wurde ein bisher unbekanntes Allergen, die Dipeptidylpeptidase IV, im Gift der *Polistes dominula* nachgewiesen und als Majorallergen identifiziert. Außerdem wuden rekombinante Giftallergene genutzt um Werkzeuge zur Untersuchung von Therapiepräparaten, die zur Therapie von Allergien gegen das Gift von *Apis mellifera* verwendet werden, herzustellen. Dabei wurde festgestellt, dass verschiedene Allergene in manchen Therapiepräparaten, möglicherweise aufgrund ihrer Instabilität, fehlen, was manche Therapieversager erklären könnte.

List of Abbreviations

Symbols

kIU/L - international kilo units per liter . . . 13

A

AAAAI - American Academy of Allergy, Asthma and Clinical Immunology . . . 18, 20
AIT - allergen-specific immunotherapy . . . 8, 11, 12
APC - antigen-presenting cell . . . 4, 8–10
AUC - area under dose curve . . . 16

C

CCD - cross-reactive carbohydrate determinant . . . i, 21, 22, 26, 28, 34, 38, 39
CD - cluster of differentiation . . . 4, 5, 8, 9, 15, 41
cDNA - complementary deoxyribonucleic acid . . . 31, 34, 35, 40
CRD - component-resolved diagnosis . . . 14
CTLA-4 - cytotoxic T-lymphocyte-associated protein 4 . . . 12

E

E. coli - *Escherichia coli* . . . 22, 38
EAACI - European Academy of Allergy and Clinical Immunology . . . 14, 18, 20, 38
ELIFAB - enzyme-linked immunosorbent facilitated antigen binding . . . 29
ELISA - enzyme-linked immunosorbent assay . . . 31, 34, 35, 40

F

FAB - IgE-facilitated antibody binding . . . 29
Fc - fragment crystallizable region . . . 6–8
FcϵRI - Fcϵ-receptor I . . . XXIII, 8–11, 15
Foxp3 - forkhead-box protein 3 . . . 6, 41

G

Gata-3 - GATA binding protein 3 . . . 6, 11

I

IFNγ - interferon γ . . . 6, 11, 30
Ig - immunoglobulin . . . 6
IgE - immunoglobulin E . . . XXIII, 2, 6–15, 20–22, 27, 39
IgG - immunoglobulin G . . . 6–8, 12, 14, 30
IL - interleukin . . . 6, 9–12, 30, 41
ITAM - immunoreceptor tyrosine-based activation motif . . . 10
IUIS - International Union of Immunological Societies . . . 13, 27, 35, 40

L

LLR - large local reaction . . . 17–19

M

MHC - major histocompatibility complex . . . 5, 9

N

NK - natural killer 4

P

PD-1 - programmed cell death protein 1 12

R

RAST - radio-allergosorbent test 13

S

SDS-PAGE - sodium dodecyl sulfate polyacrylamide gel electrophoresis 22, 25
sIgE - specific immunoglobulin E. i, 12–15, 18, 20–22, 24, 26–29, 31, 32, 34, 35, 38–41, 43
sIgG - specific immunoglobulin G 14, 29
Sf9 - *Spodoptera frugiperda* 31, 32, 34, 35
spp. - species 21

T

T-bet - T-box transcription factor TBX21 6
T_{fh} - T follicular helper cells 6
T_h1 - T helper cell type 1 . . . . 5, 6, 9, 11, 12
T_h2 - T helper cell type 2 . . . . 5, 6, 8, 9, 11, 12, 41
T_{regs} - regulatory T cells 5, 6, 9, 11, 12, 41
TCR - T cell receptor 4, 5
TGFβ - tumor growth factor β 6, 12
Th - T helper 30
TLR - toll-like receptor 9
TNFα - tumor necrosis factor α 10

V

VIT - venom immunotherapy . . . . i, 2, 11, 13, 17–19, 26, 32, 38

W

WHO - World Health Organization 13, 27, 35, 40

Contents

Abstract i

Zusammenfassung ii

I Introduction 1

1 The Immune System and Immunological Hypersensitivity Reactions 3

- 1.1 Immune System . . . 3
  - 1.1.1 Innate Immunity . . . 3
  - 1.1.2 Adaptive Immunity . . . 4
- 1.2 Allergic Diseases . . . 7
  - 1.2.1 Patophysiology of Allergy . . . 8
  - 1.2.2 Clinical Manifestation of Allergy . . . 9
  - 1.2.3 Therapy of Allergic Diseases . . . 11
- 1.3 Allergy Diagnosis . . . 12
  - 1.3.1 *In vivo* Allergy Diagnosis . . . 12
  - 1.3.2 *In vitro* Diagnosis and Molecular Allergology . . . 13
  - 1.3.3 Cellular Tests . . . 14
- 1.4 Insect Venom Allergy . . . 16
  - 1.4.1 Clinical Manifestation and Therapy of Insect Venom Allergy . . . 17
  - 1.4.2 Epidemiology . . . 19
  - 1.4.3 Venom Allergens and the Diagnosis of Hymenoptera Venom Allergy 20

2 Aim of the Study 31

II Results 33

- 2.1 Application of recombinant antigen 5 allergens from seven allergy-relevant Hymenoptera species in diagnostics. . . . 34
  - 2.1.1 Summary . . . 34
  - 2.1.2 Individual Contributions . . . 34
- 2.2 The high molecular weight dipeptidyl peptidase IV Pol d 3 is a major allergen of *Polistes dominula* venom. . . . 35
  - 2.2.1 Summary . . . 35
  - 2.2.2 Individual Contributions . . . 35

III Discussion 36

- 2.3 Unsolved Problems of *Polistes dominula* Venom Allergy Diagnosis . . . 38
- 2.4 Improved *Polistes dominula* Venom Allergy Diagnosis . . . 40
- 2.5 Component-Resolved Analysis of *Apis mellifera* Venom Immunotherapy Products . . . 42
- 2.6 Summary and Conclusion . . . 43

References I

List of Figures XXIII

List of Tables XXIV

Acknowledgement XXV

Part I

Introduction

Evolution equipped vertebrates with a specialized network of proteins and cells to fight off foreign intruders like viral particles or bacteria, called immune system. In some cases, the immune system is activated by harmless environmental particles or food components. This hypersensitivity reaction is generally known as allergy and is mostly experienced as itchiness of the eyes or skin and mucus overproduction in nose or the lungs, leading to difficulties in breathing. Systemic reactions to harmless particles can eventually lead to death in severe cases. The term allergy originates from works of Clemens von Pirquet in 1906. He observed a divergent reactions of an individual immune reactions, in most cases more sensitive, to foreign (greek: ἀλλος, allos) substances used for vaccination, like tuberculin. This was only the case, if the individual encounters the agent for the second time. At the same time he also used the term allergen to name the foreign substance able to induce this reaction (von Pirquet, 1906).

Patients experiencing hypersensitivity reactions to venoms of species out of the order of Hymenoptera, like honeybees (*Apis mellifera*), yellow jackets (*Vespula* species) or paper wasps (i.e. *Polistes* species) can experience severe and in some cases fatal reactions after a sting. The only available treatment, able to prevent anaphylactic insect sting reactions is VIT, whereby patients are treated by repetitive injections with increasing amounts of venom. In order to choose the right venom for treatment, clinicians are dependent on different diagnostic approaches. Besides skin testing, the determination of serum immunoglobulin E (IgE) against crude venom is used for allergy diagnosis. Very recently, single venom components have been introduced to improve serological tests and have launched the field of molecular or component-resolved diagnosis. Several venom components, the so called marker allergens, are currently commercially available to facilitate the discrimination of *Apis mellifera* and *Vespula vulgaris* venom allergy, but distinguishing venom components are missing for the diagnosis of *Polistes dominula* venom allergy.
This work will demonstrate the lack of venom components to correctly discriminate *Polistes dominula* venom-allergic patients from patients allergic to *Vespula vulgaris* venom and present newly identified *Polistes dominula* venom components useful for an improved Hymenoptera venom allergy diagnosis. Additionally, recombinantly produced honeybee venom allergens were used to generate tools to analyze therapeutic venom extracts, uncovering putative reasons for several treatment failures.

1 The Immune System and Immunological Hypersensitivity Reactions

The human body can protect itself against foreign pathogens and parasites not only through physical barriers but also with the help of a specialized immune system. On the one hand, the human body is equipped with an unspecific system, providing a fast but undirected immune response called innate immunity. On the other hand, the adaptive immunity enables the body to specifically react against almost all foreign structures with the help of stochastically newly built receptors. Adaptive immunity also includes the generation of long-term responses to pathogens, also called immunological memory. If the adaptive immune system reacts inappropriate to foreign structures or structures of the own organism, this can lead to a malfunction, namely hypersensitivity and auto-immunity, respectively (Murphy et al., 2017).

1.1 Immune System

The skin, mucosal sites, the intestinal tract and other areas are protected from harmful microbes such as bacteria, fungi or parasites, viruses, toxins and cancer cells by the cells and proteins of the immune system. The immune system is able to detect pathogens with two complementary systems: The innate (humoral) immunity, that reacts against conserved pathogenic structures and the adaptive (cell-mediated) immunity that can, as the name suggests, adapt its tools to be able to react against unknown foreign structures and additionally memorizes previous encounters of pathogens to react even faster upon re-infection (Murphy et al., 2017).

1.1.1 Innate Immunity

Cells of the innate immunity patrol the body and detect foreign substances, dead cells and antibody complexes bound to unfamiliar structures and promote their clearance. Upon encountering a pathogen, preformed molecules like antimicrobial enzymes and peptides, able to destroy the bacterial cell wall, are released. Furthermore, antibodies, glycoproteins and plasma proteins from the complement system target pathogens to be lysed, but also coat (opsonize) foreign intruders. This opsonization enables phagocytes, cells that ingest and clear particles of different sizes and origin, to recognize those foreign substances. If this mechanism fails, cells of the innate immunity can also be activated by different inborn pattern recognition receptors (PRRs), detecting pathogen-associated molecular patterns

(PAMPs) that are typical for microbes. The most prominent phagocytes are macrophages. Neutrophils are other important phagocytes, additionally able to release preformed proteins and enzymes that are stored in granules to help in the elimination of pathogenic bacteria or fungi. Once activated, cells of the innate immune system produce cytokines, small proteins responsible for cell-cell signaling, and chemokines, proteins able to attract further cells, and induce inflammation. Parasites that are to big to be phagocytosed are destroyed by eosinophils, cells with preformed proteins stored in granules, toxic for many tissues. Additionally, T lymphocytes like natural killer (NK) and T cells are important effector cells of the innate immune system, able to kill and fight off tumor cells or cells infected by bacteria or viruses. Other important phagocytes are dendritic cells. Moreover, dendritic cells have the ability to link the innate with the adaptive immune system by their antigen-presenting capacity. This enables them to digest pathogens or molecules derived thereof in order to present small parts (peptides) to the cells of the adaptive immune system (Murphy et al., 2017).

1.1.2 Adaptive Immunity

When pathogens adapt their structures to evade the innate immune system, the adaptive immune system starts to develop tools that react specifically with structures (antigens) either on the surface or contained in the pathogens. This antigen-dependent immune activation implies a delay between the exposure to the antigen or pathogen and the maximal immune response, as effector cells and molecules have to be generated. The generation of antigen-specific responses also allows the body to develop a memory against pathogens, which is one of the most important features of adaptive immunity. Important cells in this process are the antigen-presenting cells (APCs), like dendritic cells, as well as T and B cells. T and B cells share an important feature called somatic recombination and hypermutation. Somatic recombination is a process leading to the rearrangement of immune receptor-encoding genes from gene fragments, which makes it possible to generate receptors and molecules capable of binding almost every structure in a stochastic manner. Different selection mechanisms are needed during T and B cell development to permit these cells to correctly recognize foreign antigens in the presence of self-antigens.

T Cells

T cells arise from bone marrow from hematopoietic stem cell precursors, stem cells that give rise to blood cells. The name T cell originates from the fact that these cells mature in the thymus. The most essential feature of T cells is the T cell receptor (TCR). The TCR is build by a heterodimer of two different subunits, both containing variable regions for the recognition of different peptides presented by antigen-presenting cells (APCs). The TCR heterodimer, mostly composed of an α and a β subunit, associates with the cluster of differentiation (CD)3 molecule and the protein ζ, both used for intracellular signaling. The TCR heterodimer is encoded by different gene segments that are joined together

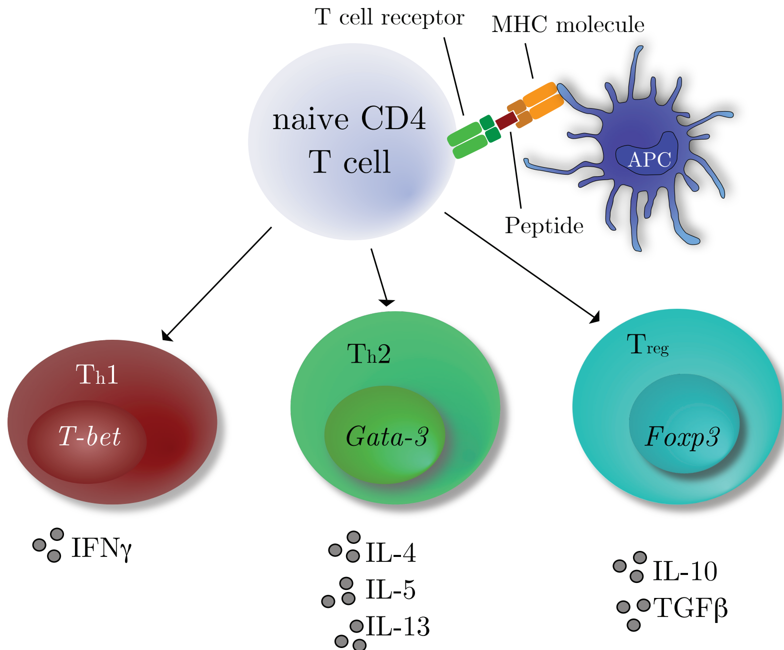

Figure 1 Peptides Presented to Naive T Cells and T Cell Subsets Relevant for Insect Venom Allergy. Peptides are presented in a complex with major histocompatibility complex (MHC) molecules. Important cluster of differentiation (CD)4^+ T helper cell subsets for insect venom allergy are T helper cell type 1 (T_h1), T helper cell type 2 (T_h2) and regulatory T cells (T_{regs}) cells. APC, antigen-presenting cell; T-bet, T-box transcription factor TBX21; Gata-3, GATA binding protein 3; Foxp3, forkhead-box protein 3; IFNγ, interferon γ; IL, interleukin; TGFβ, tumor growth factor β.

during the maturation of T lymphocytes. The TCR complexes are only able to recognize foreign peptides that are presented on major histocompatibility complex (MHC) molecules (Abbas et al., 2015).

There are two different classes of MHCs molecules, which are expressed by different cell types throughout the body and each molecule induces different immune responses. MHC class I molecules are expressed on almost all nucleated cells and present peptides from antigens that were encountered intracellularly. In most cases, intracellular encounters with foreign antigens arise from tumors or viral and bacterial infected cells. Consequently, the α polypeptide chain of MHC class I molecules has a recognition site for CD8 cytotoxic T cells. CD8 positive cells ($CD8^+$) have the function to destroy infected cells by releasing molecules and enzymes that lyse target cells or induce apoptosis (Murphy et al., 2017).

MHC class II molecules are exclusively expressed on specialized cells, able to ingest extracellular intruders and present the peptides of digested proteins, such as dendritic cells, macrophages and B cells. In contrast to MHC class I, MHC class II molecules have a binding site for CD4, hence peptides presented by cells expressing MHC class II molecules activate $CD4^+$ T helper cells. $CD4^+$ T helper cells activate of macrophages, that in turn eliminate extracellular microbes and additionally help B lymphocytes to generate antibodies against the antigen (Murphy et al., 2017).

$CD4^+$ T helper cells show a high plasticity and can be grouped into different subsets according to their cytokine expression profile and/or differentially expressed transcription factors. An overview of different $CD4^+$ T helper cells, their cytokine expression profiles

and expressed transcription factors is given in Figure 1. T helper cell type 1 (T_h1) and T helper cell type 2 (T_h2) are the most studied T helper cell subsets. The key cytokine of T_h1 cells is interferon γ (IFNγ) and the key transcription factor is T-box transcription factor TBX21 (T-bet) (Glimcher, 2007). T_h1 cells help to activate macrophages and induce immunoglobulin G (IgG) production in B cells. T_h2 cells mainly produce interleukin (IL)-4, IL-5 and IL-13 as key cytokines and are regulated by the transcription factor GATA binding protein 3 (Gata-3) (Georas et al., 2005). They mediate the defense mechanisms in the fight against helminths by the activation and recruitment of eosinophils and mast cells. IL4 additionally leads to a class switch of antibody subtype in B cells to IgE. T_h2 cells also play a central role in allergic diseases. Another important subset of T cells are the regulatory T cells (T_{regs}). This subpopulation limits, controls and suppresses immune responses. The key cytokines expressed in T_{regs} are IL-10 and tumor growth factor β (TGFβ). T_{regs} are regulated by the transcription factor forkhead-box protein 3 (Foxp3) (Hawrylowicz et al., 2005).

B Cells

B cells arise in the bone marrow from hematopoetic stem cells percursors and, in contrast to T cells, are able to recognize free and unprocessed antigens. For this purpose, B cells produce unique antigen-receptors that are generated by somatic hypermutations. Upon binding to an antigen, B cells migrate to the next lymph node. Here, the co-stimulation by T follicular helper cells (T_{fh}) initiates the process of affinity maturation. During the subsequent and tightly regulated steps, the antigen receptor is sequentially modified in order to improve its affinity. The activation is regulated by several cytokines and B cells can undergo differentiation either to memory B cells, generating a long lasting antigen memory or to plasma cells, releasing a soluble form of the antigen-receptor, called immunoglobulin (Ig) or antibody (Warrington et al., 2011). Mature B cells produce antibodies of the subclasses IgM and IgD. Depending on the antigen stimulation, cytokine microenvironment and the interaction with T cells, an additional gene rearrangement can lead to isotype class-switching and the subsequent production of antibodies of the subclasses IgG, IgA or IgE (Looney et al., 2016).

The overall structure of an antibody is “Y”-shaped, with two identical heavy chains and two identical light chains, each consisting of a constant C-terminal region and one variable N-terminal region (Figure 2). Heavy and light chains are connected by disulfide bridges. The only difference to the corresponding B cell receptor is the missing transmembrane region, anchoring the antibody to the cell membrane. Gene rearrangements of the variable region enables the antibody to bind to almost all antigens. The constant region (also called fragment crystallizable region (Fc) region) enables the antibody to bind to its specific receptor, in order to fulfill effector functions such as activation of complement or effector cells. The producers of antibody molecules are plasma cells that arise from (class-switched) B cells (Murphy et al., 2017).

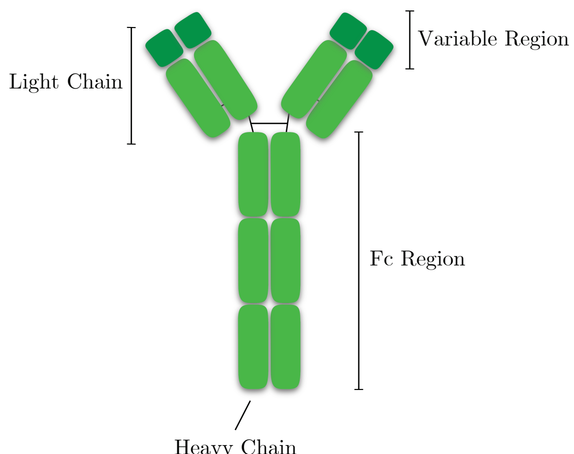

Figure 2 Structure of an Antibody Molecule. An antibody molecule consists of two identical heavy chains and two identical light chains, each consisting of a constant C-terminal region and one variable N-terminal region. Heavy and light chains are connected by disulfide bridges. Fc, fragment crystallizable.

1.2 Allergic Diseases

In some individuals the immune system, especially the adaptive immunity, reacts inappropriately to harmless substances, including but not limited to antigens of environmental origin such as pollen or food and antigens released during animal stings or bites. Malfunctions of the innate or adaptive immune system, leading to illness or disease, are categorized into three groups. If the body reacts inappropriate to self-antigens, this is designated autoimmunity. In the case of an ineffective immune response, this is termed immunodeficiency. An overreactive immune response is called hypersensitivity reaction or allergy. Hypersensitivity reactions were categorized into four groups by Gell and Coombs. Type I hypersensitivity reactions are IgE-driven allergic reactions, including allergic rhinitis and allergic asthma and are also called immediate hypersensitivity reactions or allergy. Type II reactions are cytotoxic or antibody-dependent hypersensitivity reactions. In this case, the cause of inflammation are antibodies (IgG and IgM) that bind to the patient's own cell-surface molecules. The immune complex disease or type III hypersensitivity reaction occurs, when serum antibodies (IgG) bind to soluble proteins in the blood and the formed immune complexes are deposited in the tissue. Subsequent complement activation and immune cell recruitment then causes local damage. Type IV hypersensitivity reactions are also called delayed or cell-mediated hypersensitivity reaction. In this case, T cells induce inflammation when they recognize a previously identified antigen (Gell et al., 1963; Rajan, 2003).

Immunoglobulins E and G in Allergy

The immunoglobulin type E was first described in one of the works of Ishizaka et al., 1967 and parallel in works of Johansson, 1967. The IgE antibody has the same overall structure as other antibody isotypes and has three constant region of the heavy chain (Figure2). The Fc region of IgE can bind two different receptors, the high affinity IgE receptor

Fcϵ-receptor I (FcϵRI) and the low affinity IgE receptor CD23. CD23 is expressed on mature B cells, activated macrophages, eosinophils, follicular dendritic cells and platelets (Gould et al., 2008). The FcϵRI is expressed as tetrameric molecule on basophils and mast cells, as trimer on APCs such as dendritic cells and Langerhans cells as well as on eosinophils, monocytes, platelets and smooth muscle cells. Cell signaling is executed upon cross-linking of receptor bound IgE by an antigen (Figure 4; Kraft et al., 2007). The evolutionary biological function of IgE and the induced T_h2 response is to fight of helminth infections (Pearce et al., 1991). In patients sensitized to otherwise harmless antigens (in this case allergens), IgE is the key molecule responsible for the typical symptoms of allergy following allergen contact (Section 1.2.2; Wedemeyer et al., 2000; Galli et al., 1999).

In contrast to IgE ($< 1\,\mu g/mL$) (Amarasekera, 2011), IgG antibodies are highly abundant in human serum ($13.5\,mg/mL$) (Abbas et al., 2015) and exist as four different sub-types (IgG1-4). The induction of IgGs is part of the natural exposure to daily allergens, especially to those taken up through the gastrointestinal tract. Therefore, the composition of IgGs depends on the individual diet and environment. Elevated IgG is found in patients suffering from autoimmune inflammatory diseases like rheumatoid arthritis or systemic lupus erythematosus (Scott-Taylor et al., 2017). Depending on the Fc region of the different IgG subtypes, they can bind different Fcγ-receptors. The different receptors are categorized according to their affinity to the respective immunoglobulins. FcγRI, expressed on macrophages, neutrophils and eosinophils, binds IgG with the highest affinity and activates the cells for phagocytosis. FcγRIIA and B are also expressed mainly on phagocytes, bind IgG with lower affinity and also signal for cell activation and phagocytosis. FcγRIIB is expressed on myeloid cells including macrophages, neutrophils and mast cells and is the only IgG receptor on B cells. It binds IgG (preferably IgG_4) with low affinity and has an inhibitory signaling function and might play a role in immunotherapy by its ability to dampen the immune system (Abbas et al., 2015; Murphy et al., 2017). IgG induced by immunotherapy can cross-link the FcϵRI with FcγRIIB via the allergen and inhibit the FcϵRI activation signaling (Kraft et al., 2007). IgG_4 is the most important IgG-subclass antibody and is thought to arise by a class-switch of B cells induced by allergen-specific immunotherapy (AIT). In conclusion IgG_4 is thought to be anti-inflammatory and able to block allergen induced allergic reactions (Flicker et al., 2003; Wachholz et al., 2004; Scadding et al., 2010; Möbs et al., 2012).

1.2.1 Patophysiology of Allergy

The priming of the immune system towards an allergic reaction, including the production of IgE, is also termed sensitization. Sensitiziation occurs when harmless antigens reach the immune system either through the gastrointestinal tract (foods and drugs), epithelial tissues in nose, lung and skin (airborne allergens) or by injection (insect venoms and drugs). At the respective contact site, the antigens are taken up by APCs and captured by B cells via their receptors. Once taken up by APCs and transported to the next draining

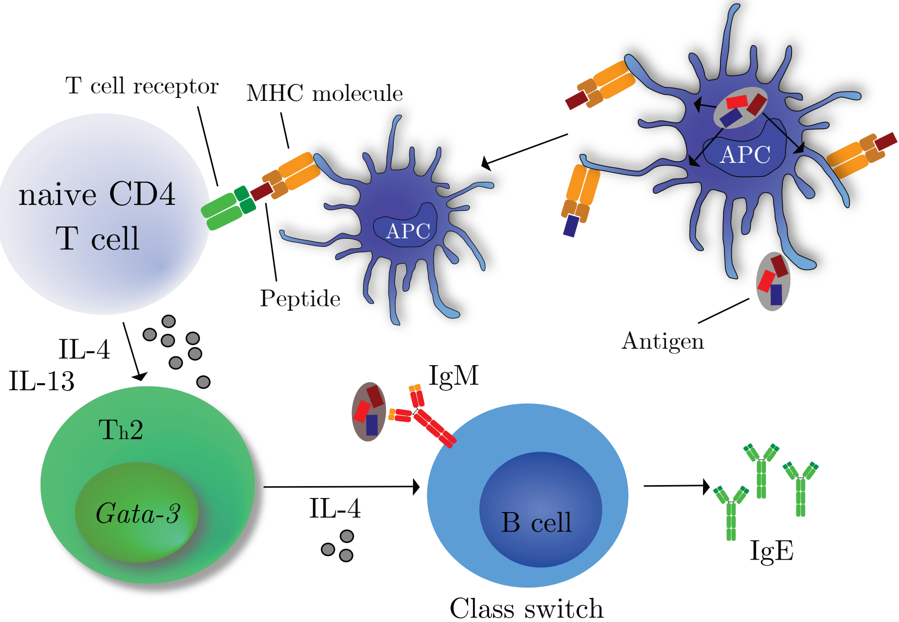

Figure 3 Mechanisms of Allergic Sensitization. Antigens are taken up by antigen-presenting cell (APC), presented in a complex with major histocompatibility complex (MHC) molecules to naive cluster of differentiation (CD)4$^+$ T helper cells. Additional stimuli in the microenvironment lead to the activation of naive T helper cells and to their differentiation into T helper cell type 2 (T_h2) cells which secrete interleukin (IL)-4 and IL-13. IL-4 leads to a class switch of B cells towards immunoglobulin E (IgE) producing plasma cells. Gata-3, GATA binding protein 3; IgM, immunoglobulin M.

lymph node, the antigen is processed and peptides thereof are presented on MHC class II molecules to naive CD4$^+$ T cells (Hammad et al., 2008). Specific T cells recognize the presented peptides and additional stimuli in the microenvironment, in particular IL-4, lead to the activation of the naive CD4$^+$ T helper cells and to their differentiation into T_h2 cells (Rotmagnani, 1998). These T_h2 cells secrete IL-4 and IL-13, that in turn contribute to allergen sensitization and support a class-switch of B cells towards IgE producing plasma cells (Shimoda et al., 1996; Geha et al., 2003) and the generation of allergen-specific memory B cells (Niederberger et al., 2002) (Figure 3). The mechanisms and reasons why some individuals do and some do not develop T_h2 skewed cells and IgE against otherwise harmless antigens are barely established. Besides possible genetic (Vercelli, 2008) and co-stimulatory factors (Romagnani, 2000), western lifestyle, including reduced microbial burden and thus toll-like receptor (TLR) stimulation is supposed to skew the balance between T_h1 and T_h2 cells (Romagnani, 2004). Furthermore, it might not only be the balance between T_h1 and T_h2 cells, but also the disbalance of regulatory mechanisms through T_{regs} might play a role in the establishment of allergy, especially in developed countries (Yazdanbakhsh et al., 2004; Romagnani, 2007).

1.2.2 Clinical Manifestation of Allergy

In sensitized individuals, the re-exposure with an allergen leads to an immediate type I hypersensitivity reaction, induced by the cross-linking of the FcϵRI on mast cells and

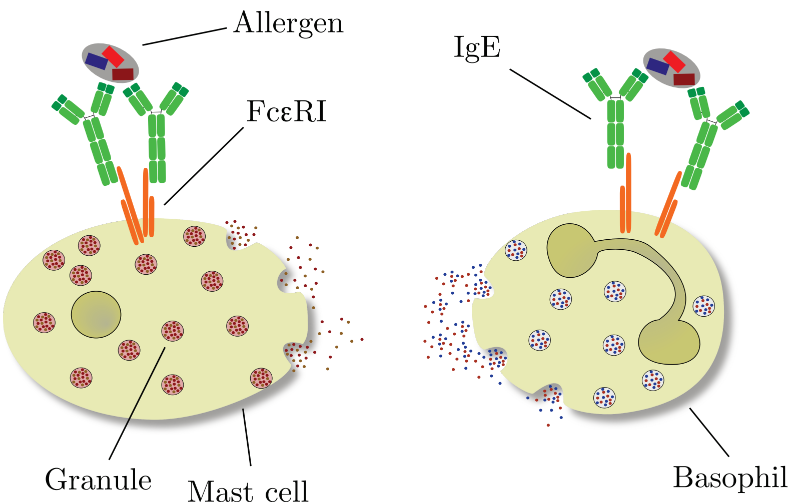

Figure 4 Mast Cell and Basophil Activation by Cross-Linked IgE on FcεRI. IgE, immunoglobulin E; FcεRI, Fcε-receptor I.

basophils. Allergen-specific IgE, bound on FcεRI, is cross-linked by the allergen and intracellular signaling is induced by the intracellular located immunoreceptor tyrosine-based activation motif (ITAM) on FcεRI (Marshall, 2004). The biologically active products, preformed and stored in granules, are released directly upon activation and include biogenic amines such as histamine, lipid mediators, enzymes, cytokines and chemokines (Galli et al., 2005; Gilfillan et al., 2006; Kraft et al., 2007; Rivera et al., 2006; Dvorak, 2005) (Figure 4). The acute allergy symptoms are vasodilation, increased vacular permeabiliy, contraction of smooth muscle cells, increased mucus secretion, conjunctivitis and asthma (Wills-Karp, 1999) and are the consequences of released histamine, proteoglycans, prostaglandins, leukotrienes and serine proteases like tryptases and chymases (Stevens et al., 2007; Pejler et al., 2007; Caughey, 2007; Boyce, 2005). Allergen-specific T cells, activated by peptides of the allergen presented by APCs, release cytokines like tumor necrosis factor α (TNFα), IL-5, IL-7, IL-10 and IL-13. The recruited T cells, neutrophils, monocytes, eosinophils, mast cells and basophils conttribute to inflammation as part of the late-phase allergic reaction (Kraft et al., 2007; Rivera et al., 2006; Galli et al., 2008a; Holgate et al., 2008). The inflammation can become chronic and cytotoxic proteins designed to kill parasites lead to tissue damage, typically observed in chronic allergic asthma (Galli et al., 2008b; Marone et al., 2005).

Particularly, allergic reactions upon the ingestion of food or as a consequence of insect stings can become systemic. These generalized reactions are also called anaphylaxis and include cutaneous urticaria, angioedema, pruritus, flush, nephropathy, central and peripheral neurologic syndromes, idiopathic thrombocytopenic purpura, rhabdomyolysis, vascular or respiratory symptoms, bradycardia, arrhythmia, angina, myocardial infarction, abdominal cramps, gastrointestinal tract, uterine smooth muscle contraction, unconsciousness and/or respiratory and cardiac arrest (Golden, 2015).

1.2.3 Therapy of Allergic Diseases

Currently available treatments of allergic diseases either address clinical symptoms (pharmacotherapy) or aim to induce long-lasting allergen tolerance (allergen-specific immunotherapy (AIT)).

Pharmacotherapy

Standard symptomatic treatment of allergic rhinitis or asthma includes systemic administered H1-antihistamines which block the action of histamine released by mast cells and basophils (Del Cuvillo et al., 2006). Corticosteroids can be used either systemically or locally to prevent the activation of inflammatory cells by suppressing the expressions of cytokines, chemokines and adhesion molecules (Barnes et al., 2003) and β-adenoreceptor agonists are used for smooth-muscle relaxation in asthmatic patients (Holgate et al., 2008). Furthermore, leukotriene modifiers or receptor antagonists are available (Grayson et al., 2003; Nathan, 2003). Several novel treatment modalities are used in research, clinical trials or already in the clinics and include inhibitors of T_h2 cell transcription factors like Gata-3 (Holgate et al., 2008; Bosnjak et al., 2011), monoclonal blocking antibodies for IL-4, IL-4-receptor, IL-13 and IL-5 (Bagnasco et al., 2016; Menzella et al., 2015) or the administration of typical T_h1 cell-inducing cytokines like IFNγ, IL-12 and IL-10 (Bosnjak et al., 2011). Monoclonal antibodies targeting IgE decrease free IgE and are able to treat severe cases of asthma (Bosnjak et al., 2011). Additionally, anti-IgE treatment has been shown to reduce side-effects during AIT in several case reports (Galera et al., 2009; da Silva et al., 2013; Palgan et al., 2014; Ricciardi, 2016). Severe anaphylactic reactions observed in venom and food allergic patients can only be treated by emergency medication like adrenaline/ephinephrine auto-injectors as well as anti-histamines and corticosteroids taken orally or administered intravenously by emergency doctors. Therefore and to improve quality of life in patients at risk of severe reactions, AIT and VIT is favoured.

Allergen-Specific Immunotherapy

Allergen-specific immunotherapy (AIT) exists in two different forms, either as subcutaneous immunotherapy (SCIT) or sublingual immunotherapy (SLIT). However, immunotherapy for venom allergy (VIT) is only administered subcutaneously. The therapy can be divided into two different phases, the up-dosing and maintenance phase. Immunotherapy is induced with the administration of low doses of allergen extract, which is increased until the maximum or maintenance dose is reached. In most cases, this maintenance dose is given in intervals of one to six weeks (depending on the formulation) for a total duration of three to five years (Burks et al., 2013). During the up-dosing, an increase and during maintenance a decrease of IgE has been observed, but this cannot be correlated to clinical symptoms (Akdis et al., 2011). It is thought, that during early stages of immunotherapy, the FcϵRI on mast cells and basophils is desensitized (Novak et al., 2012) and later on the fine balance of T_h1 and T_h2 allergen-specific T cells (Akdis et al., 2004) is shifted towards IL-10 producing T_{regs} (Francis et al., 2003; Radulovic et al., 2008; Wambre et al., 2012).

These cells are able to suppress T_h2 and T_h1 cells not only due to the release of IL-10 but also TGFβ, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1) (Akdis et al., 2004). Additionally, a reduced activation of mast cells, basophils, decreased histamine release, downregulation of IL5 and eosinophil function as well as a dampened inflammation has been observed during AIT (Akdis et al., 2015; Pierkes et al., 1999; Schandené et al., 1994). Furthermore, during AIT a switch from IgE to IgG_4 producing B cells with a regulatory phenotype, characterized by IL-10 and TGFβ secretion, has been observed (van de Veen et al., 2016). These regulatory B cells are able to suppress T cell proliferation (van de Veen et al., 2013) and induce additional T_{regs} (Kessel et al., 2012; Lee et al., 2014). IgG_4 is thought to be anti-inflammatory and able to block allergen induced allergic reactions (Möbs et al., 2012; Scadding et al., 2010; Wachholz et al., 2004; Flicker et al., 2003). AIT appears to be protective for up to 12 years, but the immune system probably needs to be continuously exposed to allergens (Meiler et al., 2008; Burks et al., 2013).

1.3 Allergy Diagnosis

Allergy diagnosis starts with a thorough anamnesis of the patient by the clinician, not only for the diagnosis but also to determine the relevant allergen source. Hereby, it is important to evaluate the time and symptoms of allergic reactions. Moreover, the frequency, duration, seasonality and effects upon the avoidance can give hints to the allergen source (Rusznak et al., 1998). If the inducing allergen or allergen source cannot be identified by the patients' history, different diagnostic methods are available. A complete overview of allergy diagnosis is given in the latest practice parameter update of Bernstein et al., 2008. Methods for the diagnosis can be divided into two groups, namely the *in vivo* provocation testing, including different kinds of skin tests and organ challenges and *in vitro* testing, including cellular tests as well as the measurement of sIgE levels in serum.

1.3.1 *In vivo* Allergy Diagnosis

In vivo provocation testing includes skin testing but also nasal, conjunctival, oral and bronchial provocation tests (Bernstein et al., 2008). For skin prick testing, commercially available allergen solutions are used, applied onto the cleaned skin and brought under the outer layer of the skin with a commercially available prick device. For more sensitive testing, diluted allergen solutions are injected into the skin (intracutaneous or intradermal skin testing). Besides being more sensitive, intracutaneous skin testing can lead to more false-positive test results and implies a higher risk for anaphylactic reactions. To interpret the results of skin testing, the size of the wheal and erythema is measured in *millimeter* and graded according to available diagnostic tables (Bernstein et al., 2008).
Organ challenge testing is only suggested, if all non-invasive allergy diagnostic tests turn out negative, despite convincing history of allergic reactions, to confirm allergy diagnosis

or to evaluate the efficacy of therapy (Agache et al., 2015; Bernstein et al., 2008). The site of organ challenge depends on the history of allergic reactions (Bernstein et al., 2008). *In vivo* allergy testing includes challenges of the conjunctiva, bronchi and nasal mucosa. Different formulation of allergens or extracts are applied in increasing concentrations to the selected organ (Abelson et al., 2003; Akerlund et al., 2005). Itching, tear volume, mucus production, hyperemia or erythema, edema, nasal and lung function tests serve as subjective and objective measurements of the allergic reaction (Friedlaender, 2004; Nathan et al., 2005; Agache et al., 2015). Additionally, it is also possible to measure inflammatory cells, mediators, cytokines and locally produced sIgE (Leonardi et al., 2000; Monteseirín et al., 2004; Ciprandi et al., 2005). Another, more physiologic organ challenge was introduced in 1987 as allergen exposure unit or challenge chambers (Horak et al., 1987) and is mostly used in academic settings or research facilities to asses the efficacy of anti-allergic treatments or basic research questions. Challenge chambers are additionally approved for phase II and phase III clinical trials (Agache et al., 2015). Provocation tests with suspected foods that induce allergic reactions are still gold standard for food allergy diagnosis (Agache et al., 2015). Challenges with drugs are only advised, if skin or in vitro tests cannot support the diagnosis and if there are no possible alternative drugs to substitute (Agache et al., 2015). Sting challenge tests with living insects are not used for the primary diagnosis of insect venom allergy because of the high risk and negative predictive value. Nevertheless, they are used to determine if VIT has been successful when the maintenance dose was tolerated repeatedly (Agache et al., 2015).

1.3.2 *In vitro* Diagnosis and Molecular Allergology

In the same year of the discovery of IgE and its importance in allergy (1967), the first measurement of IgE, called radio-allergosorbent test (RAST) was published (Wide et al., 1967). Since then, technical improvements including the World Health Organization (WHO) 75/502 international human IgE reference serum, made quantification of the results possible. Since then sIgE values are calculated as international kilo units per liter (kIU/L) (Bernstein et al., 2008). Direct (laboratory) or indirect (skin prick test) measurements of sIgE have to be evaluated with caution, as the simple presence of sIgE only demonstrates sensitization to the specific allergen (source) which does not necessarily have to be of clinical relevance (Tourlas et al., 2016).

Allergens can originate from various sources including, fungi, insect venoms, animal epithelia, foods, plant products like pollen and drugs. Most of the allergen sources consist of more than one single protein and in 1986 a sub-committee out of the International Union of Immunological Societies (IUIS) decided on an international nomenclature to name those allergens. To name an allergen, the abbreviation of its source (3-4 letters of the genus), followed by a space, the first letter of the species name and an Arabic numeral (Marsh et al., 1986). An example would be Der p 1, the first allergen described from the house dust mite *Dermatophagoides pteronyssinus*. At the beginning, new allergens of the same source were given consecutive numbers, but as newer findings categorized several allergens into

protein families that have similar sequences and structures, homologous allergens within a taxonomic order or family are assigned corresponding numbers (Radauer et al., 2014). In the past, single allergens were prepared from natural sources to be able to measure their sIgE reactivity. As this costly preparation was not feasible for routine measurements, only new technologies like recombinant protein production made it possible to use single allergens. Since then, the use of single allergen molecules for diagnosis was termed "component-resolved diagnosis (CRD)" (Valenta et al., 1999). With increasing numbers of identified allergens, several databases have emerged (Mari, 2005) and a recently published handbook, the "Molecular Allergology User's Guide" from the European Academy of Allergy and Clinical Immunology (EAACI), gives a profound overview of the state of the art techniques and allergens (molecules) used for component-resolved diagnosis (Matricardi et al., 2016b).
These developments made it possible for researchers and clinicians to gain an extended knowledge and important additional information about the sensitization profiles of patients by the use of molecular allergen testing next to sIgE measurements to allergen extracts. On the one hand, CRD profits from increased test sensitivity, in the case of under-represented or missing allergens in extract based tests. On the other hand, the measurement of sIgE to single allergens can give additional information concerning sensitization profiles of the patients, implying the potential to identify patients at risk for severe reactions, uncover cross-reactivity and possibility determine the primary sensitization (Kleine-Tebbe et al., 2015). Nevertheless, there can always be divergent test results when extract based and single allergen based IgE measurements are compared. This is probably due to the fact, that not all molecules present in the extracts are known or available for single allergen testing, important allergens are under-represented, cross-reactive, or lacking in the allergen extracts (Matricardi et al., 2016a). Due to these discrepancies and a 10 % higher cost of allergen molecule based sIgE measurements, both *in vitro* methods to determine sIgE will co-exist and have to be balanced in order to limit the resources of public health systems (Matricardi et al., 2016b). Examples of situations where CRD is superior to extract based sIgE diagnosis are given for insect venom allergy diagnosis in section 1.4.3.
Due to the availability of monoclonal antibodies against IgG subclasses (Lowe et al., 1982; Jefferis et al., 1992), specific immunoglobulin G (sIgG)-subtype serum levels to allergen extracts and molecular allergens can be measured in a similar manner (Movérare et al., 2017).

1.3.3 Cellular Tests

Cellular allergy tests reside somewhere between *in vitro* determination of sIgE and organ challenges and can therefore also be considered as *ex vivo* challenge tests. One important and easily accessible mediator of allergic inflammation are blood basophils (see section 1.2.2). In order to measure basophil activation, whole heparinized blood or washed leukocytes are incubated with allergens (Diamant et al., 1982). Hereby, stimulation with buffer

alone and an anti-FcϵRI or anti-IgE antibody serve as negative and positive controls (Hoffmann et al., 2015). The activation can be measured by the up-regulation of cell surface markers or the release of inflammatory mediators, like histamine and leukotrienes. In the case of food allergy diagnosis, basophil activation tests seem to be able to replace inconvenient oral food challenges, which can cause severe allergic reactions (Perry et al., 2004). Additionally, basophil activation testing can even be more accurate than skin testing and measurements of sIgE (Santos et al., 2014; Santos et al., 2015). For inhalant allergens, measurements of sIgE and skin testing are mostly sufficient (Khan et al., 2012) and basophil activation testing can only be advantageous, if there is no detectable sIgE and skin test results are negative (Gómez et al., 2013). Nevertheless, studies with crude allergen extracts and modified or recombinant allergens could show good correlations with nasal and bronchial provocation tests (Hoffmann et al., 2015). Moreover, some studies could show, that basophil activation tests can probably be used to monitor the efficacy of allergen-specific immunotherapy for birch- and grass-pollen allergy (Nopp et al., 2009; Ceuppens et al., 2009; Lalek et al., 2010; Schmid et al., 2014). Noteworthy, basophils of 6-17 % of patients studied do not respond to positive controls or allergen stimulation, most probably because of differences in intracellular signaling pathways (Hoffmann et al., 2015; Knol et al., 1992; Kepley et al., 1999).

The chemical detection method of histamine in various body-fluids was published in 1959 (Shore et al., 1959). Later, antibody based detection methods were developed and shown to be more sensitive, easier to use and automatable (Chevrier et al., 1986; Hammar et al., 1990). Additionally, histamine release of allergen-stimulated basophils was correlated to clinical symptoms, skin test results and sIgE levels in serum (Lichtenstein et al., 1964) and could be used as a novel tool to measure clinical relevant hypersensitivity. Equally, leukotriene C4, a mediator of inflammation can be used to determine the level of basophil activation (Moneret-Vautrin et al., 1999) and is measured in supernatants of stimulated basophils with the help of antibodies.

The membrane protein CD63, present in the same secretory lysosomal granule containing histamine, was identified in 1991. Upon basophil activation, CD63 is transported to the cell membrane and the amount of CD63 on the cell surface correlates well with histamine release (Knol et al., 1991). Up-regulated CD63 can be analyzed by flow cytometry (Hausmann et al., 2011; Eberlein et al., 2015) and has been shown to correspond to the result of IgE activated basophil degranulation (MacGlashan, 2010). CD63 has been clinically verified as basophil activation markers in several studies (Sainte-Laudy et al., 2000; Sturm et al., 2004; Erdmann et al., 2004; Eberlein-König et al., 2006b; Ocmant et al., 2007; Abuaf et al., 2008). For the interpretation of basophil activation tests, basophil reactivity and sensitivity are important parameters. Basophil reactivity indicates the number of basophils that respond to allergen stimulation, whereas basophil sensitivity correlates the allergen concentration to basophil activation (Hoffmann et al., 2015). Basophil sensitivity measurements require the measurement of several allergen concentrations and a plotted curve of reactivity versus allergen concentration. By this, the concentration where 50 % of basophils respond (EC50) can be determined. By inversion and multiplication by 100, the CD-sens value gives results easier to interpret. Higher CD-sens values, stand for higher

Order	Hymenoptera				
	Apocrita				
	Aculeata				
Superfamily	Apoidea		Vespoidea		
Family	Apidae		Vespidae		Formicidae
	Apinae	Bombinae	Vespinae	Polistinae	Myrmicinae
	Apini	Bombini		Polistini	Solenopsidini
Genus	Apis	Bombus	Vespula	Polistes	Solenopsis
Species	*Apis mellifera*	*Bombus terrestris*	*Vespula vulgaris*	*Polistes dominula*	*Solenopsis invicta*

Figure 5 Taxonomy of Allergy-Relevant Species of the Order of Hymenoptera (Sayers et al., 2009). "Apis mellifera" by Carnat Joel, "Buff-tailed bumblebee" by Jonas Myrenås, "Common Wasp (Vespula (Paravespula) vulgaris)" by Martin Cooper, licensed under CC BY 2.0. "Polistes dominula" by Ferran Turmo Gort, licensed under CC BY-NC-SA 2.0. "Red Imported Fire Ant (Solenopsis invicta)" by stevenw12339, licensed under CC BY-NC 2.0. All pictures were cropped and rearranged to create this overview.

basophil sensitivity to the allergen tested (Johansson et al., 2005). A different way of interpretation is the generation of allergen dose-response curves or the area under dose curve (AUC) (Patil et al., 2012). Basophil activation testing is elaborative and costly, thus not used in clinical routine diagnosis. Nevertheless, basophil activation tests can increase test sensitivity in difficult to diagnose patients and safety in patients where challenge tests cannot be avoided otherwise (Hoffmann et al., 2015).

1.4 Insect Venom Allergy

A prominent example of a type I hypersensitivity reaction is the adverse reaction to insect stings. Stings from insects out of the order of Hymenoptera can lead to severe and in the worst case fatal systemic reactions in allergic patients. The only curative treatment is venom immunotherapy, but accurate diagnosis including the identification of the insect causing the allergic reaction has to be unequivocal. Therefore, clinicians rely on various diagnostic options besides standard skin testing with venom extracts. Serum and cellular tests have improved in the last years, as the composition of different venoms has been investigated in detail and several allergens, which can be of immense help for diagnosis, became available in pure form by recombinant techniques.

An overview of allergy-relevant species of the order of Hymenoptera, including their taxonomy, is given in Figure 5. In the US, wasp species like *Vespula vulgaris* (yellow jacket), paper wasp (*Polistes*) species like *Polistes annularis*, honeybees (*Apis mellifera*) (Golden et al., 2017) and africanized honeybees (*Apis mellifera scutellata*) (Schumacher et al., 1992; Moffitt, 2003) as well as imported fire ants (*Solenopsis invicta* and *Solenopsis rich-*

teri) (Freeman et al., 1992; Kemp et al., 2000; Steigelman et al., 2013) are important elucidators of allergic reactions. In contrast, allergy-relevant Hymenoptera in Europa are *Apis mellifera* and wasp species like *Vespula vulgaris* (yellow jacket) (Worm et al., 2014; Sturm et al., 2017). Paper wasps, like *Polistes dominula* are more relevant in the Mediterranean area (Biló et al., 2005). The invasive species *Polistes dominula*, historically known to be domestic in south Europe (Höcherl et al., 2015), started to populate the US from the north-east to the west coast in the 1970s and 1990s, respectively (Cervo et al., 2000) and South Africa since 2008 (Eardley et al., 2009). Furthermore, as a consequence of global warming, it is very likely that *Polistes dominula* will continue to spread into previously colder climate zones and *Polistes dominula* venom allergy could also become relevant in northern areas of Europe. Another example of venom allergy spreading due to climatic changes are reports of allergic sting reactions to yellow jacket venom in Alaska (Freeman, 2008; Demain et al., 2009).

In addition, non aggressive bumblebees (*Bombus terrestris*) is an important trigger of occupational allergic reactions, as these insects are used for pollination in green houses in Spain and the Netherlands (Cruz et al., 2012; de Groot, 2006; Van Vaerenbergh et al., 2015b). An overview of ant species of the superfamily *Formicidae* relevant for allergic sting reactions is given in a review by Potiwat et al., 2015. In summary, the frequency of sting reactions and the responsible Hymenoptera species depend on geographic, environmental and ecological factors (Golden et al., 2017).

1.4.1 Clinical Manifestation and Therapy of Insect Venom Allergy

In contrast to pollen, that have to pass mucosal barriers, the allergens of insect venoms are injected into the skin and reach the blood as well as cellular and protein components of the immune system fast and easy. The normal response to stings of Hymenoptera includes transient pain, swelling and itching at the site of the sting and are caused by irritative and toxic venom components (Golden, 2015). Large local reactions (LLRs), edema, erythema and pruritus with a diameter bigger than 10 cm are part of slight hypersensitivity reactions that peak at day one or two and resolve three to ten days later. LLRs are cell mediated (Severino et al., 2009) and/or IgE-dependent (Wright et al., 1990). Earlier work showed, that LLRs are independent of IgE (Mauriello et al., 1984), but this could also be due to higher detection limits at that time. As LLRs are part of mild allergic reactions to venoms and the chance of severe future sting reactions is low (Mauriello et al., 1984; Schuberth et al., 1982; Graft et al., 1984; Abrecht et al., 1980; Fernandez et al., 2005; Pucci et al., 2015), there is no recommendation for VIT for those patients (Valentine et al., 1990; Golden et al., 2017). Severe hypersensitivity reactions to insect venom include systemic or generalized reactions (SR) also termed anaphylaxis. The clinical features of anaphylaxis were discussed in Section 1.2.2. Systemic reactions usually start ten to 30 minutes after the causative sting. However, in case of an underlying disease like mast cell disorders, they can even arise faster. Sometimes the onset of systemic reactions is slower (one to four hours after the sting) and normally less life threatening in this case (Golden,

2015). In the event of a severe systemic reaction, the median time until cardiorespiratory arrest and subsequent death occurs is fifteen minutes after the sting (Pumphrey, 2000).
The risk factors for severe hypersensitivity reactions include older age, male sex, medication of hypertension, diagnosed vespid allergy and preceding stings with systemic reactions (Ruëff et al., 2009). Another risk factor is mastocytosis, a mast cell activation disorder caused by the presence of too many mast cells, or elevated serum tryptase concentration independent of mast cell disorders (Haeberli et al., 2003). However, if the serum tryptase concentration is above a certain threshold (191 $\mu g/L$, or below 6.1 $\mu g/L$) the risk of severe reactions declines (Niedoszytko et al., 2014).
The risk to re-experience a systemic reaction after a subsequent sting without therapy is 56 % (Reisman, 1992) and the only available pharmacotherapy of systemic insect venom hypersensitivity is the prescription of adrenaline or epinephrine auto-injectors for emergency use as well as orally administered anti-histamines and corticosteroids. The only currently available curative therapy is venom immunotherapy (VIT).

Venom Immunotherapy

The first VIT was reported in 1925 by Braun, who administered a bee abdomen extract to a patient (Braun, 1925). Later on, clinicians realized that venom treatment in place of the usual whole body extract increases VIT efficiacy (Fackler et al., 1956). However, this increased efficacy was published almost 20 years later and only in case report studies (Lichtenstein et al., 1974; Busse et al., 1975). Only few years after these case reports, the first randomized controlled study in 1978 showed the efficacy of VIT, if whole venom extracts were used for therapy (Hunt et al., 1978). Today, venom extracts are available as aqueous (or lyophilized) extracts and depot preparations adsorbed to aluminium hydroxide from different manufactures. All therapeutic extracts are purified by standard procedures. Nevertheless, it has been shown, that different products might differ in composition or allergen activity (Blank et al., 2011; Frick et al., 2016). Venom immunotherapy guidelines published by the American Academy of Allergy, Asthma and Clinical Immunology (AAAAI) and the EAACI only suggest the treatment of patients with a history of systemic reactions when the hypersensitivity reaction is diagnosed by skin test, sIgE (Golden et al., 2017) and/or basophil activation test (Sturm et al., 2017). Both guidelines do not recommend the treatment of patients that only experience LLRs.
The general treatment protocols include an up-dosing phase with increasing amounts of venom, until a maintenance phase is reached, which is continued for three to five years (Golden et al., 2017; Sturm et al., 2017). Injections are given subcutaneously, on the extensor side of the upper arm, above the olecranon (Pfaar et al., 2014). For the up-dosing, clinicians can choose between the conventional protocol (8-16 weeks), rush protocols with durations of two to three days, ultra-rush protocols taking four to eight hours or clustered/modified rush protocols with two to three injections every 30 minutes every three to seven days (Golden et al., 2017; Sturm et al., 2002; Ludman et al., 2015). All protocols except the ultra-rush protocols have proven to be safe and tolerable by the majority of patients (Brown et al., 2012). When the maintenance dose of 100 μg venom extract

(corresponding to the dry weight of two honeybee or five wasp stings (Schumacher et al., 1994; Hoffman et al., 1984)) is reached, protection is already achieved (Goldberg et al., 2010; Goldberg et al., 2011). Nevertheless, to gain full protection, the treatment has to be continued for further three to five years (Golden et al., 2017; Sturm et al., 2017) but intervals can safely be extended from monthly injections to injections every six to eight weeks (Cavallucci et al., 2010). In some patients, for example patients with additional mastocytosis, treatment is recommended to be continued life-long (Przybilla et al., 2011). Severe side-effects of VIT are experienced by 14.2 % and 2.8 % of patients treated with *Apis mellifera* and *Vespula vulgaris* venom, respectively (Boyle et al., 2012). LLRs are experienced by 11.5-15.2 % of patients undergoing VIT (Mauriello et al., 1984). If severe reactions are observed during the initiation of VIT a change of the up-dosing protocol (Goldberg et al., 2003) or use of omalizumab as discussed earlier is recommended. The effectiveness in preventing future anaphylactic sting reactions is about 75-98 % (Golden, 2007) and the efficacy of VIT was shown to be higher in *Vespula vulgaris* than *Apis mellifera* venom allergy (Müller et al., 1992). The analysis of seven controlled clinical studies showed a reduction of systemic reactions in response to future stings and an improved quality of live in two non-blinded studies (Boyle et al., 2012). In the case of treatment failures, it has been shown that an increase of the maintenance dose can be benificial (Przybilla et al., 2011). In order to avoid *de novo* sensitizations by treating patients with the wrong therapeutic extract (Juarez et al., 1992), diagnosis of insect venom allergy has to be as precise as possible.

1.4.2 Epidemiology

There is a vast number of epidemiological studies concerning the prevalence of insect venom hypersensitivity in the general population and in children. The prevalence of systemic reactions due to any kind of insect sting in children has been reported to be 0.34 % (Novembre et al., 1998), 0.8 % (Jennings et al., 2010) or up to 2.5 % (Graif et al., 2006). The prevalence of mild systemic reactions in children ranges from 3.4 % (Jennings et al., 2010) to 6.5 % (Graif et al., 2006) and LLRs occur in 5.8 % (Jennings et al., 2010) or 11.5 % (Graif et al., 2006) of the studied children population. Data from the European Anaphylaxis Registry showed that 19 to 20.2 % of anaphylactic reactions occur as a result of insect stings in children (Grabenhenrich et al., 2016; Worm et al., 2014).
In the general adult population, the prevalence of reported systemic reactions is 1.2 % (Onbaşı et al., 2008), 2.3 % (Fernandez et al., 1999; Navarro et al., 2004), 2.8 % (Fernandez et al., 2005), 3.3 % (Bokanovic et al., 2011) or even up to 8.9 % (Nittner-Marszalska et al., 2004). LLRs occur in 4.6 to 31.5 % of adults in the studied populations (Fernandez et al., 1999; Navarro et al., 2004; Nittner-Marszalska et al., 2004; Fernandez et al., 2005; Onbaşı et al., 2008; Bokanovic et al., 2011). The risk of developing a severe systemic reaction in children and adults who experienced LLRs is only 5 and 7 %, respectively (Graft et al., 1984; Golden et al., 2004). A study of the general population showed, that only 2.8 % were allergic to insect venoms, whereas the prevalence of food allergy (4.7 %), asthma

(8.6 %) and hay fever (14.8 %) was higher (Bergmann et al., 2016). The evaluation of the European Network of Severe Allergic Reactions showed that 48.2 % of anaphylactic reactions in adults occurred after insect stings. Of those, 70.6 % were caused by yellow jacket, 23.4 % by honeybee and 4.1 % by hornet stings (Worm et al., 2014). To estimate the per year incidences of anaphylaxis due to insect stings, emergency department visits were evaluated in different countries and ranged from 1.5 to 8.7 per 100 000 inhabitants (Diniz et al., 2016; Beyer et al., 2012; Harduar-Morano et al., 2011). The incidence of fatal reactions range from 0.03 to 0.48 deaths per 1 000 000 inhabitants per year (Bilò et al., 2008) but the estimated deaths due to insect stings might be underestimated, as post-mortem serum samples contained venom-specific IgE in many cases (Schwartz et al., 1988).
The sensitization, meaning the mere presence of sIgE to at least one common allergen, is found in the serum of 48.6 % of adults and the sensitization to insect venom components is existent in 22.6 % (Haftenberger et al., 2013). Nevertheless, it has also been shown, that there is no difference in the frequency of insect venom allergy in the population with or without predisposition of hypersensitivity reactions to other allergen sources (Pastorello et al., 1988; Settipane et al., 1972).

1.4.3 Venom Allergens and the Diagnosis of Hymenoptera Venom Allergy

The AAAAI and EAACI regularly publish updated guidelines for the diagnosis and therapy of Hymenoptera venom allergy. The first guideline from the AAAAI was published in 1999 and the last update was released in 2017 (Portnoy et al., 1999; Moffitt et al., 2004; Golden et al., 2011; Golden et al., 2017). Guidelines from the EAACI were published as early as 1987 and the recent updated version appeared online in 2017 (Bousquet et al., 1987; Müller et al., 1993; Biló et al., 2005; Sturm et al., 2017). The evaluation of the clinical history includes previous sting reactions, their time course, severity, as well as symptoms and treatment, together with the assessment of individual risk factors. The severity of sting reactions is often under- or overestimated, as fear, panic, exercise, heat, alcohol, underlying cardiorespiratory disease or stings that happened long time ago can affect the experience or memory of venom stings (Golden, 2015). It is additionally beneficial, if the insect causing the reaction can be identified, but this is difficult for most of the patients and clinicians, as there are many insects with similar phenotype (Baker et al., 2014; Baker et al., 2016). In conclusion, if the sting reaction has been severe as well as under special circumstances like frequent exposure, further tests are needed to prove the sensitization to the insect venom that caused the allergic reaction (Golden et al., 2017; Sturm et al., 2017). These tests include skin tests, the determination of sIgE in serum and/or basophil activation tests.
The gold standard and starting point of insect venom allergy diagnosis is skin testing. Commercially available venom extracts are used for skin prick testing and for higher sensitivity, dilutions of skin prick test solutions are used for intradermal skin testing. Skin testing is a safe procedure (Bilò et al., 2011) and systemic reactions are rare. Venom ex-

tracts are commercially available, depending on geographical regions, for *Apis mellifera*, *Vespula species (spp.)*, *Polistes spp.* and *Dolichuvespula spp.* and are highly purified by dialysis in Europe but not in the USA. Test extracts for *Apis mellifera* are standardized according to the content of phospholipases A2 and extracts for *Vespula vulgaris* to the content of hyaluronidases (Bernstein et al., 2008). For the diagnosis of imported fire ant venom allergy, commercially available extracts are composed of whole-body extracts even though studies showed a higher skin test sensitivity with venom preparations (Strom et al., 1983; Butcher et al., 1988). Ideally, skin testing is performed again after six weeks, if the first skin test results are negative shortly after the sting reaction (Goldberg et al., 1997; Ludman et al., 2015). Of note, 10 to 30 % of patients with a history of systemic reactions and positive sting challenges show negative skin test results (Kontou-Fili, 2002) and several other studies showed test variability due to differences in commercially available venom extracts (Jeep et al., 1992). On the other hand, 15 % of a population without having a history of sting reactions were tested positive in skin tests with venom extracts (Golden et al., 1997). Thus, in the case of negative skin test results and a history of an allergic sting reaction, determination of venom-sIgE is recommended (Golden et al., 2017; Sturm et al., 2017), even though test sensitivity is a little lower than intradermal skin testing (Ludman et al., 2015). Before the late 1970s, whole insect body extracts (Müller, 2002) were used to determine sIgE in the serum of patients. Later, it has been shown, that the sensitivity can be increased by using venom extracts and even more by using natural or recombinant single allergens (Vachová et al., 2016). Additionally, single recombinant allergens are also used to spike venom extracts used for *in vitro* diagnosis (Vos et al., 2013). Nevertheless, the assertion of *in vitro* sIgE diagnosis is limited, since other studies showed sensitization to venom allergens in 15, 16.4 or even 22.6 % of the studied individuals (Fernandez et al., 1999; Haftenberger et al., 2013; Mosbech et al., 2016). However, only 31 % (Mosbech et al., 2016) or 5.3 % (Sturm et al., 2014) of sensitized individuals examined in the study, have had previous sting reactions. On the other hand, sIgE could only be detected in 38 or 57.6 % of patients with a history of an allergic sting reaction (Fernandez et al., 1999; Mosbech et al., 2016). Nontheless, the increasing knowledge of venom components and the use of component-resolved diagnosis continuously improves *in vitro* diagnostic sensitivity and specificity (Blank et al., 2011; Köhler et al., 2014).

Another limitation of *in vitro* insect venom diagnosis is the possibility of double-positive sIgE results with *Apis mellifera* and *Vespula vulgaris* venom. In addition to true allergy to both venoms and cross-reactivity of homologous allergens, it has been shown, that this can be the result of sIgE directed against the insect specific glycan structure (Hemmer et al., 2001). When the sIgE against the glycan structure on venom proteins (glycosylation) was identified, this glycosylation was also termed cross-reactive carbohydrate determinant (CCD). Even though it has been shown, that sIgEs to CCDs are highly affine (Jin et al., 2008), they do not seem to have any clinical relevance (Altmann, 2016). In fact, up to 75 % of double-positve *in vitro* test results to *Apis mellifera* and *Vespula vulgaris* venom are caused by IgE against CCDs (Jappe et al., 2006). To prove the presence of CCD-reactive

IgE antibodies, sIgE to CCD markers like MUXF3, horseradish peroxidase, bromelain or ascorbate oxidase can be measured (Jappe et al., 2006). Nevertheless, this does not exclude clinically relevant sensitization to both venoms. However, those patients can profit from the test specificity of component-resolved diagnostic approaches. Here, venom allergens are produced by recombinant techniques either in *Escherichia coli* (*E. coli*) without glycosylation or *Spodoptera frugiperda* (Sf9) insect cells with glycosylation that lacks CCD-reactivity (Seismann et al., 2010a). The absence of CCDs was also observed in venom preparations of a mix of analyzed *Polistes* species (Blank et al., 2013a).

Insect Venom Allergens

As the knowledge of the insect venom composition is crucial for the improvements of insect venom allergy diagnosis, there has been a vast amount of research analyzing the protein components of different venoms in the last years. The dry weight of *Apis mellifera* venom injected in one sting is around 140 μg (Schumacher et al., 1994) and the protein content was shown to be 59 μg (Hoffman et al., 1984; Schumacher et al., 1994), whereas the amount of protein emitted by a sting of *Vespula* and *Polistes* species is only 1.7 to 3.1 and 17 μg, respectively (Schumacher et al., 1994). In addition to various, mostly enzymatic active proteins, venoms consist of low molecular substances, for example biogenic amines like histamine, (basic) peptides and toxins (Habermann, 1972). The best characterized venom up to date is the venom of *Apis mellifera*. With the help of genomic data (Weinstock et al., 2006) and intensive mass spectrometry analyses (Peiren et al., 2005; Van Vaerenbergh et al., 2014) various proteins have been identified. Even seasonal changes in venom components have been discovered (Van Vaerenbergh et al., 2013). Recently, the genomic data of *Polistes dominula* was published (Standage et al., 2016) but proteomic data of venom proteins are still missing. Additional genomic data of further important members of the superfamiliy of *Vespoidea* is available for *Polybia paulista* (dos Santos et al., 2010), *Solenopsis invicta* (Wurm et al., 2011) and *Polistes canadensis* (Patalano et al., 2015). Also very recently, the proteome of *Bombus terrestris* has been unraveled with the help of available genomic data (Sadd et al., 2015; Van Vaerenbergh et al., 2015b). An overview of identified proteins with proven IgE-reactivity is given in Tables 1 and 2.

Table 1 Overview of Hymenoptera venom allergens currently listed in the WHO/IUIS Allergen Database (www.allergen.com) of species which have not been made available for routine diagnosis. MW, molecular weight in kDa determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) separation. Adapted from Ollert et al., 2015.

Allergen	Name/Function	MW [kDa]
Bumblebee (*Bombus pennsylvanicus*, *B. terrestris*^{t})		
Bom p 1^{t}	Phospholipase A2	16
Bom p 4^{t}	Protease	27

Table 1 (Continued) Overview of Hymenoptera venom allergens currently listed in the WHO/IUIS Allergen Database.

Allergen	Name/Function	MW [kDa]
White-faced hornet, yellow hornet (*Dolichovespula maculata, D. arenaria*[a])		
Dol m 1	Phospholipase A1	34
Dol m 2	Hyaluronidase	42
Dol m 5[a]	Antigen 5	23
Australian jumper ant (*Myrmecia pilosula*)		
Myr p 1		7.5/5.5
Myr p 2	Pilosulin-3	8.5/2.4
Myr p 2	Pilosulin-4.1	
American paper wasps (*Polistes annularis, P. exclamans*[e], *P. fuscatus*[f], *P. metricus*[m])		
Pol a 1[e]	Phospholipase A1	34
Pol a 2	Hyaluronidase	38
Pol e 4	Protease	33
Pol a 5[e,f,m]	Antigen 5	23
Polybia wasp (*Polybia paulista, P. scutellaris*[s])		
Poly p 1	Phospholipase A1	34
Poly p 2	Hyaluronidase	33
Poly p 5[s]	Antigen 5	23
Fire ants (*Solenopsis invicta, S. geminata*[g], *S. richteri*[r], *S. saevissima*[s])		
Sol i 1	Phospholipase A1	18
Sol i 2[g,r,s]		14
Sol i 3[g,r,s]	Antigen 5	26
Sol i 4[g]		12
Hornets (*Vespa crabro, V. magnifica*[ma], *V. mandarinia*[m])		
Vesp c 1[m]	Phospholipase A1	34
Vesp ma 2	Hyaluronidase	35
Vesp c 5[ma,m]	Antigen 5	23

An important group of allergens in the venoms of the superfamily of *Vespoidea* and *Apoidea* are proteins with phospholipase activity. Phospholipases in the venom of species out of

the superfamily of *Vespoidea* are phospholipases A1, whereas phospholipase A2 activity is only documented in the venoms of *Apis mellifera*, *Bombus pennsylvanicus* and *B. terrestris* (superfamily of *Apoidea*) (Habermann, 1972; Hoffman et al., 1996; Hoffman et al., 2001). The amino acid sequence of phospholipases A2 (Api m 1 and Bom p 1) were identified (Kuchler et al., 1989; Hoffman et al., 1996) and show 53.7 % sequence identity.
Proteins with phospholipase A1 activity have been identified in the venom of *Polistes annularis* (GenBank AF174527, GenBank Identifiers are given in the case of otherwise unpublished results), *P. dominula* (GenBank AY566645), *P. exclamans* (Hoffman, 1985b), *P. gallicus* (Pantera et al., 2003), *Dolichovespula maculata* (Soldatova et al., 1993; Hoffman, 1994), *Polybia paulista* (GenBank EF101736), *Solenopsis invicta*, *Vespa mandarinia*, *Vespula squamosa* (Hoffman et al., 2005), *Vespa crabro* (GenPept P0CH87), *Vespula maculifrons* (Hoffman, 1994) and *V. vulgaris* (King et al., 1996).
Hyaluronidases comprise another large group of allergens in the venom of stinging insects and were identified in the venom of *Dolichovespula maculata* (Lu et al., 1995), *Polistes annularis* (GenBank AF174528), *Vespa magnifica* (An et al., 2012), *Vespula maculifrons* (GenPept P0CH89), *Polybia paulista* (dos Santos et al., 2010) and *Apis mellifera* (Gmachl et al., 1993). Two hyaluronidase isoforms are present in the venom of *Vespula vulgaris* (King et al., 1996; Kolarich et al., 2005).
Melittin, a lytic peptide, represents 50 % of the honeybee venom dry weight (Habermann, 1972) but homologous peptides have not yet been shown to have sIgE-reactivity in the venoms of other species. Similarly, proteins in insect venoms with acid phosphatase activity were only identified in *Apis mellifera* venom (Hoffman, 1977; Hoffman et al., 1977).

A protein named antigen 5, first found in the venom of *Vespula maculata*, *V. arenaria*, *V. vulgaris*, *V. maculifrons* and *V. germanica* (King et al., 1978; Hoffman, 1993b) has no known biological function, but was speculated to be a presynaptic toxin, because of its similarity to a neurotoxin from the hornet *Vespa mandarinia* (Abe et al., 1982). Furthermore, antigens 5 were found in the venom of *Dolichovespula maculata*, *Vespula squamosa*, *Polistes exclamans* (Hoffman, 1985a), *D. arenaria*, *P. annularis* (Lu et al., 1993), *Solenopsis invicta* (Hoffman, 1993a), *P. gallicus* (Pantera et al., 2003) and *Vespa magnifica* (An et al., 2012). Until recently, antigens 5 were only found in species of the superfamily *Vespoidea*, but a homologous protein was found in the venom of *Apis mellifera* which is only expressed in winter, thus it seems to have no relevance for honeybee venom allergy (Van Vaerenbergh et al., 2013).
Other identified allergens include the antigen C, which was already identified as *Apis mellifera* allergen by Hoffman et al., 1977 and has later been described as dipeptidyl peptidase IV not only in the venom of *Apis mellifera* but also of *Vespula vulgaris* (Blank et al., 2010). A protease inhibitor (Kettner et al., 2001), a carboxyesterase and carboxypeptidase, both unpublished (GenBank EU564833 and FJ765738) and two major royal jelly proteins (Blank et al., 2012) were only identified in the venom of *Apis mellifera.* A protease with CUB domain was identified in the venom of *Apis mellifera*, *Polistes dominula*

(Winningham et al., 2004) as well as *Bombus pennsylvanicus* and *B. terrestris* (Hoffman et al., 1996; Hoffman et al., 2001). Shared between the venoms of *Apis mellifera* and *Vespula vulgaris* are the vitellogenins (Blank et al., 2013a) and the carbohydrate-rich protein, also called icarapin because of its instability, was only identified in the venom of *Apis mellifera* (Peiren et al., 2006). Later on, nine different isoforms of this carbohydrate rich protein were identified in a more detailed proteome analysis (Van Vaerenbergh et al., 2015a).

Table 2 Overview of Hymenoptera venom allergens currently listed in the WHO/IUIS Allergen Database (www.allergen.com) of species, where single allergens are available for routine diagnosis. Allergens available for routine diagnosis are set bold. CRP, carbohydrate-rich protein; DPP IV, dipeptidyl peptidase IV; MRJP, major royal jelly protein; MW, molecular weight in kDa determined by SDS-PAGE separation; % Sensitization, percentage of allergic patients sensitized to the single allergen. Adapted from Ollert et al., 2015.

Allergen	Name/Function	MW [kDa]	% Sensitization
Honeybees (*Apis mellifera*, *Apis cerana*[c], *Apis dorsata*[d])			
Api m 1[c,d]	Phospholipase A2	16	72.2 (Köhler et al., 2014)
Api m 2	Hyaluronidase	39	47.9 (Köhler et al., 2014)
Api m 3	Acid phosphatase	43	50.0 (Köhler et al., 2014)
Api m 4	Melittin	3	22.9 (Köhler et al., 2014)
Api m 5	DPP IV	100	58.3 (Köhler et al., 2014)
Api m 6	Protease inhibitor	6	26 (Michel et al., 2012)
Api m 7	Protease	39	
Api m 8	Carboxylesterase	70	46 (*unpublished*)
Api m 9	Carboxypeptidase	60	
Api m 10	CRP/Icarapin	50-55	61.8 (Köhler et al., 2014)
Api m 11.0101	MRJP 8	65	15 (Blank et al., 2012)
Api m 11.0201	MRJP 9	50	34 (Blank et al., 2012)
Api m 12	Vitellogenin	200	44 (Blank et al., 2013b)
European paper wasps (*Polistes dominula*, *P. gallicus*[g])			
Pol d 1[g]	Phospholipase A1	34	
Pol d 4	Protease	33	
Pol d 5[g]	Antigen 5	23	
Yellow jackets (*Vespula vulgaris*, *V. flavopilosa*[f], *V. germanica*[g], *V. maculifrons*[m], *V. pensylvanica*[p], *V. squamosa*[s], *V. vidua*[vi])			
Ves v 1[m,s]	Phospholipase A1	34	55 (Binder et al., 2002)
Ves v 2.0101[m]	Hyaluronidase	38	74 (Binder et al., 2002)
Ves v 2.0201	Hyaluronidase (inactive)	38	74 (Binder et al., 2002)
Ves v 3	DPP IV	100	57 (Blank et al., 2010)
Ves v 5[f,g,m,p,s,vi]	Antigen 5	23	84 (Binder et al., 2002)
Ves v 6	Vitellogenin	200	39 (Blank et al., 2013b)

The Role of Insect Venom Allergens for Diagnosis

Apis mellifera phospholipase A2 (Api m 1) was one of the first allergen produced by recombinant expression techniques (Dudler et al., 1992) and used for routine allergy diagnosis (Müller et al., 1995a; Müller et al., 1997). Soon, it was recognized that recombinant allergens like Api m 1 and *Vespula vulgaris* antigen 5 (Ves v 5) can increase *in vitro* test specificity and help in the diagnosis of possibly double sensitized patients with reactivity to the venoms of *Apis mellifera* and *Vespula vulgaris* (Müller et al., 2009; Mittermann et al., 2010; Hofmann et al., 2011b). However, a controversial discussion started, whether recombinant or native Api m 1 has the better test specificity (Sturm et al., 2011; Korošec et al., 2011), concluding with same reactivity of both proteins in the selected patient populations (Jakob et al., 2012). At that time CCD-reactivity was only a little concern, as recombinant allergens were produced in insect cells which have been proven to produce glycosylated proteins without CCD-reactive structures (Seismann et al., 2010a). Nevertheless, Hofmann et al. raised the idea, that even more recombinant allergens are needed for a correct discrimination of *Apis mellifera* and *Vespula vulgaris* venom allergy (Hofmann et al., 2011a). In a subsequent study, where the *Apis mellifera* venom allergens recombinant Api m 1, recombinant hyaluronidase (Api m 2), recombinant acid phosphatase (Api m 3), recombinant dipeptitdyl peptidase IV (Api m 5) and recombinant icarapin (Api m 10) together with synthetic melittin (Api m 4) were used, sIgE to one or more allergens could be detected in 94.4 % *Apis mellifera* venom allergic patients (Köhler et al., 2014).
Vespula vulgaris and *Apis mellifera* hyaluronidases (Ves v 2 and Api m 2) were discussed in a similar manner concerning their relevance for insect venom allergy diagnosis. After identification and recombinant expression of Api m 2 (Gmachl et al., 1993) and Ves v 2 (King et al., 1996), studies have shown that *Vespula vulgaris* venom contains an additional inactive hyaluronidase isoform (Kolarich et al., 2005) which is even more frequent (Kolarich et al., 2007). Studies of the native *Vespula vulgaris* venom hyaluronidase as well as the recombinant inactive hyaluronidase isoform showed that only very few (10-15 %) allergic patients show sIgE-reactivity to these hyaluronidases (Jin et al., 2010; Seismann et al., 2010a). Additionally, structural studies of *Apis mellifera* and *Vespula vulgaris* hyaluronidases showed differences in topology and electrostatic surface potentials, suggesting only little sIgE cross-reactivity in the serum of *Apis mellifera* and *Vespula vulgaris* venom allergic patients (Hemmer, 2008; Skov et al., 2006).
Apis mellifera venom acid phosphatase was identified, cloned and expressed in insect cells by Grunwald et al., 2006 and identified as major allergen, with sIgE-reactivity in 50 % of allergic patients (Köhler et al., 2014). sIgE to the carbohydrate-rich *Apis mellifera* venom protein (icarapin, Api m 10) was shown to be present in approximately 50-70 % patients (Blank et al., 2011; Köhler et al., 2014; Frick et al., 2016), confirming its role as a major allergen. Additionally, Api m 10 was demonstrated to be underrepresented in some *Apis mellifera* venom extracts used for VIT (Blank et al., 2011) and that therapeutic treatment failures can be correlated with a predominant sensitization to Api m 10 (Frick et al., 2016).
Even though melittin (Api m 4) is the major component in the venom of *Apis mellifera* (Habermann, 1972; Paull et al., 1977), it is an allergen of minor clinical relevance and sIgE is only detected in the serum of 22.9 % of allergic patients (Köhler et al., 2014).

Similarly, the *Apis mellifera* protease inhibitor (Api m 6) and protease (Api m 7) were identified as allergens of minor clinical relevance (Kettner et al., 2001; Michel et al., 2012; Winningham et al., 2004). Only $< 50\,\%$ of allergic patients react to the *Apis mellifera* venom carboxyesterase (Api m 8) and carboxypeptidase (Api m 9), but these results were only submitted to the WHO/IUIS allergen database. Furthermore, the major royal jelly proteins 8 and 9 (Api m 11) also represent minor allergens (Blank et al., 2012). Vitellogenins were identified in the venom of *Apis mellifera* and *Vespula vulgaris* (Api m 12 and Ves v 6) and are of minor clinical relevance but show cross-reactivity in *Apis mellifera* and *Vespula vulgaris* venom allergic patients (Blank et al., 2013a).

Molecular allergology also improved the diagnosis of *Vespula vulgaris* venom allergic patients and the discrimination of patients sensitized to the venom of *Vespula vulgaris* and *Apis mellifera*. Following the identification and recombinant production of *Vespula vulgaris* venom phospholipase A1 (Ves v 1) (King et al., 1996) a different study found that the basophils of *Vespula vulgaris* venom allergic patients could be stimulated by natural Ves v 1 in 50 % of the cases (Binder et al., 2002). Measuring sIgE-reactivity against recombinant *Vespula vulgaris* antigen 5 (Ves v 5) (Lu et al., 1993; Monsalve et al., 1999) together with Ves v 1 increased test sensitivity to over 90 % in *Vespula vulgaris* venom allergic patients double-sensitized to the venoms of *Apis mellifera* and *Vespula vulgaris* (Seismann et al., 2010b). This was also confirmed by an additional study (Müller et al., 2012) but it has also been shown, that measuring IgE-reactivity against Ves v 1 and Ves v 5 only, still misses up to 8 % of a tested patient population (Korošec et al., 2012). Furthermore, the addition of recombinant Ves v 5 to *Vespula vulgaris* venom extract is able to increase sensitivity of *in vitro* extract based diagnosis 96.8 % (Vos et al., 2013).
As described before, antigens 5 were found in various species out of the family of *Vespoidea* and their cross-reactivity is known since more than 30 years (Hoffman, 1985c). Partially known antigen 5 sequences showed sequence similarity and studies with polyclonal mouse antibodies confirmed this cross-reactivity of antigens 5 from similar *Vespoidea* species (King et al., 1987). Complete knowledge of antigen 5 amino acid sequences for several *Vespula* and one *Vespa* species showed high sequence similarity, the most probable cause for immunologic cross-reactivity (Hoffman, 1993b). The *in vitro* observed cross-reactivity with *Polistes dominula* and *Vespula vulgaris* venom extracts (Caruso et al., 2007) is of particular interest, as these insects live alongside in the south of Europe and allergy to *Polistes dominula* venom is frequent in these regions (Severino et al., 2006). Nevertheless, it has been shown that venom extract cross-inhibition experiments could correctly identify 60 % of tested patients (Caruso et al., 2007). In accordance to that, measurements of sIgE against Ves v 5 and Pol d 5, in combination with Pol d 1 and Ves v 5 are claimed to identify the most probable sensitizing insect and discriminate between *Polistes dominula* and *Vespula vulgaris* venom allergy (Monsalve et al., 2012; Caruso et al., 2016). Nevertheless, the probability of double sensitization cannot be excluded in a reliable manner.

In conclusion, the study and identification of allergenic proteins in venom extracts can aid in insect venom *in vitro* diagnosis in many cases, especially when potential double sensitizations are observed either between venoms of *Apis mellifera* and *Vespula vulgaris* or *Polistes dominula* and *Vespula vulgaris.* Following these findings, many allergens were introduced for various diagnostic test systems in the past years, including the spiked *Vespula vulgaris* venom extract (Vos et al., 2013). As of 2017, these single allergens are Api m 1, 2, 3, 4, 5, 10, Pol d 5 (Pol d 1 is only available on a semi-quantitative system) and Ves v 1 and 5. All venom allergens available for routine diagnosis are marked in bold in Table 2.

Basophil Activation Test for the Diagnosis of Insect Venom Allergy

Since the finding, that *in vitro* activation of basophils by allergens can be measured either by the release of mediators or by detection of up-regulated cellular markers, a vast amount of studies evaluated the use of basophil activation tests for the diagnosis of Hymenoptera venom allergy. Over the years, all studies could demonstrate a sensitivity between 67-75 % in a pediatric population and 75-100 % in a general population of *Apis mellifera* and *Vespula vulgaris* venom allergic patients. The specificity of basophil activation tests in the studied patient population was between 84-100 % (Sainte-Laudy et al., 2000; Platz et al., 2001; Binder et al., 2002; Lambert et al., 2003; Sturm et al., 2004; Erdmann et al., 2004; Eberlein-König et al., 2006b; Eberlein-König et al., 2006a; Sainte-Laudy et al., 2007; Ebo et al., 2007b; Scherer et al., 2008; Ott et al., 2011; Žitnik et al., 2012). Furthermore, the basophil activation test can be used in addition to other *in vitro* diagnostic methods to discriminate between *Apis mellifera* and *Vespula vulgaris* venom allergy (Eberlein-König et al., 2004; Ebo et al., 2007a; Eberlein et al., 2012). Additionally, 60 % of intradermally negative tested patients could be diagnosed by basophil activation (Korosec et al., 2009) and 81 % of 21 patients without detectable sIgE were shown to have positive basophil activation tests (Korošec et al., 2013). However, some basophil activation tests with whole venoms have to be discussed with care, because they can be positive in the case of sIgE against CCDs (Mertens et al., 2010). In the same manner, basophil activation tests of patients suffering from systemic mastocytosis have to be discussed thoroughly. On the one hand, insect venom allergy diagnosis in those patients can be improved by basophil activation testing (González-De-Olano et al., 2011; Bidad et al., 2014b; Bidad et al., 2014a), but on the other hand, this finding could not be reproduced in similar patient populations (Bonadonna et al., 2012; Rietveld et al., 2016). Equally, the basophil activation test cannot be used to predict the severity of a sting reaction, as study results cannot be validated with sting challenges due to ethical concerns (Eberlein et al., 2012).
Until today, recombinant allergens for basophil activation tests were only used for research purposes and in the identification of novel allergens (Seismann et al., 2010b; Blank et al., 2010; Blank et al., 2011). Nevertheless, the diagnostic specificity could possibly be increased by using a panel of recombinant allergens in addition to natural venoms, as it was shown for the diagnosis of *Vespular vulgaris* venom allergy (Balzer et al., 2014).
The guidelines for diagnosis and therapy of Hymenoptera venom allergy therefore only

recommend the use of basophil activation tests if other diagnostic methods fail to detect the sensitization, despite of a convincing history of systemic sting reactions (Golden et al., 2017; Sturm et al., 2017).

Monitoring of Venom Immunotherapy

So far, there are no *in vitro* tests available that predict the safe endpoint of venom immunotherapy. Sting challenges are used in some centers to determine the induced tolerance (Van Halteren et al., 1997; Ruëff et al., 1996; Ruëff et al., 2014) and a negative sting challenge has been shown to increase the health-related quality of life in patients at risk for severe sting reactions (Koschel et al., 2014). Additionally, there is no risk of re-sensitization due to the challenge stings (Golden et al., 1996). Even though the outcome of venom immunotherapy could be predicted in 95 % of patients (van Halteren et al., 1996), another study showed low reproducibility of sting challenges (Franken et al., 1994). The only advance in this field is a good outcome of a first study trying to standardize and replace live stings by challenges with a micro-syringe (Cortellini et al., 2012). Nevertheless, several studies using basophil activation tried to investigate the outcome of venom immunotherapy by this non invasive *in vitro* method. A first study identified several cellular basophil markers to show baseline activation in *Vespula vulgaris* venom allergic individuals and these markers were shown to decrease after immunotherapy (Siegmund et al., 2000). Several other studies either found no change in basophil activation (Erdmann et al., 2004; Brown et al., 2004), a decrease of basophil sensitivity when activated with submaximal stimulating concentrations (Peternelj et al., 2008; Kucera et al., 2010; Žitnik et al., 2012; Čelesnik Smodiš et al., 2014; Trabado et al., 2016; Sainte-Laudy et al., 2016) or lower blood basophil counts (Mikkelsen et al., 2010) after or during successful venom immunotherapy. A similar study found, that the per cell amount of released histamine of activated basophils decreased at maintenance therapy (Nullens et al., 2013). Overall, basophil activation tests differ too much between individuals to be predictive (Neis et al., 2011) but they have been shown to predict side-effects of therapy (Kosnik et al., 2005).
Studies using the measurements of skin test sensitivity or sIgE and sIgG/sIgG$_4$ are less conclusive, due to the low number of treatment failures of venom immunotherapy (Keating et al., 1991). Nevertheless, most of the studies detect the increase of sIgG$_4$ and sIgE antibodies at the beginning of treatment and during maintenance injections whereas the sIgE titers start to decrease during maintenance therapy (Urbanek et al., 1986; Golden et al., 1996; Van Halteren et al., 1997; Lerch et al., 1998; Ruëff et al., 2004; Neis et al., 2011; Eržen et al., 2012). Even though not statistically significant, some studies suggest that the decreasing sIgE and increasing sIgG/sIgG$_4$ concentrations can predict the safe outcome of immunotherapy (Urbanek et al., 1986; Van Halteren et al., 1997) and low induction of sIgG/sIgG$_4$ or the missing decline of sIgE can predict a higher risk of treatment failure (Golden et al., 1992; Golden et al., 1996). Tests measuring the blocking capacity of immunoglobulins, either conducted with cells (IgE-facilitated antibody binding (FAB) (Shamji et al., 2006)), or *in vitro* (enzyme-linked immunosorbent facilitated antigen binding (ELIFAB) (Shamji et al., 2013)), could show increased blocking capacity

during venom immunotherapy, but this blocking capacity was not persistent after several years of therapy (Varga et al., 2009; Möbs et al., 2015).
For skin test sensitivity the same observations can be made. In most of the studied patients, skin test sensitivity decreases after (successful) venom immunotherapy and the chance of experiencing systemic sting reactions after completing venom immunotherapy was higher in patients where skin test sensitivity did not change (Müller et al., 1995b; Lerch et al., 1998; Golden et al., 1998; Graft et al., 1998).
Several groups studied changes on cellular level before and after venom immunotherapy. The most prominent observed changes were a shift from T helper (Th)2 (IL-4 secreting T cells) to Th1 (IFNγ secreting T cells) and an increase of IL-10 production either by regulatory T cells or regulatory B cells (Schuerwegh et al., 2001; Mamessier et al., 2006; Neis et al., 2011; Boonpiyathad et al., 2017) and lower T cell proliferation upon allergen stimulation (Neis et al., 2011). The only clinical correlation of those studies was a faster change on cellular levels in patients that had less severe sting reactions (Mamessier et al., 2006).The same observation has been made in non-allergic beekeepers after multiple stings (Meiler et al., 2008). Moreover, an increase of allergen-specific memory B cells and IgG_4 switched memory B cells was observed (Boonpiyathad et al., 2017). Together, these cellular data could be promising, yet difficult to obtain biomarkers of tolerance induction after venom immunotherapy. Nevertheless, no study has tried to correlate these markers with the outcome of immunotherapy until today.

2 Aim of the Study

Increased knowledge of *Apis mellifera* and *Vespula vulgaris* venom components, notably improved molecular or component-resolved diagnosis. Nevertheless, a review of the literature shows that the venom of *Polistes dominula* has not been studied in this detail. It is additionally known, that in the Mediterranean regions of Europe, *Polistes dominula* and *Vespula vulgaris* live alongside, hence double-sensitization to both species is often observed in the clinics (Severino et al., 2006; Caruso et al., 2007). Until now, there is no valuable diagnostic test to discriminate between sensitization to venoms of *Polistes dominula* and *Vespula vulgaris*. The only option to identify the insect that is most probably inducing the allergic sting reaction, is the determination of sIgE against crude venoms or antigens 5 (Ves v 5 and Pol d 5) together with additional and complicated cross-inhibition experiments (Caruso et al., 2007; Monsalve et al., 2012; Caruso et al., 2016).
The first aim of the presented study, was to analyse and demonstrate the diagnostic cross-reactivity between the venoms of *Polistes dominula* and *Vespula vulgaris* by the structural, comprehensive and extended analysis of antigen 5 cross-reactivity. Therefore, the antigens 5 of the yellow jacket *Vespula vulgaris* (Ves v 5) and European paper wasp *Polistes dominula* (Pol d 5) as well as of the American paper wasp *Polistes annularis* (Pol a 5), the hornet *Vespa crabro* (Vesp c 5), the white-faced hornet *Dolichovespula maculata* (Dol m 5), the fire ant *Solenopsis invicta* (Sol i 3) and the wasp *Polybia scutellaris* (Poly s 5) were produced as recombinant proteins in insect cells. Insect cell expression was mediated by virus infection of *Spodoptera frugiperda* (Sf9) insect cells with recombinant baculovirus. Genes for antigens 5 were cloned from venom gland complementary deoxyribonucleic acid (cDNA) or synthetic genes and recombinant baculovirus was generated by co-transfection of antigens 5 encoding plasmids together with the baculovirus DNA. His-tagged antigens 5 were isolated from cell culture supernatants by nickel affinity chromatography. Purified antigens 5 were used to analyze sIgE in serum of *Vespula vulgaris* and *Polistes dominula* venom allergic patients from Germany and Spain by enzyme-linked immunosorbent assay (ELISA). Additionally, the recombinant antigens 5 were used for basophil activation tests in a group of *Vespula vulgaris* venom allergic patients.
Furthermore, it was aimed to improve *Polistes dominula* venom allergy diagnosis by detailed analysis of *Polistes dominula* venom components. Therefore, crude *Polistes dominula* venom was separated by isoelectric point and/or size and single protein bands or spots, together with crude venom, were analyzed by mass spectrometry. A newly identified venom component, together with its homologues from *Apis mellifera* and *Vespula vulgaris* venom was produced recombinantly in insect cells as described before. The new *Polistes dominula* venom protein was analyzed for its clinical relevance by measuring sIgE reactivity (ELISA) and by basophil activation test from *Polistes dominula* and/or *Vespula*

vulgaris and/or *Apis mellifera* venom allergic patients.
Besides the diagnostic discrimination of *Apis mellifera* and *Vespula vulgaris* venom allergic patients, molecular allergology can be used to evaluate the efficacy of venom immunotherapy (VIT) in great detail. In this manner, serum sIgE levels in a group *Apis mellifera* venom allergic patients was analyzed during the course of VIT to all available allergens (Api m 1, 2, 3, 5 and 10). Hereby, sensitization to Api m 10 was identified as risk for treatment failure (Frick et al., 2016) and analysis of treatment products could probably explain this failure by missing Api m 10 in some of the available products (Blank et al., 2011; Frick et al., 2016). In this study, detailed analysis of therapeutic products were aimed. Therefore, recombinant *Apis mellifera* allergens were expressed in Sf9 cells and used for the generation of polyclonal antibodies. These antibodies were then used for Western Blot analysis of all therapeutic products available in Germany.
An overview of the aim of this study is given in Figure 6.

Review of literature

Component-Resolved Analysis

Extensive study of the cross-reactivity of veonm antigen 5 from seven different Hymenoptera species

Component-resolved analysis of therapeutic products used for venom immunotherapy

Improved Diagnosis and Therapy of Insect Venom Allergy

Identification of novel Polistes dominula venom allergens

Figure 6 Schematic Overview of the Aim of the Study.

Part II

Results

2.1 Application of recombinant antigen 5 allergens from seven allergy-relevant Hymenoptera species in diagnostics.

Schiener, M., Eberlein, B., Moreno-Aguilar, C., Pietsch, G., Serrano, P., Mcintyre, M., Schwarze, L., Russkamp, D., Biedermann, T., Spillner, E., Darsow, U., Ollert, M., Schmidt-Weber, C. B., and Blank, S. (2017a). *Allergy*, *72*, 98–108. doi:10.1111/all.13000

2.1.1 Summary

The cross-reactivity of the major allergen, antigen 5, known to be present in the venom of species of the superfamily of *Vespoidea* was analyzed in two groups of Hymenoptera venom allergic patients. Therefore, the antigens 5 of the seven most allergy-relevant species *Vespula vulgaris* (Ves v 5), *Vespa crabro* (Vesp c 5), *Polistes dominula* (Pol d 5), *Polistes annularis* (Pol a 5), *Dolichovespula maculata* (Dol m 5), *Solenopsis invicta* (Sol i 3) and *Polybia scutellaris* (Poly s 5) were cloned from cDNA or synthetic genes, expressed in *Spodoptera frugiperda* (Sf9) cells, devoid of CCD-reactivity and purified by affinity chromatography. Specific immunoglobulin E (sIgE)-reactivity in the serum of venom allergic patients to the recombinant allergens was analyzed by ELISA. Patients were chosen on the basis of clinical history, skin testing and extract based sIgE measurements and divided into *Vespula vulgaris* venom allergic patients from the south of Germany (Munich) and patients sensitized to the venom of *Polistes dominula* from the south of Spain (Córdoba). Furthermore, basophil activation tests were implemented in a subgroup of *Vespula vulgaris* venom allergic patients.
Analysis of amino acid sequences and structural models of all antigens 5 revealed high sequence homology and suggested similar B cell epitopes, thus high cross-reactivity in patient's serum was expected. This cross-reactivity was confirmed by the presence of cross-reactive sIgE against one or more allergy-irrelevant antigen 5 in the serum of 30 to 75 % of allergic patients. Basophil activation tests in the subgroup of *Vespula vulgaris* venom allergic patients showed similar cross-reactivity. Nevertheless, highest basophil activation sensitivity was observed with recombinant Ves v 5 in many cases.

2.1.2 Individual Contributions

I generated the recombinant baculoviruses, expressed and purified all allergens and performed the ELISA for sIgE determination (Figure 1 and 2). Patient recruitment and basophil activation tests were performed with the help of cooperation partners at the Department of Dermatology and Allergy, Technical University Munich and Hospital Universitario Reina Sofía, Córdoba, Spain. Data was analyzed and the manuscript was written with the help of my direct supervisor Simon Blank and supervisor of this thesis Carsten Schmidt-Weber.

2.2 The high molecular weight dipeptidyl peptidase IV Pol d 3 is a major allergen of *Polistes dominula* venom.

Schiener, M., Hilger, C., Eberlein, B., Pascal, M., Kuehn, A., Revets, D., Planchon, S., Pietsch, G., Serrano, P., Moreno-Aguilar, C., de la Roca, F., Biedermann, T., Darsow, U., Schmidt-Weber, C. B., Ollert, M., and Blank, S. (2018). *Sci. Rep.* *8*(1), 1318. doi:10.1038/s41598-018-19666-7

2.2.1 Summary

In order to expand the panel of known *Polistes dominula* venom allergens, crude venom was analyzed by separation of protein components by isoelectric point and/or size and subsequent mass spectrometry. The band of the protein with a size of around 100 kDa was *de novo* sequenced and the amino acid sequences of six peptides were obtained. A database search of the peptides, identified the protein as dipeptidyl peptidase IV and the protein was cloned from *Polistes dominula* venom gland cDNA. Recombinant baculovirus of dipeptidyl peptidases IV from *Polistes dominula* and additionally of *Apis mellifera* (Api m 5) and *Vespula vulgaris* venom (Ves v 3) was generated. All three proteins were expressed in Sf9 cells and purified by affinity chromatography. Specific immunoglobulin E (sIgE) reactivity was measured to *Polistes dominula* venom dipeptidyl peptidase IV in serum of *Polistes dominula* venom sensitized patients from the area of Córdoba (Spain) by ELISA. Thereby, *Polistes dominula* venom dipeptidyl peptidase IV was determined to be a major allergen, as sIgE was found in the serum of around 66 % of sensitized patients and the allergen was registered as Pol d 3 at the IUIS/WHO allergen database. The analysis of sIgE of Pol d 3 and the homologous dipeptidyl peptidases IV Api m 5 and Ves v 3 demonstrated cross-reactivity in serum of *Apis mellifera*, *Vespula vulgaris* or *Polistes dominula* sensitized patients. Additionally, basophil activation tests with the recombinant allergens in *Apis mellfera*, *Vespula vulgaris* and/or *Polistes dominula* sensitized patients confirmed the observed cross-reactivity. Additionally, the analysis of clinical data obtained by skin testing, sIgE measurements to crude venom and antigens 5 correlated well with the data generated by sIgE measurements to Pol d 3 and basophil activation profiles by stimulation with Pol d 3.

2.2.2 Individual Contributions

I analyzed crude *Polistes dominula* venom (Figure 1). Mass spectrometry data were obtained in cooperation with the Luxemburg Institute of Health. I designed primers, cloned the protein and generated recombinant baculovirus, expressed, purified recombinant proteins and analyzed sIgE reactivity by ELISA (Figure 3 and 5). Basophil activation tests were conducted by cooperation partners in Munich (Germany) and Barcelona (Spain). I analyzed the data, designed the figures and wrote the manuscript with the help of my direct supervisor Simon Blank and my supervisor Carsten Schmidt-Weber.

Part III

Discussion

This thesis demonstrates the application of component-resolved approaches and how it can improve the diagnosis and therapy of Hymenoptera venom allergic patients. In the first part of this thesis, a component-resolved diagnosis concept was used to point out and analyze the limitations in the diagnosis of *Polistes dominula* venom allergy. Double-positive diagnostic test results with *Polistes dominula* and *Vespula vulgaris* venom are frequently observed due to cross-reactive homologous venom allergens, in regions where these species coexist. The only recombinant *Polistes dominula* venom allergen available for routine diagnosis, the antigen 5, together with six additional allergy-relevant antigens 5 from other Hymenoptera species, were used in the first part of this thesis for a detailed analysis of venom cross-reactivity between insects out of the superfamily of *Vespoidea* (Section 2.1). The next part of the thesis aimed at the improvement of current available *Polistes dominula* venom allergy diagnosis, by extending the panel of available venom allergens for component-resolved diagnosis (Section 2.2). In the last part, a component-resolved approach was used to analyze the composition of licensed venom preparations used for *Apis mellifera* venom immunotherapy. The results of this study imply a possible explanation for the infrequently observed treatment failures of *Apis mellifera* venom allergy (Blank et al., 2017). An overview of the different parts of this thesis is given in Figure 7.

Allergen-specific immunotherapy of Hymenoptera venom allergy – also a matter of diagnosis.

Review of literature

Component-Resolved Analysis

Application of recombinant antigen 5 allergens from seven allergy-relevant Hymenoptera species in diagnostics.

Extensive study of the cross-reactivity of veonm antigen 5 from seven different Hymenoptera species

Component-resolved evaluation of the content of major allergens in therapeutic extracts for specific immunotherapy of honeybee venom allergy.

Component-resolved analysis of therapeutic products used for venom immunotherapy

Improved Diagnosis and Therapy of Insect Venom Allergy

Identification of a novel Polistes dominula venom allergen

The high molecular weight dipeptidyl peptidase IV Pol d 3 is a major allergen of Polistes dominula venom

Figure 7 Schematic Overview of the Results of this Study Together with the Titles of Published Articles.

The available literature covering the current knowledge of venom immunotherapy (VIT) and the importance of molecular allergy diagnosis was reviewed and published as part of the "Special Focus on Allergen Immunotherapy" in the journal *"Human Vaccines & Immunotherapeutics"* (Schiener et al., 2017b), together with the analysis of *Apis mellifera* venom immunotherapy products (Blank et al., 2017). The analysis of antigens 5 of seven different Hymenoptera species was published in *"Allergy"*, the official journal of the European Academy of Allergy and Clinical Immunology (EAACI) (Section 2.1) and the newly identified *Polistes dominula* venom allergen Pol d 3 was published in *"Scientific Reports"*, an open access journal belonging to the Nature publishing group (Section 2.2). An overview of published articles is given in Figure 7.

2.3 Unsolved Problems of *Polistes dominula* Venom Allergy Diagnosis

Specific immunoglobulin E (sIgE) based diagnosis of Hymenoptera venom allergy is frequently hampered by double-positive test results to more than one venom (Hoffman et al., 1980; Egner et al., 1998). In many cases, clinically irrelevant sIgE against cross-reactive carbohydrate determinants (CCDs) is the cause of false double-positive test results (Hemmer et al., 2001; Jappe et al., 2006; Altmann, 2016). Many insects like *Apis mellifera* and *Vespula vulgaris* produce glycosylated proteins with CCD epitopes, thus *in vitro* diagnostic discrimination between the sensitization to one or both venoms is often difficult. After several venom components were identified and made available by recombinant techniques in *E. coli* or *Spodoptera frugiperda* insect cells, cells that have been shown to produce proteins devoid of CCDs, sIgE could be measured against single components (component-resolved diagnosis) (Valenta et al., 1999; Seismann et al., 2010a) without interference by CCDs. However, double-positive test results can additionally be explained by sIgE antibodies directed against homologous protein epitopes, as described for example for the homologous hyaluronidases (Api m 2 and Ves v 2), dipeptidyl peptidases (Api m 5 and Ves v 3) as well as the vitellogenins (Api m 12 and Ves v 6) (Hemmer, 2008; Blank et al., 2010; Blank et al., 2013b). Nevertheless, if venom extract-based sIgE diagnosis does not allow the identification of the insect causing the allergic sting reaction, molecular diagnosis using recombinant and species specific marker allergens have a notable potential to assist in the diagnosis of *Apis mellifera* and *Vespula vulgaris* venom allergy.

Polistes dominula and *Vespula vulgaris* live alongside in Mediterranean regions of Europe. Sensitization to one or both venoms is frequently observed but cannot be analyzed in detail, because the only commercially available *Polistes dominula* venom allergen (antigen 5, Pol d 5) cross-reacts with the homologous *Vespula vulgaris* antigen 5 (Ves v 5) (Caruso et al., 2007; Severino et al., 2006). Nevertheless, it was suggested, that time consuming, expensive, challenging to interpret and rarely used cross-inhibition experiments and subsequent sIgE measurements can detect primary venom sensitization in most patients (Caruso et al., 2007). Additionally, the determination of the level of sIgE against antigens 5 (Ves v 5 and Pol d 5) and the phospholipases A1 (Ves v 1 and Pol d 1), of which Pol d 1 is not available for the most commonly used assay platforms, was shown to be sufficient for a

correct diagnosis in many cases (Monsalve et al., 2012). In order to extend the knowledge of antigen 5 cross-reactivity, a comprehensive analysis of serum sIgE reactivity and cellular activation by antigens 5 was implemented in this study. Recombinant techniques and the therefore availability of correctly folded allergens without CCD-reactivity made it possible to analyze the antigens 5 of seven allergy-relevant *Vespoidea* species, namely *Vespula vulgaris*, *Vespa crabro*, *Polistes dominula*, *Polistes annularis*, *Dolichovespula maculata*, *Solenopsis invicta* and *Polybia scutellaris* in two different patient groups. One patient group was from the south of Germany (Munich) hence exclusively sensitized to the venom of *Vespula vulgaris.* The other patient group was from the south of Spain (Córdoba) and sensitized to the venom of *Polistes dominula.* The sensitization to the venom of *Polistes dominula* can be excluded in the patient group from Germany, as this species is not present in this area. Due to the coexistence of *Polistes dominula* and *Vespula vulgaris*, allergic sensitization to the stings of both insects cannot be excluded in the Spanish patient group.

The analysis of amino acid sequences of all antigens 5 revealed high sequence homology, ranging from 45 to 85 %. Structural models of the allergens showed similar protein epitopes of all antigens 5, suggesting shared B cell epitopes. Taken together, this hints to high cross-reactivity of all examined antigens 5 in diagnostic tests and this observation could be confirmed by the presence of cross-reactive sIgE in the serum of 30 to 75 % *Polistes venom* and *Vespula vulgaris* venom allergic patients, determined by positive sIgE to allergy-irrelevant antigens 5. Moreover, cross-inhibition experiments with Pol d 5 and Ves v 5 could confirm diagnosis or give hints to the insect causing the allergic reaction, in cases where diagnosis was not clear. Taken together, the study of antigens 5 clearly demonstrates the drawbacks of component-resolved diagnosis in this case. Nevertheless, antigens 5 are an important group of allergens in the superfamily of *Vespoidea* and the major content of the venoms of most species. Additionally, Ves v 5 is not only used to spike the *Vespula vulgaris* venom extract to increase diagnostic test sensitivity (Vos et al., 2013), but it is also used in component-resolved diagnosis as discriminating allergen (among Ves v 1) for the diagnosis of *Vespula vulgaris* and *Apis mellifera* venom sensitized patients. Interestingly, sIgE reactivity of the potentially hypoallergenic Poly s 5, the antigen 5 of *Polybia scutellaris*, was observed in almost 60 % and 70 % of *Vespula vulgaris* and *Polistes dominula* venom allergic patients, respectively (Vinzón et al., 2012). This observed difference could originate from the different expression systems used. In this study, insect cells were used for recombinant protein production, whereas the yeast *Pichia pastoris* was used to produce Poly s 5 by Vinzon et al.. The putative differences in protein folding or glycosylation of Poly s 5 recombinantly produced in these two expression systems could possibly explain the observed differences by different IgE epitope accessibility. This underlines, that insect venom allergens produced in insect cells seem to be the most authentic way to obtain allergens for component-resolved diagnosis for routine measurements. Additionally, basophil activation experiments with the antigens 5 in a subgroup of *Vespula vulgaris* venom allergic patients showed the highest basophil

activation with recombinant Ves v 5 in many cases. This demonstrated, that basophil activation testing could possibly be used in the future to discriminate allergy to stings of *Vespula vulgaris* and *Polistes dominula* more accurately, but further experiments, also in a group of *Polistes dominula* venom allergic patients and bigger patient groups are needed to confirm this. The analysis of antigen 5 sIgE cross-reactivity in the groups of *Vespula vulgaris* and *Polistes dominula* venom allergic patients, together with the review of available literature on this topic clearly demonstrates the need for additional *Polistes dominula* venom allergens for routine diagnosis of venom allergy.

2.4 Improved *Polistes dominula* Venom Allergy Diagnosis

The recent insights into *Apis mellifera* venom components remarkably improved specificity and sensitivity of *in vitro* venom allergy diagnosis (Köhler et al., 2014). Contradictory, the only known *Polistes dominula* venom allergens are the phospholipase A1 (Pol d 1), a protease (Pol d 4) and the antigen 5 (Pol d 5). All these allergens have been shown to be cross-reactive in diagnostic tests (Monsalve et al., 2012, Section 2.1 and unpublished results observed during this thesis). Moreover, the only commercially available allergens for allergy diagnosis are Pol d 5 for the most commonly used assay systems and Pol d 1 for a semi-quantitative sIgE detection platform. To expand the panel of *Polistes dominula* venom allergens and to possibly increase the diagnostic test sensitivity and specificity comparable to *Apis mellifera* venom allergy diagnosis, *Polistes dominula* venom was analyzed thoroughly in this study. Peptides, found in the venom of *Polistes dominula*, separated by size and/or isoelectric point, were de novo sequenced by mass spectrometry. Peptide sequences were used to search the database of the published genome and predicted proteins of *Polistes dominula* (Standage et al., 2016). De novo sequenced peptides of a protein found at around 100 kDa were identified to be part of the secreted dipeptidyl peptidase IV. Additional peptides of this protein were also found by mass spectrometry in whole *Polistes dominula* venom (unpublished data). The dipeptidyl peptidase IV was successfully cloned from *Polistes dominula* venom gland cDNA and was obtained by baculovirus mediated expression in insect cells. Furthermore, using the recombinant *Polistes dominula* venom dipeptidyl peptidase IV to measure sIgE (ELISA), showed sIgE reactivity in around 66 % of *Polistes dominula* venom sensitized patients from the area of Córdoba and Barcelona, Spain, suggesting the protein to be a major allergen. Additionally, *Polistes dominula* venom dipeptidyl peptidase IV was able to activate the basophils of *Polistes dominula* venom sensitized individuals from Barcelona, Spain. Therefore, *Polistes dominula* venom dipeptidyl peptidase IV was registered at the IUIS/WHO allergen database as Pol d 3. The allergen number was chosen according to the homologous dipeptidyl peptidase IV from *Vespula vulgaris* (Ves v 3) venom (Blank et al., 2010). Additional analysis with the recombinant dipeptidyl peptidases IV from all three species (Pol d 3, Api m 5 and Ves v 3) showed sIgE-based cross-reactivity in three patient groups consisting of either *Apis mellifera*, *Vespula vulgaris* and/or *Polistes dominula* venom allergic patients. This analysis provides an additional molecular explanation of the observed *Vespula vulgaris* and

Polistes dominula venom cross-reactivity. Moreover, the cross-reactivity between *Polistes dominula* and *Apis mellifera* (Grant et al., 1983) could be explained on molecular level for the first time. Nevertheless, basophil activation profiles correlated well to clinical data obtained by skin testing and sIgE measurements to crude venoms and antigens 5, indicating the usefulness of basophil activation tests with recombinant allergens in clinical practice. In conclusion, the newly identified *Polistes dominula* venom allergen can possibly improve *in vitro* venom allergy diagnosis not only in component-resolved analyses of serum sIgE but also in diagnostic basophil activation tests with recombinant allergens.

The identification of yet another enzyme in the list of Hymenoptera venom components underlines the relevance and importance of studies investigating the role of enzymatic activity and putative molecular mechanisms of enzymatic allergens and proteins in allergy sensitization or immunotherapy. Reports of the immunologic activity of *Apis mellifera* venom and *Apis mellifera* phospholipase A2 (Api m 1) showed contradictory mechanisms, probably depending on route of exposure and allergy models. On the one hand, *Apis mellifera* venom has been shown to enhance the generation of Foxp3 expressing T_{regs} capable of effector cell suppression (Caramalho et al., 2015). On the other hand, active phospholipase A2 was shown to induce a T_h2 immune response, characterized by the expression of IL-4, IL-5 and IL-13 in mice (Palm et al., 2013). Then again, the intratracheally application of phospholipase A2 in an allergy mouse model showed a reduced T_h2 response (Jung et al., 2016). A human *in vitro* study additionally suggested that *Apis mellifera* venom phospholipase A2 generates lipid neoantigens. These neoantigens, presented to T cells, might possibly enhance the generation of allergy (Bourgeois et al., 2015).
Similar studies of Hymenoptera venom dipeptidyl peptidase IV (Api m 5) still have to be conducted. Nevertheless, human membrane anchored dipeptidyl peptidase IV or its soluble form, CD26 and sCD26, respectively, are elaborative discussed molecules in T cell immunology. Besides sequence identity between human and Hymenoptera venom dipeptidyl peptidases IV in the range of 32 %, they possess similar peptidase activity (Blank et al., 2010, Section 2.2) and might trigger the same immunologic mechanisms as human (s)CD26. CD26 is present on $CD4^+$ and $CD8^+$ T cells and the peptidase activity is supposed to activate and inactivate proteins by cleaving dipeptides at the N-terminus. Additionally, CD26 has inhibitory as well as co-stimulatory functions, like the induction of IL-10 production and the induction of T_{regs} (Klemann et al., 2016; Hatano et al., 2015). In this manner, CD26 was shown to be anti-inflammatory and less CD26 expression on T cells was observed in psoriasis and atopic dermatitis patients (Bock et al., 2001). On the other hand, elevated sCD26 and CD26 expression on T cells was found in patients with allergic asthma (Nieto-Fontarigo et al., 2016) and patients with autoimmune diseases (Hatano et al., 2015).

Taken together, component-resolved approaches are not only able to identify novel Hy-

menoptera venom allergens, but the knowledge of enzymatic active proteins in venoms might also be able to explain immunologic mechanisms of sensitization or immunotherapy. Further studies should be made regarding enzymatically active venom components in order to elucidate their contribution to allergic sensitization. Moreover, active enzymes present in Hymenoptera venoms like dipeptidyl peptidase IV, able to modulate T cell responses, might also influence efficacy of venom immunotherapy.

2.5 Component-Resolved Analysis of *Apis mellifera* Venom Immunotherapy Products

Component-resolved *Apis mellifera* diagnosis revealed the carbohydrate-rich protein icarapin, Api m 10, to be part of the sensitization profile in 50 to 70 % of *Apis mellifera* venom allergic patients (Blank et al., 2011; Köhler et al., 2014; Frick et al., 2016). With the available *Apis mellifera* allergens in hand, it was possible to generate monoclonal or polyclonal antibodies against these proteins. The analysis of therapeutic products used for *Apis mellifera* venom immunotherapy with these antibodies revealed that Api m 3 and Api m 10 are underrepresented in some of the analyzed products used for venom immunotherapy (Blank et al., 2011). This fact could not fully be interpreted, until it was published very recently, that a predominant sensitization to Api m 10 increases the risk of treatment failures. Similarly to the former study, this study also showed a lack of Api m 10 in some therapeutic products used for venom immunotherapy (Frick et al., 2016). In order to generate highly sensitive antibodies for extended component-resolved analyses of available therapeutic products, the panel of available recombinant proteins produced for this thesis was extended. Subsequently, the *Apis mellifera* venom allergens Api m 2, 3 and 10 were produced and polyclonal antibodies were generated by the immunization of rabbits. Additionally, a recombinant anti-Api m 5 antibody was available (Blank et al., 2010). All antibodies were able to specifically detect low amounts of the corresponding allergen down to $4\,\mu g$ total crude venom. Analyses of therapeutic venom preparations showed that some products or batches lack detectable Api m 3, Api m 5 and/or Api m 10. Additional analysis of recombinant Api m 10, dissolved crude venom and dissolved therapy preparations with and without the addition of human serum albumin found the instability of Api m 10 to probably be responsible for its absence in some products. Nevertheless, human serum albumin, present in all therapeutic venom products, was shown to stabilize Api m 10 over the course of more than four weeks. Taken together, component-resolved analyses of therapeutic products used for venom immunotherapy might be able to explain treatment failures and make a step towards personalized immunotherapy. Additionally, the results of such analyses can be used by companies to further improve therapeutic products and give advice on the correct use and storage.

2.6 Summary and Conclusion

In conclusion, the work of this thesis demonstrated the successful use of component-resolved approaches for an improved management of Hymenoptera venom allergy. First, it was shown that the only available allergen for *Polistes dominula* venom allergy diagnosis, the antigen 5 (Pol d 5) is highly highly cross-reactive (Section 2.1 and Schiener et al., 2017a). Even though, some studies suggest to use the amount of sIgE detected against the different species-specific antigens 5 and phospholipases A1 or cross-inhibition experiments of sIgE in the serum of suspected *Polistes dominula* or *Vespula vulgaris* venom allergic patients (Caruso et al., 2007; Monsalve et al., 2012), these techniques are costly and hard to include in routine diagnosis. Therefore, it was aimed to analyze the unknown components of *Polistes dominula* venom, in order to expand the posibilities of component-resolved diagnosis in the case of *Polistes dominula* venom allergy diagnosis. This thesis therefore suggest an improved *in vitro* venom allergy diagnosis by the expansion of the available venom allergens with the introduction of the newly identified dipeptidyl peptidase IV (Pol d 3) (Section 2.2 and Schiener et al., 2018). Nevertheless, it was also shown that the novel allergen Pol d 3 is cross-reactive with the dipeptidyl peptidases IV, known to be present in the venom of *Apis mellifera* and *Vespula vulgaris*. Most probably, the expanded panel of *Polistes dominula* allergens used for component-resolved diagnosis will increase sensitivity and specificity in the same manner as it was demonstrated in the work of Köhler et al., but the interpretation of test results will still be difficult due to the present cross-reactivity (Köhler et al., 2014). However, mass spectrometric analysis of crude *Polistes dominula* venom conducted in the course of this thesis revealed additional proteins (unpublished results). For some of these proteins, there are no known homologues in the venom of related insect venoms. In the future, these proteins need to be investigated and might serve as marker allergens in the discrimination between sensitization to the venoms of *Polistes dominula* and *Vespula vulgaris*. The analysis of commercially available and novel identified venom allergens clearly demonstrate the power and pitfalls of component-resolved diagnosis. Nevertheless, it also demonstrates that it might be possible to improve the diagnosis of *Polistes dominula* venom allergic patients, as the known information of venom components is still not complete.

By the use of component-resolved approaches in the analysis of therapeutic products, this thesis also demonstrates its power to explain and possibly increase the efficacy of venom immunotherapy. With the knowledge of venom components, in this case components of *Apis mellifera* venom, and the possibility to produce these allergens as recombinant and highly pure proteins, the generation of specific antibodies against these allergens is possible. These antibodies can then be used to analyze commercially available therapy products. In the case of the analyzed therapy products used for the treatment of *Apis mellifera* venom allergic patients, it could be demonstrated that some products lack one or more allergens (Blank et al., 2017). These missing allergens have previously shown to be major and important allergens (Blank et al., 2011; Köhler et al., 2014; Frick et al., 2016). Additionally, one allergen that is missing in some available therapeutic products (Api m 10) has previously been shown to be the reason for treatment failure in patients

sensitized to Api m 10 (Frick et al., 2016). Similar studies might be needed to standardize and improve all available venom immunotherapy products. Furthermore, tools like specific antibodies that are generated by component-resolved approaches can (and should) be used by companies during the necessary manufacturing processes needed to generate therapeutic venom preparations, starting from crude venoms to final products. In this manner, the management of Hymenoptera venom allergic patients can benefit from improvements of component-resolved approaches not only in diagnosis but also in therapy.

References

Abbas, A. K., Lichtman, A. H., & Pillai, S. (2015). *Cellular and Molecular Immunology* (8. Edition). Canada: Elsevier Saunders, Philadelphia, USA.

Abe, T., Kawai, N., & Niwa, A. (1982). Purification and properties of a presynaptically acting neurotoxin, mandaratoxin, from hornet (Vespa mandarinia). *Biochemistry*, *21*(7), 1693–1697. doi:10.1021/bi00536a034

Abelson, M. B. & Loeffler, O. (2003). Conjunctival allergen challenge: Models in the investigation of ocular allergy. *Curr. Allergy Asthma Rep. 3*(4), 363–368. doi:10.1007/s11882-003-0100-z

Abrecht, I., Eichler, G., Müller, U., & Hoigné, R. (1980). On the significance of severe local reactions to Hymenoptera stings. *Clin. Allergy*, *10*(6), 675–82. doi:10.1111/j.1365-2222.1980.tb02151.x

Abuaf, N., Rostane, H., Rajoely, B., Gaouar, H., Autegarden, J. E., Leynadier, F., & Girot, R. (2008). Comparison of two basophil activation markers CD63 and CD203c in the diagnosis of amoxicillin allergy. *Clin. Exp. Allergy*, *38*(6), 921–928. doi:10.1111/j.1365-2222.2008.02960.x

Agache, I., Bilò, M., Braunstahl, G.-J., Delgado, L., Demoly, P., Eigenmann, P., Gevaert, P., Gomes, E., Hellings, P., Horak, F., Muraro, A., Werfel, T., & Jutel, M. (2015). In vivo diagnosis of allergic diseases-allergen provocation tests. *Allergy*, *70*(4), 355–365. doi:10.1111/all.12586

Akdis, C. A. & Akdis, M. (2011). Mechanisms of allergen-specific immunotherapy. *J. Allergy Clin. Immunol. 127*(1), 18–27. doi:10.1016/j.jaci.2010.11.030

Akdis, C. A. & Akdis, M. (2015). Mechanisms of allergen-specific immunotherapy and immune tolerance to allergens. *World Allergy Organ. J. 8*(1), 17. doi:10.1186/s40413-015-0063-2

Akdis, M., Verhagen, J., Taylor, A., Karamloo, F., Karagiannidis, C., Crameri, R., Thunberg, S., Deniz, G., Valenta, R., Fiebig, H., Kegel, C., Disch, R., Schmidt-Weber, C. B., Blaser, K., & Akdis, C. A. (2004). Immune Responses in Healthy and Allergic Individuals Are Characterized by a Fine Balance between Allergen-specific T Regulatory 1 and T Helper 2 Cells. *J. Exp. Med. 199*(11), 1567–1575. doi:10.1084/jem.20032058

Akerlund, A., Andersson, M., Leflein, J., Lildholdt, T., & Mygind, N. (2005). Clinical trial design, nasal allergen challenge models, and considerations of relevance to pediatrics, nasal polyposis, and different classes of medication. *J. Allergy Clin. Immunol. 115*(3 SUPPL.), 460–482. doi:10.1016/j.jaci.2004.12.016

Altmann, F. (2016). Coping with cross-reactive carbohydrate determinants in allergy diagnosis. *Allergo J. Int. 25*(4), 98–105. doi:10.1007/s40629-016-0115-3

Amarasekera, M. (2011). Immunoglobulin E in health and disease. *Asia Pac Allergy*, *1*, 12–15. doi:10.5415/apallergy.2011.1.12

An, S., Chen, L., Wei, J.-F., Yang, X., Ma, D., Xu, X., Xu, X., He, S., Lu, J., & Lai, R. (2012). Purification and Characterization of Two New Allergens from the Venom of Vespa magnifica. *PLoS One*, *7*(2), e31920. doi:10.1371/journal.pone.0031920

Bagnasco, D., Ferrando, M., Varricchi, G., Passalacqua, G., & Canonica, G. W. (2016). A Critical Evaluation of Anti-IL-13 and Anti-IL-4 Strategies in Severe Asthma. *Int. Arch. Allergy Immunol. 170*(2), 122–131. doi:10.1159/000447692

Baker, T. W., Forester, J. P., Johnson, M. L., Sikora, J. M., Stolfi, A., & Stahl, M. C. (2016). Stinging insect identification: Are the allergy specialists any better than their patients? *Ann. Allergy, Asthma Immunol. 116*(5), 431–434. doi:10.1016/j.anai.2016.01.025

Baker, T. W., Forester, J. P., Johnson, M. L., Stolfi, A., & Stahl, M. C. (2014). The HIT study: Hymenoptera Identification Test - How accurate are people at identifying stinging insects? *Ann. Allergy, Asthma Immunol. 113*(3), 267–270. doi:10.1016/j.anai.2014.05.029

Balzer, L., Pennino, D., Blank, S., Seismann, H., Darsow, U., Schnedler, M., McIntyre, M., Ollert, M. W., Durham, S. R., Spillner, E., Ring, J., & Cifuentes, L. (2014). Basophil Activation Test Using Recombinant Allergens: Highly Specific Diagnostic Method Complementing Routine Tests in Wasp Venom Allergy. *PLoS One*, *9*(10), e108619. doi:10.1371/journal.pone.0108619

Barnes, P. J. & Adcock, I. M. (2003). How Do Corticosteroids Work in Asthma? *Ann. Intern. Med. 139*(5, Part 1), 359. doi:10.7326/0003-4819-139-5_Part_1-200309020-00012

Bergmann, K.-C., Heinrich, J., & Niemann, H. (2016). Current status of allergy prevalence in Germany. *Allergo J. Int. 25*(1), 6–10. doi:10.1007/s40629-016-0092-6

Bernstein, I. L., Li, J. T., Bernstein, D. I., Hamilton, R., Spector, S. L., Tan, R., Sicherer, S., Golden, D. B., Khan, D. A., Nicklas, R. A., Portnoy, J. M., Blessing-Moore, J., Cox, L., Lang, D. M., Oppenheimer, J., Randolph, C. C., Schuller, D. E., Tilles, S. A., Wallace, D. V., Levetin, E., & Weber, R. (2008). Allergy Diagnostic Testing: An Updated Practice Parameter. *Ann. Allergy, Asthma Immunol. 100*(3), S1–S148. doi:10.1016/S1081-1206(10)60305-5

Beyer, K., Eckermann, O., Hompes, S., Grabenhenrich, L., & Worm, M. (2012). Anaphylaxis in an emergency setting - elicitors, therapy and incidence of severe allergic reactions. *Allergy, 67*(11), 1451–1456. doi:10.1111/all.12012

Bidad, K., Nawijn, M. C., Van Oosterhout, A. J. M., Van Der Heide, S., & Oude Elberink, J. N. G. (2014a). Basophil activation test in the diagnosis and monitoring of mastocytosis patients with wasp venom allergy on immunotherapy. *Cytom. Part B - Clin. Cytom. 86*(3), 183–190. doi:10.1002/cyto.b.21148

Bidad, K., Nawijn, M. C., van Oosterhout, A. J. M., van der Heide, S., & Elberink, J. N. G. O. (2014b). Basophil activation test in the diagnosis and monitoring of mastocytosis patients with wasp venom allergy on immunotherapy. *Cytometry B. Clin. Cytom. 86*(3), 183–90. doi:10.1002/cyto.b.21148

Biló, B. M., Rueff, F., Mosbech, H., Bonifazi, F., Oude-Elberink, J. N. G., Birnbaum, J., Bucher, C., Forster, J., Hemmer, W., Incorvaia, C., Kontou-Fili, K., Gawlik, R., Muller, U., Fernandez, J., Jarish, R., Jutel, M., & Wuthrich, B. (2005). Diagnosis of Hymenoptera venom allergy. *Allergy, 60*(11), 1339–1349. doi:10.1111/j.1398-9995.2005.00963.x

Bilò, B. M. & Bonifazi, F. (2008). Epidemiology of insect-venom anaphylaxis. *Curr. Opin. Allergy Clin. Immunol. 8*, 330–337. doi:10.1097/ACI.0b013e32830638c5

Bilò, B. M. & Bonifazi, F. (2011). Hymenoptera venom immunotherapy. *Immunotherapy, 3*(2), 229–246. doi:10.2217/imt.10.88

Binder, M., Fierlbeck, G., King, T. P., Valent, P., & Bühring, H.-J. (2002). Individual Hymenoptera Venom Compounds Induce Upregulation of the Basophil Activation Marker Ectonucleotide Pyrophosphatase/Phosphodiesterase 3 (CD203c) in Sensitized Patients. *Int. Arch. Allergy Immunol. 129*(2), 160–168. doi:10.1159/000065875

Blank, S., Seismann, H., Michel, Y., McIntyre, M., Cifuentes, L., Braren, I., Grunwald, T., Darsow, U., Ring, J., Bredehorst, R., Ollert, M., & Spillner, E. (2011). Api m 10, a genuine A. mellifera venom allergen, is clinically relevant but underrepresented in therapeutic extracts. *Allergy, 66*(10), 1322–1329. doi:10.1111/j.1398-9995.2011.02667.x

Blank, S., Bantleon, F. I., McIntyre, M., Ollert, M., & Spillner, E. (2012). The major royal jelly proteins 8 and 9 (Api m 11) are glycosylated components of Apis mellifera venom with allergenic potential beyond carbohydrate-based reactivity. *Clin. Exp. Allergy, 42*(6), 976–985. doi:10.1111/j.1365-2222.2012.03966.x

Blank, S., Etzold, S., Darsow, U., Schiener, M., Eberlein, B., Russkamp, D., Wolf, S., Graessel, A., Biedermann, T., Ollert, M., & Schmidt-Weber, C. B. (2017). Component-resolved evaluation of the content of major allergens in therapeutic extracts for specific immunotherapy of honeybee venom allergy. *Hum. Vaccin. Immunother. 13*(10), 2482–2489. doi:10.1080/21645515.2017.1323603

Blank, S., Neu, C., Hasche, D., Bantleon, F. I., Jakob, T., & Spillner, E. (2013a). Polistes species venom is devoid of carbohydrate-based cross-reactivity and allows interference-free diagnostics. *J. Allergy Clin. Immunol. 131*(4), 1239–1242. doi:10.1016/j.jaci.2012.10.047

Blank, S., Seismann, H., Bockisch, B., Braren, I., Cifuentes, L., McIntyre, M., Ruhl, D., Ring, J., Bredehorst, R., Ollert, M. W., Grunwald, T., & Spillner, E. (2010). Identification, Recombinant Expression, and Characterization of the 100 kDa High Molecular Weight Hymenoptera Venom Allergens Api m 5 and Ves v 3. *J. Immunol. 184*(9), 5403–5413. doi:10.4049/jimmunol.0803709

Blank, S., Seismann, H., McIntyre, M., Ollert, M., Wolf, S., Bantleon, F. I., & Spillner, E. (2013b). Vitellogenins Are New High Molecular Weight Components and Allergens (Api m 12 and Ves v 6) of Apis mellifera and Vespula vulgaris Venom. *PLoS One, 8*(4), e62009. doi:10.1371/journal.pone.0062009

Bock, O., Kreiselmeyer, I., & Mrowietz, U. (2001). Expression of dipeptidyl-peptidase IV (CD26) on CD8+ T cells is significantly decreased in patients with psoriasis vulgaris and atopic dermatitis. *Exp. Dermatol. 10*(6), 414–419. doi:10.1034/j.1600-0625.2001.100604.x

Bokanovic, D., Aberer, W., Griesbacher, A., & Sturm, G. J. (2011). Prevalence of hymenoptera venom allergy and poor adherence to immunotherapy in Austria. *Allergy*, *66*(10), 1395–1396. doi:10.1111/j.1398-9995.2011.02659.x

Bonadonna, P., Zanotti, R., Melioli, G., Antonini, F., Romano, I., Lenzi, L., Caruso, B., & Passalacqua, G. (2012). The role of basophil activation test in special populations with mastocytosis and reactions to hymenoptera sting. *Allergy*, *67*(7), 962–965. doi:10.1111/j.1398-9995.2012.02849.x

Boonpiyathad, T., Meyer, N., Moniuszko, M., Sokolowska, M., Eljaszewicz, A., Wirz, O. F., Tomasiak-Lozowska, M. M., Bodzenta-Lukaszyk, A., Ruxrungtham, K., & van de Veen, W. (2017). High-dose bee venom exposure induces similar tolerogenic B-cell responses in allergic patients and healthy beekeepers. *Allergy*, *72*(3), 407–415. doi:10.1111/all.12966

Bosnjak, B., Stelzmueller, B., Erb, K. J., & Epstein, M. M. (2011). Treatment of allergic asthma: Modulation of Th2 cells and their responses. *Respir. Res. 12*(1), 114. doi:10.1186/1465-9921-12-114

Bourgeois, E. A., Subramaniam, S., Cheng, T.-y., Jong, A. D., Layre, E., Ly, D., Salimi, M., Legaspi, A., Modlin, R. L., Salio, M., Cerundolo, V., Moody, D. B., & Ogg, G. (2015). Bee venom processes human skin lipids for presentation by CD1a. *J. Exp. Med. in Press.* doi:10.1084/jem.20141505

Bousquet, J., Müller, U. R., Dreborg, S., Jarisch, R., Malling, H.-J., Mosbech, H., Urbanek, R., & Youlten, L. (1987). Immunotherapy with Hymenoptera venoms. *Allergy*, *42*(6), 401–413. doi:10.1111/j.1398-9995.1987.tb00355.x

Boyce, J. A. (2005). Eicosanoid mediators of mast cells: receptors, regulation of synthesis, and pathobiologic implications. *Chem. Immunol. Allergy*, *87*, 59–79. doi:10.1159/000087571

Boyle, R. J., Elremeli, M., Hockenhull, J., Cherry, M. G., Bulsara, M. K., Daniels, M., & Oude Elberink, J. N. G. (2012). Venom immunotherapy for preventing allergic reactions to insect stings. *Cochrane database Syst. Rev. 10*(10), CD008838. doi:10.1002/14651858.CD008838.pub2

Braun, D. (1925). Notes on Desensitisation of a Patient Hypersensitive to Bee Stings. *South African Med. J. 18*, 408–409. doi:10.1080/0005772X.1926.11096103

Brown, S. G. A., Haas, M. A., Black, J. A., Parameswaran, A., Woods, G. M., & Heddle, R. J. (2004). In vitro testing to diagnose venom allergy and monitor immunotherapy: a placebo-controlled, crossover trial. *Clin. Exp. Allergy*, *34*(5), 792–800. doi:10.1111/j.1365-2222.2004.01949.x

Brown, S. G., Wiese, M. D., van Eeden, P., Stone, S. F., Chuter, C. L., Gunner, J., Wanandy, T., Phillips, M., & Heddle, R. J. (2012). Ultrarush versus semirush initiation of insect venom immunotherapy: A randomized controlled trial. *J. Allergy Clin. Immunol. 130*(1), 162–168. doi:10.1016/j.jaci.2012.02.022

Burks, A. W., Calderon, M. A., Casale, T., Cox, L., Demoly, P., Jutel, M., Nelson, H., & Akdis, C. A. (2013). Update on allergy immunotherapy: American Academy of Allergy, Asthma & Immunology/European Academy of Allergy and Clinical Immunology/PRACTALL consensus report. *J. Allergy Clin. Immunol. 131*(5), 1288–1296.e3. doi:10.1016/j.jaci.2013.01.049

Busse, W. W., Reed, C. E., Lichtenstein, L. M., & Reisman, R. E. (1975). Immunotherapy in Bee-Sting Anaphylaxis. *JAMA*, *231*(11), 1154. doi:10.1001/jama.1975.03240230028014

Butcher, B. T., DeShazo, R. D., Ortiz, A. A., & Reed, M. A. (1988). Superiority of Solenopsis invicta Venom to Whole-Body Extract in RAST for Diagnosis of Imported Fire Ant Allergy. *Int. Arch. Allergy Immunol. 85*(4), 458–461. doi:10.1159/000234551

Caramalho, I., Melo, A., Pedro, E., Barbosa, M. M. P., Victorino, R. M. M., Pereira Santos, M. C., & Sousa, A. E. (2015). Bee venom enhances the differentiation of human regulatory T cells. *Allergy*, *70*(10), 1340–1345. doi:10.1111/all.12691

Caruso, B., Bonadonna, P., Severino, M. G., Manfredi, M., Dama, A., Schiappoli, M., Rizzotti, P., Senna, G., & Passalacqua, G. (2007). Evaluation of the IgE cross-reactions among vespid venoms. A possible approach for the choice of immunotherapy. *Allergy*, *62*(5), 561–564. doi:10.1111/j.1398-9995.2007.01353.x

Caruso, B., Bonadonna, P., Bovo, C., Melloni, N., Lombardo, C., Senna, G., & Lippi, G. (2016). Wasp venom allergy screening with recombinant allergen testing. Diagnostic performance

of rPol d 5 and rVes v 5 for differentiating sensitization to Vespula and Polistes subspecies. *Clin. Chim. Acta*, *453*, 170–173. doi:10.1016/j.cca.2015.12.026

Caughey, G. H. (2007). Mast cell tryptases and chymases in inflammation and host defense. *Immunol. Rev. 217*(1), 141–154. doi:10.1111/j.1600-065X.2007.00509.x

Cavallucci, E., Ramondo, S., Renzetti, A., Turi, M. C., Di Claudio, F., Braga, M., Incorvaia, C., Schiavone, C., Ballone, E., & Di Gioacchino, M. (2010). Maintenance venom immunotherapy administered at a 3-month interval preserves safety and efficacy and improves adherence. *J. Investig. Allergol. Clin. Immunol. 20*(1), 63–8.

Čelesnik Smodiš, N., Šilar, M., Eržen, R., Rijavec, M., Košnik, M., & Korošec, P. (2014). Down-Regulation of FcϵRI-Mediated CD63 Basophil Response during Short-Term VIT Determined Venom-Nonspecific Desensitization. *PLoS One*, *9*(4), e94762. doi:10.1371/journal.pone.0094762

Cervo, R., Zacchi, F., & Turillazzi, S. (2000). Polistes dominulus (Hymenoptera, Vespidae) invading North America: some hypotheses for its rapid spread. *Insectes Soc. 47*(2), 155–157. doi:10.1007/PL00001694

Ceuppens, J. L., Bullens, D., Kleinjans, H., & van der Werf, J. (2009). Immunotherapy with a modified birch pollen extract in allergic rhinoconjunctivitis: clinical and immunological effects. *Clin. Exp. Allergy*, *39*(12), 1903–1909. doi:10.1111/j.1365-2222.2009.03379.x

Chevrier, D., Guesdon, J.-L., Mazié, J.-C., & Avrameas, S. (1986). Enzyme immunoassay for the measurement of histamine. *J. Immunol. Methods*, *94*(1-2), 119–125. doi:10.1016/0022-1759(86)90223-1

Ciprandi, G., Vizzaccaro, A., Cirillo, I., Tosca, M., Massolo, A., & Passalacqua, G. (2005). Nasal Eosinophils Display the Best Correlation with Symptoms, Pulmonary Function and Inflammation in Allergic Rhinitis. *Int. Arch. Allergy Immunol. 136*(3), 266–272. doi:10.1159/000083953

Cortellini, G., Severino, M., Francescato, E., Turillazzi, S., Spadolini, I., Rogkakou, A., & Passalacqua, G. (2012). Evaluation and validation of a bee venom sting challenge performed by a micro-syringe. *Ann. Allergy, Asthma Immunol. 109*(6), 438–441. doi:10.1016/j.anai.2012.09.003

Cruz, S., Vega, A., Fernandez, S., Marqués, L., Baltasar, M., Alonso, A., Jorro, G., Moreno, A., Sánchez-Morillas, L., Miranda, A., Soriano, V., Fernández, J., & Guspí, R. (2012). Report From the Hymenoptera Committee of the Spanish Society of Allergology and Clinical Immunology: Immunotherapy With Bumblebee Venom. *J. Investig. Allergol. Clin. Immunol. 22*(5), 390–391.

da Silva, E. N. & Randall, K. L. (2013). Omalizumab mitigates anaphylaxis during ultrarush honey bee venom immunotherapy in monoclonal mast cell activation syndrome. *J. Allergy Clin. Immunol. Pract. 1*(6), 687–688. doi:10.1016/j.jaip.2013.07.004

de Groot, H. (2006). Allergy to bumblebees. *Curr. Opin. Allergy Clin. Immunol. 6*(4), 294–297. doi:10.1097/01.all.0000235905.87676.09

Del Cuvillo, A., Mullol, J., Bartra, J., Dávila, I., Jáuregui, I., Montoro, J., Sastre, J., & Valero, A. L. (2006). Comparative pharmacology of the H1 antihistamines. *J. Investig. Allergol. Clin. Immunol. 16*(SUPPL. 1), 3–12.

Demain, J. G., Gessner, B. D., McLaughlin, J. B., Sikes, D. S., & Foote, J. T. (2009). Increasing insect reactions in Alaska: Is this related to changing climate? *Allergy Asthma Proc. 30*(3), 238–243. doi:10.2500/aap.2009.30.3231

Diamant, B. & Patkar, S. (1982). Histamine Release from Washed Whole Blood. *Int. Arch. Allergy Immunol. 67*(1), 13–17. doi:10.1159/000232981

Diniz, A. G. Q., Belmino, J. F. B., de Araújo, K. A. M., Vieira, A. T., & Leite, R. d. S. (2016). Epidemiology of Honeybee Sting Cases in the State of Ceará, Northeastern Brazil. *Rev. Inst. Med. Trop. Sao Paulo*, *58*, 40. doi:10.1590/S1678-9946201658040

dos Santos, L. D., Santos, K. S., Pinto, J. R. A., Dias, N. B., Souza, B. M. D., dos Santos, M. F., Perales, J., Domont, G. B., Castro, F. M., Kalil, J. E., & Palma, M. S. (2010). Profiling the Proteome of the Venom from the Social Wasp Polybia paulista: A Clue to Understand the Envenoming Mechanism. *J. Proteome Res. 9*(8), 3867–3877. doi:10.1021/pr1000829

Dudler, T., Chen, W.-Q., Wang, S., Schneider, T., Annand, R. R., Dempcy, R. O., Crameri, R., Gmachl, M., Suter, M., & Gelb, M. H. (1992). High-level expression in Escherichia coli and rapid purification of enzymatically active honey bee venom phospholipase A2. *Biochim. Biophys. Acta - Lipids Lipid Metab. 1165*(2), 201–210. doi:10.1016/0005-2760(92)90188-2

Dvorak, A. M. (2005). Ultrastructural Studies of Human Basophils and Mast Cells. *J. Histochem. Cytochem. 53*(9), 1043–1070. doi:10.1369/jhc.5R6647.2005

Eardley, C., Koch, F., & Wood, A. (2009). Polistes dominulus (Christ, 1791) (Hymenoptera: Polistinae: Vespidae) Newly Recorded from South Africa. *African Entomol. 17*(2), 226–227. doi:10.4001/003.017.0214

Eberlein, B., Hann, R., Eyerich, S., Pennino, D., Ring, J., Schmidt-Weber, C. B., & Buters, J. (2015). Optimizing of the basophil activation test: Comparison of different basophil identification markers. *Cytom. Part B Clin. Cytom. 88*(3), 183–189. doi:10.1002/cyto.b.21203

Eberlein, B., Krischan, L., Darsow, U., Ollert, M., & Ring, J. (2012). Double positivity to bee and wasp venom: Improved diagnostic procedure by recombinant allergen-based IgE testing and basophil activation test including data about cross-reactive carbohydrate determinants. *J. Allergy Clin. Immunol. 130*(1), 155–161. doi:10.1016/j.jaci.2012.02.008

Eberlein-König, B., Schmidt-Leidescher, C., Rakoski, J., Behrendt, H., & Ring, J. (2006a). In vitro basophil activation using CD63 expression in patients with bee and wasp venom allergy. *J. Investig. Allergol. Clin. Immunol. 16*(1), 5–10.

Eberlein-König, B., Varga, R., Mempel, M., Darsow, U., Behrendt, H., & Ring, J. (2006b). Comparison of basophil activation tests using CD63 or CD203c expression in patients with insect venom allergy. *Allergy*, *61*(9), 1084–1085. doi:10.1111/j.1398-9995.2006.01122.x

Eberlein-König, B., Rakoski, J., Behrendt, H., & Ring, J. (2004). Use of CD63 expression as marker of in vitro basophil activation in identifying the culprit in insect venom allergy. *J. Investig. Allergol. Clin. Immunol. 14*(1), 10–6.

Ebo, D. G., Hagendorens, M. M., Bridts, C. H., De Clerck, L. S., & Stevens, W. J. (2007a). Hymenoptera venom allergy: Taking the sting out of difficult cases. *J. Investig. Allergol. Clin. Immunol. 17*(6), 357–360.

Ebo, D. G., Hagendorens, M. M., Schuerwegh, A. J., Beirens, L. M.-N., Bridts, C. H., De Clerck, L. S., & Stevens, W. J. (2007b). Flow-assisted quantification of in vitro activated basophils in the diagnosis of wasp venom allergy and follow-up of wasp venom immunotherapy. *Cytom. Part B Clin. Cytom. 72B*(3), 196–203. doi:10.1002/cyto.b.20142

Egner, W., Ward, C., Brown, D. L., & Ewan, P. W. (1998). The frequency and clinical significance of specific IgE to both wasp (Vespula) and honey-bee (Apis) venoms in the same patient. *Clin. Exp. Allergy*, *28*(1), 26–34. doi:10.1046/j.1365-2222.1998.00176.x

Erdmann, S. M., Sachs, B., Kwiecien, R., Moll-Slodowy, S., Sauer, I., & Merk, H. F. (2004). The basophil activation test in wasp venom allergy: sensitivity, specificity and monitoring specific immunotherapy. *Allergy*, *59*(10), 1102–1109. doi:10.1111/j.1398-9995.2004.00624.x

Eržen, R., Košnik, M., Šilar, M., & Korošec, P. (2012). Basophil response and the induction of a tolerance in venom immunotherapy: A long-term sting challenge study. *Allergy*, *67*(6), 822–830. doi:10.1111/j.1398-9995.2012.02817.x

Fackler, W. & Loveless, M. (1956). Wasp venom allergy and immunity. *Ann. Allergy*, *14*(5), 347–366.

Fernandez, J., Soriano, V., Mayorga, L., & Mayor, M. (2005). Natural history of Hymenoptera venom allergy in Eastern Spain. *Clin. Exp. Allergy*, *35*(2), 179–85. doi:10.1111/j.1365-2222.2005.02169.x

Fernandez, Blanca, Soriano, Sanchez, & Juarez. (1999). Epidemiological study of the prevalence of allergic reactions to Hymenoptera in a rural population in the Mediterranean area. *Clin. Exp. Allergy*, *29*(8), 1069–1074. doi:10.1046/j.1365-2222.1999.00614.x

Flicker, S. & Valenta, R. (2003). Renaissance of the Blocking Antibody Concept in Type I Allergy. *Int. Arch. Allergy Immunol. 132*(1), 13–24. doi:10.1159/000073260

Francis, J. N., Till, S. J., & Durham, S. R. (2003). Induction of IL-10+CD4+CD25+ T cells by grass pollen immunotherapy. *J. Allergy Clin. Immunol. 111*(6), 1255–1261. doi:10.1067/mai.2003.1570

Franken, H. H., Dubois, A., Minkema, H., van der Heide, S., & de Monchy, J. G. (1994). Lack of reproducibility of a single negative sting challenge response in the assessment of anaphylactic risk in patients with suspected yellow jacket hypersensitivity. *J. Allergy Clin. Immunol. 93*(2), 431–436. doi:10.1016/0091-6749(94)90351-4

Freeman, T. M., Hylander, R., Ortiz, A., & Martin, M. E. (1992). Imported fire ant immunotherapy: effectiveness of whole body extracts. *J. Allergy Clin. Immunol. 90*(2), 210–5. doi:10.1016/0091-6749(92)90073-B

Freeman, T. M. (2008). Cold-blooded. *Curr. Opin. Allergy Clin. Immunol. 8*(4), 308–309. doi:10.1097/ACI.0b013e32830656ec

Frick, M., Fischer, J., Helbling, A., Ruëff, F., Wieczorek, D., Ollert, M., Pfützner, W., Müller, S., Huss-Marp, J., Dorn, B., Biedermann, T., Lidholm, J., Ruecker, G., Bantleon, F., Miehe, M., Spillner, E., & Jakob, T. (2016). Predominant Api m 10 sensitization as risk factor for treatment failure in honey bee venom immunotherapy. *J. Allergy Clin. Immunol. 138*(6), 1663–1671.e9. doi:10.1016/j.jaci.2016.04.024

Friedlaender, M. H. (2004). Objective measurement of allergic reactions in the eye. *Curr. Opin. Allergy Clin. Immunol. 4*(5), 447–453. doi:10.1097/00130832-200410000-00019

Galera, C., Soohun, N., Zankar, N., Caimmi, S., Gallen, C., & Demoly, P. (2009). Severe anaphylaxis to bee venom immunotherapy: Efficacy of pretreatment and concurrent treatment with omalizumab. *J. Investig. Allergol. Clin. Immunol. 19*(3), 225–229.

Galli, S. J., Grimbaldeston, M., & Tsai, M. (2008a). Immunomodulatory mast cells: negative, as well as positive, regulators of immunity. *Nat. Rev. Immunol. 8*(6), 478–486. doi:10.1038/nri2327

Galli, S. J., Kalesnikoff, J., Grimbaldeston, M. A., Piliponsky, A. M., Williams, C. M. M., & Tsai, M. (2005). Mast cells as "tunable" effector and immunoregulatory cells: recent advances. *Annu. Rev. Immunol. 23*(1), 749–86. doi:10.1146/annurev.immunol.21.120601.141025

Galli, S. J., Maurer, M., & Lantz, C. S. (1999). Mast cells as sentinels of innate immunity. *Curr. Opin. Immunol. 11*(1), 53–59. doi:10.1016/S0952-7915(99)80010-7

Galli, S. J., Tsai, M., & Piliponsky, A. M. (2008b). The development of allergic inflammation. *Nature*, *454*(7203), 445–454. doi:10.1038/nature07204

Geha, R. S., Jabara, H. H., & Brodeur, S. R. (2003). The regulation of immunoglobulin E class-switch recombination. *Nat. Rev. Immunol. 3*(9), 721–732. doi:10.1038/nri1181

Gell, P. & Coombs, R. (1963). *Clinical aspects of immunology.* Blackwell Science Ltd.

Georas, S. N., Guo, J., De Fanis, U., & Casolaro, V. (2005). T-helper cell type-2 regulation in allergic disease. *Eur. Respir. J. 26*(6), 1119–1137. doi:10.1183/09031936.05.00006005

Gilfillan, A. M. & Tkaczyk, C. (2006). Integrated signalling pathways for mast-cell activation. *Nat. Rev. Immunol. 6*(3), 218–230. doi:10.1038/nri1782

Glimcher, L. H. (2007). Trawling for treasure: tales of T-bet. *Nat. Immunol. 8*(5), 448–450. doi:10.1038/ni0507-448

Gmachl, M. & Kreil, G. (1993). Bee venom hyaluronidase is homologous to a membrane protein of mammalian sperm. *Proc. Natl. Acad. Sci. 90*(8), 3569–3573. doi:10.1073/pnas.90.8.3569

Goldberg, A. & Confino-Cohen, R. (1997). Timing of venom skin tests and IgE determinations after insect sting anaphylaxis. *J. Allergy Clin. Immunol. 100*(2), 182–4. doi:10.1016/S0091-6749(97)70222-7

Goldberg, A. & Confino-Cohen, R. (2010). Bee venom immunotherapy - how early is it effective? *Allergy*, *65*(3), 391–395. doi:10.1111/j.1398-9995.2009.02198.x

Goldberg, A. & Confino-Cohen, R. (2003). Rush venom immunotherapy in patients experiencing recurrent systemic reactions to conventional venom immunotherapy. *Ann. Allergy, Asthma Immunol. 91*(4), 405–410. doi:10.1016/S1081-1206(10)61689-4

Goldberg, A., Yogev, A., & Confino-Cohen, R. (2011). Three Days Rush Venom Immunotherapy in Bee Allergy: Safe, Inexpensive and Instantaneously Effective. *Int. Arch. Allergy Immunol. 156*(1), 90–98. doi:10.1159/000322258

Golden, D. B., Kwiterovich, K. A., Kagey-Sobotka, A., Valentine, M. D., & Lichtenstein, L. M. (1996). Discontinuing venom immunotherapy: outcome after five years. *J. Allergy Clin. Immunol. 97*(2), 579–87. doi:10.1016/S0091-6749(96)70302-0

Golden, D. B., Marsh, D. G., Freidhoff, L. R., Kwiterovich, K. A., Addison, B., Kagey-Sobotka, A., & Lichtenstein, L. M. (1997). Natural history of Hymenoptera venom sensitivity in adults. *J. Allergy Clin. Immunol. 100*(6 Pt 1), 760–6. doi:10.1016/S0091-6749(97)70270-7

Golden, D. B. K. (2015). Anaphylaxis to insect stings. *Immunol. Allergy Clin. North Am. 35*(2), 287–302. doi:10.1016/j.iac.2015.01.007

Golden, D. B. (2007). Insect Sting Anaphylaxis. *Immunol. Allergy Clin. North Am. 27*(2), 261–272. doi:10.1016/j.iac.2007.03.008

Golden, D. B., Demain, J., Freeman, T., Graft, D., Tankersley, M., Tracy, J., Blessing-Moore, J., Bernstein, D., Dinakar, C., Greenhawt, M., Khan, D., Lang, D., Nicklas, R., Oppenheimer, J., Portnoy, J., Randolph, C., Schuller, D., & Wallace, D. (2017). Stinging insect hypersensitivity. *Ann. Allergy, Asthma Immunol. 118*(1), 28–54. doi:10.1016/j.anai.2016.10.031

Golden, D. B., Kagey-Sobotka, A., Norman, P. S., Hamilton, R. G., & Lichtenstein, L. M. (2004). Outcomes of Allergy to Insect Stings in Children, with and without Venom Immunotherapy. *N. Engl. J. Med. 351*(7), 668–674. doi:10.1056/NEJMoa022952

Golden, D. B., Kwiterovich, K. A., Kagey-Sobotka, A., & Lichtenstein, L. M. (1998). Discontinuing venom immunotherapy: Extended observations. *J. Allergy Clin. Immunol. 101*(3), 298–305. doi:10.1016/S0091-6749(98)70239-8

Golden, D. B., Lawrence, I. D., Hamilton, R. H., Kagey-Sobotka, A., Valentine, M. D., & Lichtenstein, L. M. (1992). Clinical correlation of the venom-specific IgG antibody level during maintenance venom immunotherapy. *J. Allergy Clin. Immunol. 90*(3), 386–393. doi:10.1016/S0091-6749(05)80019-3

Golden, D. B., Moffitt, J., Nicklas, R. A., Freeman, T., Graft, D. F., Reisman, R. E., Tracy, J. M., Bernstein, D., Blessing-Moore, J., Cox, L., Khan, D. A., Lang, D. M., Oppenheimer, J., Portnoy, J. M., Randolph, C., Schuller, D. E., Spector, S. L., Tilles, S. A., & Wallace, D. (2011). Stinging insect hypersensitivity: A practice parameter update 2011. *J. Allergy Clin. Immunol. 127*(4), 852–854.e23. doi:10.1016/j.jaci.2011.01.025

Gómez, E., Campo, P., Rondón, C., Barrionuevo, E., Blanca-López, N., Torres, M. J., Herrera, R., Galindo, L., Mayorga, C., & Blanca, M. (2013). Role of the basophil activation test in the diagnosis of local allergic rhinitis. *J. Allergy Clin. Immunol. 132*(4), 975–976.e5. doi:10.1016/j.jaci.2013.07.016

González-De-Olano, D., Álvarez-Twose, I., Morgado, J. M., López, M. I. E., Castro, A. V., Díaz De Durana, M. D. A., Sánchez-Muñoz, L., Matito, A., De La Hoz Caballer, B., Sanz, M. L., Orfao, A., & Escribano, L. (2011). Evaluation of basophil activation in mastocytosis with hymenoptera venom anaphylaxis. *Cytom. Part B - Clin. Cytom. 80 B*(3), 167–175. doi:10.1002/cyto.b.20577

Gould, H. J. & Sutton, B. J. (2008). IgE in allergy and asthma today. *Nat. Rev. Immunol. 8*(3), 205–217. doi:10.1038/nri2273

Grabenhenrich, L. B., Dölle, S., Moneret-Vautrin, A., Köhli, A., Lange, L., Spindler, T., Ruëff, F., Nemat, K., Maris, I., Roumpedaki, E., Scherer, K., Ott, H., Reese, T., Mustakov, T., Lang, R., Fernandez-Rivas, M., Kowalski, M. L., Bilò, M. B., Hourihane, J. O., Papadopoulos, N. G., Beyer, K., Muraro, A., & Worm, M. (2016). Anaphylaxis in children and adolescents: The European Anaphylaxis Registry. *J. Allergy Clin. Immunol. 137*(4), 1128–1137.e1. doi:10.1016/j.jaci.2015.11.015

Graft, D. F., Golden, D. B., Reisman, R. E., Valentine, M. D., & Yunginger, J. W. (1998). The discontinuation of Hymenoptera venom immunotherapy. *J. Allergy Clin. Immunol. 101*(5), 573–575. doi:10.1016/S0091-6749(98)70161-7

Graft, D. F., Schuberth, K. C., Kagey-Sobotka, A., Kwiterovich, K. A., Niv, Y., Lichtenstein, L. M., & Valentine, M. D. (1984). A prospective study of the natural history of large local reactions after Hymenoptera stings in children. *J. Pediatr. 104*(5), 664–668. doi:10.1016/S0022-3476(84)80940-3

Graif, Y., Romano-Zelekha, O., Livne, I., Green, M. S., & Shohat, T. (2006). Allergic reactions to insect stings: results from a national survey of 10,000 junior high school children in Israel. *J. Allergy Clin. Immunol. 117*(6), 1435–9. doi:10.1016/j.jaci.2006.03.004

Grant, J. A., Rahr, R., Thueson, D. O., Lett-Brown, M. A., Hokanson, J. A., & Yunginger, J. W. (1983). Diagnosis of Polistes wasp hypersensitivity. *J. Allergy Clin. Immunol. 72*(4), 399–406. doi:10.1016/0091-6749(83)90506-7

Grayson, M. H. & Korenblat, P. E. (2003). The Emerging Role of Leukotriene Modifiers in Allergic Rhinitis. *Am. J. Respir. Med. 2*(6), 441–450. doi:10.1007/BF03256671

Grunwald, T., Bockisch, B., Spillner, E., Ring, J., Bredehorst, R., & Ollert, M. W. (2006). Molecular cloning and expression in insect cells of honeybee venom allergen acid phosphatase (Api m 3). *J. Allergy Clin. Immunol. 117*(4), 848–54. doi:10.1016/j.jaci.2005.12.1331

Habermann, E. (1972). Bee and wasp venoms. *Science (80-.). 177*(4046), 314–22. doi:10.1126/science.177.4046.314

Haeberli, G., Brönnimann, M., Hunziker, T., & Müller, U. (2003). Elevated basal serum tryptase and hymenoptera venom allergy: Relation to severity of sting reactions and to safety and efficacy of venom immunotherapy. *Clin. Exp. Allergy, 33*(9), 1216–1220. doi:10.1046/j.1365-2222.2003.01755.x

Haftenberger, M., Laußmann, D., Ellert, U., Kalcklösch, M., Langen, U., Schlaud, M., Schmitz, R., & Thamm, M. (2013). Prävalenz von Sensibilisierungen gegen Inhalations- und Nahrungsmit-

telallergene. *Bundesgesundheitsblatt - Gesundheitsforsch. - Gesundheitsschutz*, *56*(5-6), 687–697. doi:10.1007/s00103-012-1658-1

Hammad, H. & Lambrecht, B. N. (2008). Dendritic cells and epithelial cells: Linking innate and adaptive immunity in asthma. *Nat. Rev. Immunol. 8*(3), 193–204. doi:10.1038/nri2275

Hammar, E., Berglund, A., Hedin, A., Norrman, A., Rustas, K., Ytterström, U., & Åkerblom, E. (1990). An immunoassay for histamine based on monoclonal antibodies. *J. Immunol. Methods*, *128*(1), 51–58. doi:10.1016/0022-1759(90)90462-5

Harduar-Morano, L., Simon, M. R., Watkins, S., & Blackmore, C. (2011). A population-based epidemiologic study of emergency department visits for anaphylaxis in Florida. *J. Allergy Clin. Immunol. 128*(3), 594–600.e1. doi:10.1016/j.jaci.2011.04.049

Hatano, R., Ohnuma, K., Otsuka, H., Komiya, E., Taki, I., Iwata, S., Dang, N. H., Okumura, K., & Morimoto, C. (2015). CD26-mediated induction of EGR2 and IL-10 as potential regulatory mechanism for CD26 costimulatory pathway. *J. Immunol. 194*(3), 960–72. doi:10.4049/jimmunol.1402143

Hausmann, O. V., Gentinetta, T., Fux, M., Ducrest, S., Pichler, W. J., & Dahinden, C. A. (2011). Robust expression of CCR3 as a single basophil selection marker in flow cytometry. *Allergy*, *66*(1), 85–91. doi:10.1111/j.1398-9995.2010.02431.x

Hawrylowicz, C. M. & O'Garra, A. (2005). Potential role of interleukin-10-secreting regulatory T cells in allergy and asthma. *Nat. Rev. Immunol. 5*(4), 271–283. doi:10.1038/nri1589

Hemmer, W. (2008). Kreuzreaktivität auf Bienen- und Wespengift. *Der Hautarzt*, *59*(3), 194–199. doi:10.1007/s00105-008-1485-3

Hemmer, W., Focke, M., Kolarich, D., Wilson, I. B., Altmann, F., Wöhrl, S., Götz, M., & Jarisch, R. (2001). Antibody binding to venom carbohydrates is a frequent cause for double positivity to honeybee and yellow jacket venom in patients with stinging-insect allergy. *J. Allergy Clin. Immunol. 108*(6), 1045–1052. doi:10.1067/mai.2001.120013

Höcherl, N. & Tautz, J. (2015). Nesting behavior of the paper wasp Polistes dominula in Central Europe—a flexible system for expanding into new areas. *Ecosphere*, *6*(12), 1–11. doi:10.1890/ES15-00254.1

Hoffman, D. R. (1985a). Allergens in Hymenoptera venom XIII: Isolation and purification of protein components from three species of vespid venoms. *J. Allergy Clin. Immunol. 75*(5), 599–605. doi:10.1016/0091-6749(85)90036-3

Hoffman, D. R. (1985b). Allergens in Hymenoptera venom XIV: IgE binding activities of venom proteins from three species of vespids. *J. Allergy Clin. Immunol. 75*(5), 606–10. doi:10.1016/0091-6749(85)90037-5

Hoffman, D. R. (1985c). Allergens in Hymenoptera venom XV: The immunologic basis of vespid venom cross-reactivity. *J. Allergy Clin. Immunol. 75*(5), 611–3. doi:10.1016/0091-6749(85)90038-7

Hoffman, D. R. (1993a). Allergens in hymenoptera venom XXIV: The amino acid sequences of imported fire ant venom allergens II, III, and IV. *J. Allergy Clin. Immunol. 91*(1), 71–78. doi:10.1016/0091-6749(93)90298-T

Hoffman, D. R. (1993b). Allergens in Hymenoptera venom. XXV: The amino acid sequences of antigen 5 molecules and the structural basis of antigenic cross-reactivity. *J. Allergy Clin. Immunol. 92*(5), 707–16. doi:10.1016/0091-6749(93)90014-7

Hoffman, D. R. & Jacobson, R. S. (1996). Allergens in Hymenoptera venom. XXVII: bumblebee venom allergy and allergens. *J. Allergy Clin. Immunol. 97*(3), 812–21. doi:10.1016/S0091-6749(96)80159-X

Hoffman, D. R., Miller, J. S., & Sutton, J. L. (1980). Hymenoptera venom allergy: a geographic study. *Ann. Allergy*, *45*(5), 276–9.

Hoffman, D., Shipman, W., & Babin, D. (1977). Allergens in bee venom II. Two new high molecular weight allergenic specificities. *J. Allergy Clin. Immunol. 59*(2), 147–153. doi:10.1016/0091-6749(77)90217-2

Hoffman, D. R. (1977). Allergens in bee venom. III. Identification of allergen B of bee venom as an acid phosphatase. *J. Allergy Clin. Immunol. 59*(5), 364–366. doi:10.1016/0091-6749(77)90019-7

Hoffman, D. R. (1994). Allergens in Hymenoptera Venom. *Int. Arch. Allergy Immunol. 104*(2), 184–190. doi:10.1159/000236728

Hoffman, D. R., El-Choufani, S. E., Smith, M. M., & de Groot, H. (2001). Occupational allergy to bumblebees: Allergens of Bombus terrestris. *J. Allergy Clin. Immunol. 108*(5), 855–860. doi:10.1067/mai.2001.119029

Hoffman, D. R. & Jacobson, R. S. (1984). Allergens in hymenoptera venom XII: How much protein is in a sting? *Ann. Allergy*, *52*(4), 276–278.

Hoffman, D. R., Sakell, R. H., & Schmidt, M. (2005). Sol i 1, the phospholipase allergen of imported fire ant venom. *J. Allergy Clin. Immunol. 115*(3), 611–616. doi:10.1016/j.jaci.2004.11.020

Hoffmann, H. J., Santos, A. F., Mayorga, C., Nopp, A., Eberlein, B., Ferrer, M., Rouzaire, P., Ebo, D. G., Sabato, V., Sanz, M. L., Pecaric-Petkovic, T., Patil, S. U., Hausmann, O. V., Shreffler, W. G., Korosec, P., & Knol, E. F. (2015). The clinical utility of basophil activation testing in diagnosis and monitoring of allergic disease. *Allergy Eur. J. Allergy Clin. Immunol. 70*(11), 1393–1405. doi:10.1111/all.12698

Hofmann, S., Pfender, N., Weckesser, S., Blank, S., Huss-Marp, J., Spillner, E., & Jakob, T. (2011a). Reply. *J. Allergy Clin. Immunol. 128*(1), 248. doi:10.1016/j.jaci.2011.02.020

Hofmann, S. C., Pfender, N., Weckesser, S., Huss-Marp, J., & Jakob, T. (2011b). Added value of IgE detection to rApi m 1 and rVes v 5 in patients with Hymenoptera venom allergy. *J. Allergy Clin. Immunol. 127*(1), 265–267. doi:10.1016/j.jaci.2010.06.042

Holgate, S. T. & Polosa, R. (2008). Treatment strategies for allergy and asthma. *Nat. Rev. Immunol. 8*(3), 218–230. doi:10.1038/nri2262

Horak, K. & Jager, S. (1987). The Vienna challenge chamber - a new method for allergen exposition tests. *Wien Klin Wochenschr*, *99*, 509–510.

Hunt, K. J., Valentine, M. D., Sobotka, A. K., Benton, A. W., Amodio, F. J., & Lichtenstein, L. M. (1978). A Controlled Trial of Immunotherapy in Insect Hypersensitivity. *N. Engl. J. Med. 299*(4), 157–161. doi:10.1056/NEJM197807272990401

Ishizaka, K. & Ishizaka, T. (1967). Identification of γE-Antibodies as a Carrier of Reaginic Activity. *J. Immunol. 99*(6), 1187 LP –1198.

Jakob, T., Köhler, J., Blank, S., Magnusson, U., Huss-Marp, J., Spillner, E., & Lidholm, J. (2012). Comparable IgE reactivity to natural and recombinant Api m 1 in cross-reactive carbohydrate determinant–negative patients with bee venom allergy. *J. Allergy Clin. Immunol. 130*(1), 276–278. doi:10.1016/j.jaci.2012.03.048

Jappe, U., Raulf-Heimsoth, M., Hoffmann, M., Burow, G., Hubsch-Muller, C., & Enk, A. (2006). In vitro hymenoptera venom allergy diagnosis: improved by screening for cross-reactive carbohydrate determinants and reciprocal inhibition. *Allergy*, *61*(10), 1220–1229. doi:10.1111/j.1398-9995.2006.01232.x

Jeep, S., Reiprich, G., & Kunkel, G. (1992). Yellow jacket allergy. *Allergy*, *47*(1), 35–40. doi:10.1111/j.1398-9995.1992.tb02247.x

Jefferis, R., Reimer, C., Skvaril, F., de Lange, G., Goodall, D., Bentley, T., Phillips, D., Vlug, A., Harada, S., Radl, J., Claassen, E., Boersma, J., & Coolen, J. (1992). Evaluation of monoclonal antibodies having specificity for human IgG subclasses: results of the 2nd IUIS/WHO collaborative study. *Immunol. Lett. 31*(2), 143–168. doi:10.1016/0165-2478(92)90141-A

Jennings, A., Duggan, E., Perry, I. J., & Hourihane, J. O. (2010). Epidemiology of allergic reactions to hymenoptera stings in Irish school children. *Pediatr. Allergy Immunol. 21*(8), 1166–1170. doi:10.1111/j.1399-3038.2010.01054.x

Jin, C., Focke, M., Léonard, R., Jarisch, R., Altmann, F., & Hemmer, W. (2010). Reassessing the role of hyaluronidase in yellow jacket venom allergy. *J. Allergy Clin. Immunol. 125*(1), 184–190.e1. doi:10.1016/j.jaci.2009.08.037

Jin, C., Hantusch, B., Hemmer, W., Stadlmann, J., & Altmann, F. (2008). Affinity of IgE and IgG against cross-reactive carbohydrate determinants on plant and insect glycoproteins. *J. Allergy Clin. Immunol. 121*(1), 185–190.e2. doi:10.1016/j.jaci.2007.07.047

Johansson, S. G. O., Nopp, A., Hage, M., Olofsson, N., Lundahl, J., Wehlin, L., Soderstrom, L., Stiller, V., & Oman, H. (2005). Passive IgE-sensitization by blood transfusion. *Allergy*, *60*(9), 1192–1199. doi:10.1111/j.1398-9995.2005.00870.x

Johansson, S. (1967). Raised Levels of a New Immunoglobulin Class (IgND) in Asthma. *Lancet*, *290*(7523), 951–953. doi:10.1016/S0140-6736(67)90792-1

Juarez, C., Blanca, M., Miranda, A., Sanchez, F., Carmona, M. J., Avila, M. J., Fernandez, S., Fernandez, J., & Terrados, S. (1992). Specific IgE antibodies to vespids in the course of immunotherapy with Vespula germanica administered to patients sensitized to Polistes dominulus. *Allergy*, *47*(4), 299–302. doi:10.1111/j.1398-9995.1992.tb02057.x

Jung, K.-H., Baek, H., Shin, D., Lee, G., Park, S., Lee, S., Choi, D., Kim, W., & Bae, H. (2016). Protective Effects of Intratracheally-Administered Bee Venom Phospholipase A2 on Ovalbumin-Induced Allergic Asthma in Mice. *Toxins (Basel). 8*(10), 269. doi:10.3390/toxins8100269

Keating, M., Kagey-Sobotka, A., Hamilton, R., & Yunginger, J. (1991). Clinical and immunologic follow-up of patients who stop venom immunotherapy. *J. Allergy Clin. Immunol. 88*(3), 339–348. doi:10.1016/0091-6749(91)90095-6

Kemp, S. F., DeShazo, R. D., Moffitt, J. E., Williams, D. F., & Buhner, W. A. (2000). Expanding habitat of the imported fire ant (Solenopsis invicta) : A public health concern. *J. Allergy Clin. Immunol. 105*(4), 683–691. doi:10.1067/mai.2000.105707

Kepley, C. L., Youssef, L., Andrews, R. P., Wilson, B. S., & Oliver, J. M. (1999). Syk deficiency in nonreleaser basophils. *J. Allergy Clin. Immunol. 104*(2), 279–284. doi:10.1016/S0091-6749(99)70367-2

Kessel, A., Haj, T., Peri, R., Snir, A., Melamed, D., Sabo, E., & Toubi, E. (2012). Human CD19+CD25high B regulatory cells suppress proliferation of CD4+ T cells and enhance Foxp3 and CTLA-4 expression in T-regulatory cells. *Autoimmun. Rev. 11*(9), 670–677. doi:10.1016/j.autrev.2011.11.018

Kettner, A., Hughes, G. J., Frutiger, S., Astori, M., Roggero, M., Spertini, F., & Corradin, G. (2001). Api m 6: A new bee venom allergen. *J. Allergy Clin. Immunol. 107*(5), 914–920. doi:10.1067/mai.2001.113867

Khan, F. M., Ueno-Yamanouchi, A., Serushago, B., Bowen, T., Lyon, A. W., Lu, C., & Storek, J. (2012). Basophil activation test compared to skin prick test and fluorescence enzyme immunoassay for aeroallergen-specific Immunoglobulin-E. *Allergy, Asthma Clin. Immunol. 8*(1), 1. doi:10.1186/1710-1492-8-1

King, T. P., Lu, G., Gonzalez, M., Qian, N., & Soldatova, L. (1996). Yellow jacket venom allergens, hyaluronidase and phospholipase: sequence similarity and antigenic cross-reactivity with their hornet and wasp homologs and possible implications for clinical allergy. *J. Allergy Clin. Immunol. 98*(3), 588–600. doi:10.1016/S0091-6749(96)70093-3

King, T. P., Sobotka, A. K., Alagon, A., Kochoumian, L., & Lichtenstein, L. M. (1978). Protein allergens of white-faced hornet, yellow hornet, and yellow jacket venoms. *Biochemistry, 17*(24), 5165–5174. doi:10.1021/bi00617a016

King, T. P., Kochoumian, L., & Lam, T. (1987). Immunochemical observations of antigen 5, a major venom allergen of hornets, yellowjackets and wasps. *Mol. Immunol. 24*(8), 857–864. doi:10.1016/0161-5890(87)90188-X

Kleine-Tebbe, J. & Jakob, T. (2015). Molecular allergy diagnostics using IgE singleplex determinations: methodological and practical considerations for use in clinical routine. *Allergo J. Int. 24*(6), 185–197. doi:10.1007/s40629-015-0067-z

Klemann, C., Wagner, L., Stephan, M., & von Hörsten, S. (2016). Cut to the chase: a review of CD26/dipeptidyl peptidase-4's (DPP4) entanglement in the immune system. *Clin. Exp. Immunol. 185*(1), 1–21. doi:10.1111/cei.12781

Knol, E. F., Mul, F. P., Jansen, H., Calafat, J., & Roos, D. (1991). Monitoring human basophil activation via CD63 monoclonal antibody 435. *J. Allergy Clin. Immunol. 88*(3), 328–338. doi:10.1016/0091-6749(91)90094-5

Knol, E. F., Mul, F. P., Kujipers, T. W., Verhoeven, A. J., & Roos, D. (1992). Intracellular events in anti-IgE nonreleasing human basophils. *J. Allergy Clin. Immunol. 90*(1), 92–103. doi:10.1016/S0091-6749(06)80015-1

Köhler, J., Blank, S., Müller, S., Bantleon, F., Frick, M., Huss-Marp, J., Lidholm, J., Spillner, E., & Jakob, T. (2014). Component resolution reveals additional major allergens in patients with honeybee venom allergy. *J. Allergy Clin. Immunol. 133*(5), 1383–1389.e6. doi:10.1016/j.jaci.2013.10.060

Kolarich, D., Leonard, R., Hemmer, W., & Altmann, F. (2005). The N-glycans of yellow jacket venom hyaluronidases and the protein sequence of its major isoform in Vespula vulgaris. *FEBS J. 272*(20), 5182–5190. doi:10.1111/j.1742-4658.2005.04841.x

Kolarich, D., Loos, A., Léonard, R., Mach, L., Marzban, G., Hemmer, W., & Altmann, F. (2007). A proteomic study of the major allergens from yellow jacket venoms. *Proteomics, 7*(10), 1615–1623. doi:10.1002/pmic.200600800

Kontou-Fili, K. (2002). Patients with negative skin tests. *Curr. Opin. Allergy Clin. Immunol. 2*(4), 353–357. doi:10.1097/00130832-200208000-00010

Korosec, P., Erzen, R., Silar, M., Bajrovic, N., Kopac, P., & Kosnik, M. (2009). Basophil responsiveness in patients with insect sting allergies and negative venom-specific immunoglobulin E and skin prick test results. *Clin. Exp. Allergy*, *39*(11), 1730–1737. doi:10.1111/j.1365-2222.2009.03347.x

Korošec, P., Šilar, M., Eržen, R., Čelesnik, N., Bajrović, N., Zidarn, M., & Košnik, M. (2013). Clinical routine utility of Basophil activation testing for diagnosis of hymenoptera-allergic patients with emphasis on individuals with negative venom-specific IgE antibodies. *Int. Arch. Allergy Immunol. 161*(4), 363–368. doi:10.1159/000348500

Korošec, P., Valenta, R., Mittermann, I., Čelesnik, N., Eržen, R., Zidarn, M., & Košnik, M. (2011). Low sensitivity of commercially available rApi m 1 for diagnosis of honeybee venom allergy. *J. Allergy Clin. Immunol. 128*(3), 671–673. doi:10.1016/j.jaci.2011.03.012

Korošec, P., Valenta, R., Mittermann, I., Čelesnik, N., Šilar, M., Zidarn, M., & Košnik, M. (2012). High sensitivity of CAP-FEIA rVes v 5 and rVes v 1 for diagnosis of Vespula venom allergy. *J. Allergy Clin. Immunol. 129*(5), 1406–1408. doi:10.1016/j.jaci.2011.12.975

Koschel, D. S., Schmies, M., Weber, C. N., Höffken, G., & Balck, F. (2014). Tolerated sting challenge in patients on Hymenoptera venom immunotherapy improves health-related quality of life. *J. Investig. Allergol. Clin. Immunol. 24*(4), 226–30.

Kosnik, M., Silar, M., Bajrovic, N., Music, E., & Korosec, P. (2005). High sensitivity of basophils predicts side-effects in venom immunotherapy. *Allergy*, *60*(11), 1401–1406. doi:10.1111/j.1398-9995.2005.00894.x

Kraft, S. & Kinet, J.-P. (2007). New developments in FcϵRI regulation, function and inhibition. *Nat. Rev. Immunol. 7*(5), 365–378. doi:10.1038/nri2072

Kucera, P., Cvackova, M., Hulikova, K., Juzova, O., & Pachl, J. (2010). Basophil Activation Can Predict Clinical Immunotherapy. *J. Investig. Allergol. Clin. Immunol. 20*(2), 110–116.

Kuchler, K., Gmachl, M., Sippl, M. J., & Kreil, G. (1989). Analysis of the cDNA for phospholipase A2 from honeybee venom glands. The deduced amino acid sequence reveals homology to the corresponding vertebrate enzymes. *Eur. J. Biochem. 184*(1), 249–54. doi:10.1111/j.1432-1033.1989.tb15014.x

Lalek, N., Kosnik, M., Silar, M., & Korosec, P. (2010). Immunoglobulin G-dependent changes in basophil allergen threshold sensitivity during birch pollen immunotherapy. *Clin. Exp. Allergy*, *40*(8), 1186–1193. doi:10.1111/j.1365-2222.2010.03524.x

Lambert, C., Guilloux, L., Dzviga, C., Gourgaud-Massias, C., & Genin, C. (2003). Flow cytometry versus histamine release analysis of in vitro basophil degranulation in allergy to Hymenoptera venom. *Cytometry*, *52B*(1), 13–19. doi:10.1002/cyto.b.10012

Lee, K. M., Stott, R. T., Zhao, G., SooHoo, J., Xiong, W., Lian, M. M., Fitzgerald, L., Shi, S., Akrawi, E., Lei, J., Deng, S., Yeh, H., Markmann, J. F., & Kim, J. I. (2014). TGF-β-producing regulatory B cells induce regulatory T cells and promote transplantation tolerance. *Eur. J. Immunol. 44*(6), 1728–1736. doi:10.1002/eji.201344062

Leonardi, A., Borghesan, F., Faggian, D., DePaoli, M., Secchi, A. G., & Plebani, M. (2000). Tear and serum soluble leukocyte activation markers in conjunctival allergic diseases. *Am. J. Ophthalmol. 129*(2), 151–158. doi:10.1016/S0002-9394(99)00295-0

Lerch, E. & Müller, U. R. (1998). Long-term protection after stopping venom immunotherapy: Results of re-stings in 200 patients. *J. Allergy Clin. Immunol. 101*(5), 606–612. doi:10.1016/S0091-6749(98)70167-8

Lichtenstein, L. M. & Abraham, O. G. (1964). Studies on the Mechanisms of Hypersensitivity Phenomena. IX. Histamine Release from Human Leukocytes by Ragweed Pollen Antigen. *J. Exp. Med. 120*(4), 507–30. doi:10.1084/jem.120.4.507

Lichtenstein, L. M., Valentine, M. D., & Sobotka, A. K. (1974). A Case for Venom Treatment in Anaphylactic Sensitivity to Hymenoptera Sting. *N. Engl. J. Med. 290*(22), 1223–1227. doi:10.1056/NEJM197405302902204

Looney, T. J., Lee, J.-Y., Roskin, K. M., Hoh, R. A., King, J., Glanville, J., Liu, Y., Pham, T. D., Dekker, C. L., Davis, M. M., & Boyd, S. D. (2016). Human B-cell isotype switching origins of IgE. *J. Allergy Clin. Immunol. 137*(2), 579–586.e7. doi:10.1016/j.jaci.2015.07.014

Lowe, J., Bird, P., Hardie, D., Jefferis, R., & Ling, N. R. (1982). Monoclonal antibodies (McAbs) to determinants on human gamma chains: properties of antibodies showing subclass restriction or subclass specificity. *Immunology*, *47*(2), 329–36.

Lu, G., Villalba, M., Coscia, M. R., Hoffman, D. R., & King, T. P. (1993). Sequence analysis and antigenic cross-reactivity of a venom allergen, antigen 5, from hornets, wasps, and yellow jackets. *J. Immunol. 150*(7), 2823–2830.

Lu, G., Kochoumian, L., & King, T. P. (1995). Sequence Identity and Antigenic Cross-reactivity of White Face Hornet Venom Allergen, Also a Hyaluronidase, with Other Proteins. *J. Biol. Chem. 270*(9), 4457–4465. doi:10.1074/jbc.270.9.4457

Ludman, S. W. & Boyle, R. J. (2015). Stinging insect allergy: current perspectives on venom immunotherapy. *J. Asthma Allergy*, *8*, 75–86. doi:10.2147/JAA.S62288

MacGlashan, D. (2010). Expression of CD203c and CD63 in human basophils: relationship to differential regulation of piecemeal and anaphylactic degranulation processes. *Clin. Exp. Allergy*, *40*(9), 1365–1377. doi:10.1111/j.1365-2222.2010.03572.x

Mamessier, E., Birnbaum, J., Dupuy, P., Vervloet, D., & Magnan, A. (2006). Ultra-rush venom immunotherapy induces differential T cell activation and regulatory patterns according to the severity of allergy. *Clin. Exp. Allergy*, *36*(6), 704–713. doi:10.1111/j.1365-2222.2006.02487.x

Mari, A. (2005). Importance of Databases in Experimental and Clinical Allergology. *Int. Arch. Allergy Immunol. 138*(1), 88–96. doi:10.1159/000087848

Marone, G., Triggiani, M., & de Paulis, A. (2005). Mast cells and basophils: friends as well as foes in bronchial asthma? *Trends Immunol. 26*(1), 25–31. doi:10.1016/j.it.2004.10.010

Marsh, D. G., Goodfriend, L., King, T. P., Lowenstein, H., & Platts-Mills, T. A. (1986). Allergen nomenclature. *Bull. World Health Organ. 64*(5), 767–774.

Marshall, J. S. (2004). Mast-cell responses to pathogens. *Nat. Rev. Immunol. 4*(10), 787–799. doi:10.1038/nri1460

Matricardi, P. M. & Kleine-Tebbe, J. (2016a). Molecular Allergology between Precision Medicine and the Choosing Wisely initiative. *Clin. Exp. Allergy*, *46*(5), 664–667. doi:10.1111/cea.12679

Matricardi, P. M., Kleine-Tebbe, J., Hoffmann, H., Valenta, R., & Ollert, M. (2016b). *Molecular Allergology User's Guide*. Vienna, Austria: John Wiley & Sons, Ltd.

Mauriello, P., Barde, S., Georgitis, J., & Reisman, R. (1984). Natural history of large local reactions from stinging insects. *J. Allergy Clin. Immunol. 74*(4), 494–498. doi:10.1016/0091-6749(84)90384-1

Meiler, F., Zumkehr, J., Klunker, S., Rückert, B., Akdis, C. A., & Akdis, M. (2008). In vivo switch to IL-10–secreting T regulatory cells in high dose allergen exposure. *J. Exp. Med. 205*(12), 2887–2898. doi:10.1084/jem.20080193

Menzella, F., Lusuardi, M., Galeone, C., Taddei, S., & Zucchi, L. (2015). Profile of anti-IL-5 mAb mepolizumab in the treatment of severe refractory asthma and hypereosinophilic diseases. *J. Asthma Allergy*, 105. doi:10.2147/JAA.S40244

Mertens, M., Amler, S., Moerschbacher, B. M., & Brehler, R. (2010). Cross-reactive carbohydrate determinants strongly affect the results of the basophil activation test in hymenoptera-venom allergy. *Clin. Exp. Allergy*, *40*(9), 1333–1345. doi:10.1111/j.1365-2222.2010.03535.x

Michel, Y., McIntyre, M., Ginglinger, H., Ollert, M., Cifuentes, L., Blank, S., & Spillner, E. (2012). The putative serine protease inhibitor Api m 6 from Apis Mellifera venom: Recombinant and structural evaluation. *J. Investig. Allergol. Clin. Immunol. 22*(7), 476–484.

Mikkelsen, S., Bibby, B., Dolberg, M., Dahl, R., & Hoffmann, H. (2010). Basophil sensitivity through CD63 or CD203c is a functional measure for specific immunotherapy. *Clin. Mol. Allergy*, *8*(1), 2. doi:10.1186/1476-7961-8-2

Mittermann, I., Zidarn, M., Silar, M., Markovic-Housley, Z., Aberer, W., Korosec, P., Kosnik, M., & Valenta, R. (2010). Recombinant allergen-based IgE testing to distinguish bee and wasp allergy. *J. Allergy Clin. Immunol. 125*(6), 1300–1307.e3. doi:10.1016/j.jaci.2010.03.017

Möbs, C., Müller, J., Rudzio, A., Pickert, J., Blank, S., Jakob, T., Spillner, E., & Pfützner, W. (2015). Decline of Ves v 5-specific blocking capacity in wasp venom-allergic patients after stopping allergen immunotherapy. *Allergy*, *70*(6), 715–9. doi:10.1111/all.12606

Möbs, C., Ipsen, H., Mayer, L., Slotosch, C., Petersen, A., Würtzen, P. A., Hertl, M., & Pfützner, W. (2012). Birch pollen immunotherapy results in long-term loss of Bet v 1–specific TH2 responses, transient TR1 activation, and synthesis of IgE-blocking antibodies. *J. Allergy Clin. Immunol. 130*(5), 1108–1116.e6. doi:10.1016/j.jaci.2012.07.056

Moffitt, J. E. (2003). Allergic Reactions to Insect Stings and Bites. *South. Med. J. 96*(11), 1073–1079. doi:10.1097/01.SMJ.0000097885.28467.21

Moffitt, J. E., Golden, D. B., Reisman, R. E., Lee, R., Nicklas, R., Freeman, T., DeShazo, R., Tracy, J., Bernstein, I. L., & Blessing-Moore, J. (2004). Stinging insect hypersensitivity: A practice parameter update. *J. Allergy Clin. Immunol. 114*(4), 869–886. doi:10.1016/j.jaci.2004.07.046

Moneret-Vautrin, D. A., Sainte-Laudy, J., Kanny, G., & Frémont, S. (1999). Human basophil activation measured by CD63 expression and LTC4 release in IgE-mediated food allergy. *Ann. Allergy, Asthma Immunol. 82*(1), 33–40. doi:10.1016/S1081-1206(10)62657-9

Monsalve, R. I., Vega, A., Marqués, L., Miranda, A., Fernández, J., Soriano, V., Cruz, S., Domínguez-Noche, C., Sánchez-Morillas, L., Armisen-Gil, M., Guspí, R., & Barber, D. (2012). Component-resolved diagnosis of vespid venom-allergic individuals: phospholipases and antigen 5s are necessary to identify Vespula or Polistes sensitization. *Allergy*, *67*(4), 528–536. doi:10.1111/j.1398-9995.2011.02781.x

Monsalve, R. I., Lu, G., & King, T. P. (1999). Expressions of Recombinant Venom Allergen, Antigen 5 of Yellowjacket (Vespula vulgaris) and Paper Wasp (Polistes annularis), in Bacteria or Yeast. *Protein Expr. Purif. 16*(3), 410–416. doi:10.1006/prep.1999.1082

Monteseirín, J., Fernández-Pineda, I., Chacón, P., Vega, A., Bonilla, I., Camacho, M. J., Fernández-Delgado, L., Conde, J., & Sobrino, F. (2004). Myeloperoxidase Release After Allergen-Specific Conjunctival Challenge. *J. Asthma*, *41*(6), 639–643. doi:10.1081/JAS-200026407

Mosbech, H., Tang, L., & Linneberg, A. (2016). Insect Sting Reactions and Specific IgE to Venom and Major Allergens in a General Population. *Int. Arch. Allergy Immunol. 170*(3), 194–200. doi:10.1159/000448399

Movérare, R., Blume, K., Lind, P., Crevel, R., Marknell DeWitt, Å., & Cochrane, S. (2017). Human Allergen-Specific IgG Subclass Antibodies Measured Using ImmunoCAP Technology. *Int. Arch. Allergy Immunol. 172*(1), 1–10. doi:10.1159/000455098

Müller, U. R. (2002). Recombinant Hymenoptera venom allergens. *Allergy*, *57*(7), 570–576. doi:10.1034/j.1398-9995.2002.02157.x

Müller, U. R., Dudler, T., Schneider, T., Crameri, R., Fischer, H., Skrbic, D., Maibach, R., Blaser, K., & Suter, M. (1995a). Type I skin reactivity to native and recombinant phospholipase A2 from honeybee venom is similar. *J. Allergy Clin. Immunol. 96*(3), 395–402. doi:10.1016/S0091-6749(95)70059-5

Müller, U. R., Dudler, T., Schneider, T., Crameri, R., Fischer, H., Skrbic, D., Maibach, R., Blaser, K., & Suter, M. (1995b). Type I skin reactivity to native and recombinant phospholipase A2 from honeybee venom is similar. *J. Allergy Clin. Immunol. 96*(3), 395–402. doi:10.1016/0091-6749(91)90392-2

Müller, U. R., Johansen, N., Petersen, A. B., Fromberg-Nielsen, J., & Haeberli, G. (2009). Hymenoptera venom allergy: analysis of double positivity to honey bee and Vespula venom by estimation of IgE antibodies to species-specific major allergens Api m1 and Ves v5. *Allergy*, *64*(4), 543–548. doi:10.1111/j.1398-9995.2008.01794.x

Müller, U. R. & Mosbech, H. (1993). Position Paper: Immunotherapy with Hymenoptera venoms. *Allergy*, *48*(s14), 37–46. doi:10.1111/j.1398-9995.1993.tb04755.x

Müller, U., Fricker, M., Wymann, D., Blaser, K., & Crameri, R. (1997). Increased specificity of diagnostic tests with recombinant major bee venom allergen phospholipase A2. *Clin. Exp. Allergy*, *27*(8), 915–20. doi:10.1111/j.1365-2222.1997.tb01233.x

Müller, U., Helbling, A., & Berchtold, E. (1992). Immunotherapy with honeybee venom and yellow jacket venom is different regarding efficacy and safety. *J. Allergy Clin. Immunol. 89*(2), 529–35. doi:10.1016/0091-6749(92)90319-W

Müller, U., Schmid-Grendelmeier, P., Hausmann, O., & Helbling, A. (2012). IgE to recombinant allergens Api m 1, Ves v 1, and Ves v 5 distinguish double sensitization from crossreaction in venom allergy. *Allergy*, *67*(8), 1069–1073. doi:10.1111/j.1398-9995.2012.02847.x

Murphy, K. & Weaver, C. (2017). *Janeway's immunobiology* (9. Edition). Garland Science, Taylor & Francis Group, LLC.

Nathan, R. A. (2003). Pharmacotherapy for allergic rhinitis: a critical review of leukotriene receptor antagonists compared with other treatments. *Ann. Allergy, Asthma Immunol. 90*(2), 182–191. doi:10.1016/S1081-1206(10)62138-2

Nathan, R. A., Eccles, R., Howarth, P. H., Steinsvåg, S. K., & Togias, A. (2005). Objective monitoring of nasal patency and nasal physiology in rhinitis. *J. Allergy Clin. Immunol. 115*(3 Suppl 1), S442–59. doi:10.1016/j.jaci.2004.12.015

Navarro, L. A., Peláez, A., De La Torre, F., Burillo, J. M. T., Megías, J., & Martínez, I. (2004). Epidemiological factors on Hymenoptera venom allergy in a Spanish adult population. *J. Investig. Allergol. Clin. Immunol. 14*(2), 134–141.

Neis, M., Wurpts, G., Wilbers, L., & Merk, H. (2011). In-vitro-Diagnostik und Monitoring bei Insektengifthyposensibilisierung. *Der Hautarzt*, *62*(9), 677–682. doi:10.1007/s00105-011-2160-7

Niederberger, V., Niggemann, B., Kraft, D., Spitzauer, S., & Valenta, R. (2002). Evolution of IgM, IgE and IgG1-4antibody responses in early childhood monitored with recombinant allergen components: implications for class switch mechanisms. *Eur. J. Immunol. 32*(2), 576–584. doi:10.1002/1521-4141(200202)32:2<576::AID-IMMU576>3.0.CO;2-U

Niedoszytko, M., Bonadonna, P., Elberink, J. N. G. O., & Golden, D. B. K. (2014). Epidemiology, diagnosis, and treatment of Hymenoptera venom allergy in mastocytosis patients. *Immunol. Allergy Clin. North Am. 34*(2), 365–381. doi:10.1016/j.iac.2014.02.004

Nieto-Fontarigo, J. J., González-Barcala, F. J., San José, E., Arias, P., Nogueira, M., & Salgado, F. J. (2016). CD26 and Asthma: a Comprehensive Review. *Clin. Rev. Allergy Immunol.* 1–22. doi:10.1007/s12016-016-8578-z

Nittner-Marszalska, M., Liebhart, J., Liebhart, E., Dor, A., Dobek, R., Obojski, A., & Medrala, W. (2004). Prevalence of Hymenoptera venom allergy and its immunological markers current in adults in Poland. *Med. Sci. Monit. 10*(7), CR324–9.

Nopp, A., Cardell, L. O., Johansson, S. G. O., & Öman, H. (2009). CD-sens: a biological measure of immunological changes stimulated by ASIT. *Allergy*, *64*(5), 811–814. doi:10.1111/j.1398-9995.2008.01900.x

Novak, N., Mete, N., Bussmann, C., Maintz, L., Bieber, T., Akdis, M., Zumkehr, J., Jutel, M., & Akdis, C. (2012). Early suppression of basophil activation during allergen-specific immunotherapy by histamine receptor 2. *J. Allergy Clin. Immunol. 130*(5), 1153–1158.e2. doi:10.1016/j.jaci.2012.04.039

Novembre, Cianferoni, Bernardini, Veltroni, Ingargiola, Lombardi, & Vierucci. (1998). Epidemiology of insect venom sensitivity in children and its correlation to clinical and atopic features. *Clin. Exp. Allergy*, *28*(7), 834–838. doi:10.1046/j.1365-2222.1998.00313.x

Nullens, S., Sabato, V., Faber, M., Leysen, J., Bridts, C. H., De Clerck, L. S., Falcone, F. H., Maurer, M., & Ebo, D. G. (2013). Basophilic histamine content and release during venom immunotherapy: Insights by flow cytometry. *Cytom. Part B Clin. Cytom. 84B*(3), 173–178. doi:10.1002/cyto.b.21084

Ocmant, A., Peignois, Y., Mulier, S., Hanssens, L., Michils, A., & Schandené, L. (2007). Flow cytometry for basophil activation markers: The measurement of CD203c up-regulation is as reliable as CD63 expression in the diagnosis of cat allergy. *J. Immunol. Methods*, *320*(1-2), 40–48. doi:10.1016/j.jim.2006.12.002

Ollert, M. & Blank, S. (2015). Anaphylaxis to Insect Venom Allergens: Role of Molecular Diagnostics. *Curr. Allergy Asthma Rep. 15*(5), 26. doi:10.1007/s11882-015-0527-z

Onbaşı, K., Onbaşı, O., Eminbeyli, L., & Kaynak, C. (2008). Prevalence and alternative therapy methods for bee and wasp allergy in Van. *Allergy*, *63*(2), 246–247. doi:10.1111/j.1398-9995.2007.01588.x

Ott, H., Tenbrock, K., Baron, J., Merk, H., & Lehmann, S. (2011). Basophil Activation Test for the Diagnosis of Hymenoptera Venom Allergy in Childhood: a Pilot Study. *Klin. Pädiatrie*, *223*(01), 27–32. doi:10.1055/s-0030-1268430

Palgan, K., Bartuzi, Z., & Gotz-Zbikowska, M. (2014). Treatment with a combination of omalizumab and specific immunotherapy for severe anaphylaxis after a wasp sting. *Int. J. Immunopathol. Pharmacol. 27*(1), 109–12. doi:10.1177/039463201402700114

Palm, N. W., Rosenstein, R. K., Yu, S., Schenten, D. D., Florsheim, E., & Medzhitov, R. (2013). Bee venom phospholipase A2 induces a primary type 2 response that is dependent on the receptor ST2 and confers protective immunity. *Immunity*, *39*(5), 976–985. doi:10.1016/j.immuni.2013.10.006

Pantera, B., Hoffman, D. R., Carresi, L., Cappugi, G., Turillazzi, S., Manao, G., Severino, M., Spadolini, I., Orsomando, G., Moneti, G., & Pazzagli, L. (2003). Characterization of the major allergens purified from the venom of the paper wasp Polistes gallicus. *Biochim. Biophys. Acta*, *1623*(2-3), 72–81. doi:10.1016/j.bbagen.2003.07.001

Pastorello, E. A., Incorvaia, C., Sarassi, A., Qualizza, R., Bigi, A., & Farioli, L. (1988). Epidemiological and clinical study on bee venom allergy among beekeepers. *Boll. Ist. Sieroter. Milan. 67*(5-6), 386–92.

Patalano, S., Vlasova, A., Wyatt, C., Ewels, P., Camara, F., Ferreira, P. G., Asher, C. L., Jurkowski, T. P., Segonds-Pichon, A., Bachman, M., González-Navarrete, I., Minoche, A. E., Krueger, F., Lowy, E., Marcet-Houben, M., Rodriguez-Ales, J. L., Nascimento, F. S., Balasubramanian, S., Gabaldon, T., Tarver, J. E., Andrews, S., Himmelbauer, H., Hughes, W. O. H., Guigó, R., Reik, W., & Sumner, S. (2015). Molecular signatures of plastic phenotypes in two eusocial insect species with simple societies. *Proc. Natl. Acad. Sci. 112*(45), 13970–13975. doi:10.1073/pnas.1515937112

Patil, S. U. & Shreffler, W. G. (2012). Immunology in the Clinic Review Series; focus on allergies: basophils as biomarkers for assessing immune modulation. *Clin. Exp. Immunol. 167*(1), 59–66. doi:10.1111/j.1365-2249.2011.04503.x

Paull, B. R., Yunginger, J. W., & Gleich, G. J. (1977). Melittin: an allergen of honeybee venom. *J. Allergy Clin. Immunol. 59*(4), 334–8. doi:10.1016/0091-6749(77)90056-2

Pearce, E. J., Caspar, P., Grzych, J.-M., Lewis, F. A., & Sher, A. (1991). Downregulation of Th1 cytokine production accompanies induction of Th2 responses by a parasitic helminth, Schistosoma mansoni. *J. Exp. Med. 173*(1), 159–166. doi:10.1084/jem.173.1.159

Peiren, N., de Graaf, D. C., Brunain, M., Bridts, C. H., Ebo, D. G., Stevens, W. J., & Jacobs, F. J. (2006). Molecular cloning and expression of icarapin, a novel IgE-binding bee venom protein. *FEBS Lett. 580*(20), 4895–4899. doi:10.1016/j.febslet.2006.08.005

Peiren, N., Vanrobaeys, F., de Graaf, D. C., Devreese, B., Van Beeumen, J., & Jacobs, F. J. (2005). The protein composition of honeybee venom reconsidered by a proteomic approach. *Biochim. Biophys. Acta*, *1752*(1), 1–5. doi:10.1016/j.bbapap.2005.07.017

Pejler, G., Åbrink, M., Ringvall, M., & Wernersson, S. (2007). Mast Cell Proteases. In *Adv. immunol.* (Vol. 95, *07*, pp. 167–255). doi:10.1016/S0065-2776(07)95006-3

Perry, T. T., Matsui, E. C., Conover-Walker, M. K., & Wood, R. A. (2004). Risk of oral food challenges. *J. Allergy Clin. Immunol. 114*(5), 1164–1168. doi:10.1016/j.jaci.2004.07.063

Peternelj, A., Silar, M., Erzen, R., Kosnik, M., & Korosec, P. (2008). Basophil Sensitivity in Patients Not Responding to Venom Immunotherapy. *Int. Arch. Allergy Immunol. 146*(3), 248–254. doi:10.1159/000116361

Pfaar, O., Bachert, C., Bufe, A., Buhl, R., Ebner, C., Eng, P., Friedrichs, F., Fuchs, T., Hamelmann, E., Hartwig-Bade, D., Hering, T., Huttegger, I., Jung, K., Klimek, L., Kopp, M. V., Merk, H., Rabe, U., Saloga, J., Schmid-Grendelmeier, P., Schuster, A., Schwerk, N., Sitter, H., Umpfenbach, U., Wedi, B., Wöhrl, S., Worm, M., & Kleine-Tebbe, J. (2014). Guideline on allergen-specific immunotherapy in IgE-mediated allergic diseases. *Allergo J. Int. 23*(8), 282–319. doi:10.1007/s40629-014-0032-2

Pierkes, M., Bellinghausen, I., Hultsch, T., Metz, G., Knop, J., & Saloga, J. (1999). Decreased release of histamine and sulfidoleukotrienes by human peripheral blood leukocytes after wasp venom immunotherapy is partially due to induction of IL-10 and IFN-γ production of T cells. *J. Allergy Clin. Immunol. 103*(2), 326–332. doi:10.1016/S0091-6749(99)70509-9

Platz, I. J., Binder, M., Marxer, A., Lischka, G., Valent, P., & Bühring, H.-J. (2001). Hymenoptera-Venom-Induced Upregulation of the Basophil Activation Marker Ecto-Nucleotide Pyrophosphatase/Phosphodiesterase 3 in Sensitized Individuals. *Int. Arch. Allergy Immunol. 126*(4), 335–342. doi:10.1159/000049531

Portnoy, J. M., Moffitt, J. E., Golden, D. B., Bernstein, W. E., Dykewicz, M. S., Fineman, S. M., Lee, R. E., Li, J. T., Nicklas, R. a., Schuller, D. E., & Spector, S. L. (1999). Stinging insect hypersensitivity: a practice parameter. *J. Allergy Clin. Immunol. 103*(5 Pt 1), 963–80. doi:10.1016/S0091-6749(99)70450-1

Potiwat, R. & Sitcharungsi, R. (2015). Ant allergens and hypersensitivity reactions in response to ant stings. *Asian Pacific J. allergy Immunol. 33*(4), 267–75.

Przybilla, B., Ruëff, F., Walker, A., Räwer, H.-C., Aberer, W., Bauer, C. P., Berdel, D., Biedermann, T., Brockow, K., Forster, J., Fuchs, T., Hamelmann, E., Jakob, T., Jarisch, R., Merk, H. F., Müller, U., Ott, H., Sitter, W., Urbanek, R., & Wedi, B. (2011). Diagnose und Therapie der Bienen- und Wespengiftallergie. *Allergo J. 20*(6), 318–339. doi:10.1007/BF03362543

Pucci, S., D'Alò, S., De Pasquale, T., Illuminati, I., Makri, E., & Incorvaia, C. (2015). Risk of anaphylaxis in patients with large local reactions to hymenoptera stings: a retrospective and prospective study. *Clin. Mol. Allergy*, *13*(1), 21. doi:10.1186/s12948-015-0030-z

Pumphrey, R. S. H. (2000). Lessons for management of anaphylaxis from a study of fatal reactions. *Clin. Exp. Allergy*, *30*(8), 1144–1150. doi:10.1046/j.1365-2222.2000.00864.x

Radauer, C., Nandy, A., Ferreira, F., Goodman, R. E., Larsen, J. N., Lidholm, J., Pomés, A., Raulf-Heimsoth, M., Rozynek, P., Thomas, W. R., & Breiteneder, H. (2014). Update of the WHO/IUIS Allergen Nomenclature Database based on analysis of allergen sequences. *Allergy*, *69*(4), 413–419. doi:10.1111/all.12348

Radulovic, S., Jacobson, M. R., Durham, S. R., & Nouri-Aria, K. T. (2008). Grass pollen immunotherapy induces Foxp3-expressing CD4+CD25+ cells in the nasal mucosa. *J. Allergy Clin. Immunol. 121*(6), 1467–1472.e1. doi:10.1016/j.jaci.2008.03.013

Rajan, T. V. (2003). The Gell-Coombs classification of hypersensitivity reactions: a re-interpretation. *Trends Immunol. 24*(7), 376–9. doi:10.1016/S1471-4906(03)00142-X

Reisman, R. E. (1992). Natural history of insect sting allergy: Relationship of severity of symptoms of initial sting anaphylaxis to re-sting reactions. *J. Allergy Clin. Immunol. 90*(3), 335–339. doi:10.1016/S0091-6749(05)80012-0

Ricciardi, L. (2016). Omalizumab: A useful tool for inducing tolerance to bee venom immunotherapy. *Int. J. Immunopathol. Pharmacol. 29*(4), 726–728. doi:10.1177/0394632016670920

Rietveld, M. J. A., Schreurs, M. W. J., Gerth Van Wijk, R., Van Daele, P. L. A., & Hermans, M. A. W. (2016). The Basophil Activation Test Is Not a Useful Screening Tool for Hymenoptera Venom-Related Anaphylaxis in Patients with Systemic Mastocytosis. *Int. Arch. Allergy Immunol. 169*(2), 125–129. doi:10.1159/000444996

Rivera, J. & Gilfillan, A. M. (2006). Molecular regulation of mast cell activation. *J. Allergy Clin. Immunol. 117*(6), 1214–25, quiz 1226. doi:10.1016/j.jaci.2006.04.015

Romagnani, S. (2000). The role of lymphocytes in allergic disease. *J. Allergy Clin. Immunol. 105*(3), 399–408. doi:10.1067/mai.2000.104575

Romagnani, S. (2004). Immunologic influences on allergy and the TH1/TH2 balance. *J. Allergy Clin. Immunol. 113*(3), 395–400. doi:10.1016/j.jaci.2003.11.025

Romagnani, S. (2007). Coming back to a missing immune deviation as the main explanatory mechanism for the hygiene hypothesis. *J. Allergy Clin. Immunol. 119*(6), 1511–1513. doi:10.1016/j.jaci.2007.04.005

Rotmagnani, S. (1998). The Th1/Th2 paradigm and allergic disorders. *Allergy*, *53*(Allergy), 12–15. doi:10.1111/j.1398-9995.1998.tb04951.x

Ruëff, F., Przybilla, B., Müller, U., & Mosbech, H. (1996). The sting challenge test in Hymenoptera venom allergy Position paper of the subcommittee on Insect Venom Allergy of the European Academy of Allergology and Clinical Immunology. *Allergy*, *51*(4), 216–225. doi:10.1111/j.1398-9995.1996.tb04596.x

Ruëff, F., Vos, B., Oude Elberink, J., Bender, A., Chatelain, R., Dugas-Breit, S., Horny, H. P., Küchenhoff, H., Linhardt, A., Mastnik, S., Sotlar, K., Stretz, E., Vollrath, R., Przybilla, B., & Flaig, M. (2014). Predictors of clinical effectiveness of Hymenoptera venom immunotherapy. *Clin. Exp. Allergy*, *44*(5), 736–746. doi:10.1111/cea.12275

Ruëff, F., Wolf, H., Schnitker, J., Ring, J., & Przybilla, B. (2004). Specific immunotherapy in honeybee venom allergy: A comparative study using aqueous and aluminium hydroxide adsorbed preparations. *Allergy Eur. J. Allergy Clin. Immunol. 59*(6), 589–595. doi:10.1111/j.1398-9995.2004.00505.x

Ruëff, F., Przybilla, B., Biló, M. B., Müller, U., Scheipl, F., Aberer, W., Birnbaum, J., Bodzenta-Lukaszyk, A., Bonifazi, F., Bucher, C., Campi, P., Darsow, U., Egger, C., Haeberli, G., Hawranek, T., Körner, M., Kucharewicz, I., Küchenhoff, H., Lang, R., Quercia, O., Reider, N., Severino, M., Sticherling, M., Sturm, G. J., & Wüthrich, B. (2009). Predictors of severe systemic anaphylactic reactions in patients with Hymenoptera venom allergy: Importance of baseline serum tryptase—a study of the European Academy of Allergology and Clinical Immunology Interest Group on Insect Venom Hypersensitivity. *J. Allergy Clin. Immunol. 124*(5), 1047–1054. doi:10.1016/j.jaci.2009.08.027

Rusznak, C. & Davies, R. J. (1998). ABC of allergies: Diagnosing allergy. *BMJ*, *316*(7132), 686–686. doi:10.1136/bmj.316.7132.686

Sadd, B. M., Barribeau, S. M., Bloch, G., de Graaf, D. C., Dearden, P., Elsik, C. G., Gadau, J., Grimmelikhuijzen, C. J., Hasselmann, M., Lozier, J. D., Robertson, H. M., Smagghe, G., Stolle, E., Van Vaerenbergh, M., Waterhouse, R. M., Bornberg-Bauer, E., Klasberg, S., Bennett, A. K., Câmara, F., Guigó, R., Hoff, K., Mariotti, M., Munoz-Torres, M., Murphy, T., Santesmasses, D., Amdam, G. V., Beckers, M., Beye, M., Biewer, M., Bitondi, M. M.,

Blaxter, M. L., Bourke, A. F., Brown, M. J., Buechel, S. D., Cameron, R., Cappelle, K., Carolan, J. C., Christiaens, O., Ciborowski, K. L., Clarke, D. F., Colgan, T. J., Collins, D. H., Cridge, A. G., Dalmay, T., Dreier, S., du Plessis, L., Duncan, E., Erler, S., Evans, J., Falcon, T., Flores, K., Freitas, F. C., Fuchikawa, T., Gempe, T., Hartfelder, K., Hauser, F., Helbing, S., Humann, F. C., Irvine, F., Jermiin, L. S., Johnson, C. E., Johnson, R. M., Jones, A. K., Kadowaki, T., Kidner, J. H., Koch, V., Köhler, A., Kraus, F. B., Lattorff, H. M. G., Leask, M., Lockett, G. A., Mallon, E. B., Antonio, D. S. M., Marxer, M., Meeus, I., Moritz, R. F., Nair, A., Näpflin, K., Nissen, I., Niu, J., Nunes, F. M., Oakeshott, J. G., Osborne, A., Otte, M., Pinheiro, D. G., Rossié, N., Rueppell, O., Santos, C. G., Schmid-Hempel, R., Schmitt, B. D., Schulte, C., Simões, Z. L., Soares, M. P., Swevers, L., Winnebeck, E. C., Wolschin, F., Yu, N., Zdobnov, E. M., Aqrawi, P. K., Blankenburg, K. P., Coyle, M., Francisco, L., Hernandez, A. G., Holder, M., Hudson, M. E., Jackson, L., Jayaseelan, J., Joshi, V., Kovar, C., Lee, S. L., Mata, R., Mathew, T., Newsham, I. F., Ngo, R., Okwuonu, G., Pham, C., Pu, L.-L., Saada, N., Santibanez, J., Simmons, D., Thornton, R., Venkat, A., Walden, K. K., Wu, Y.-Q., Debyser, G., Devreese, B., Asher, C., Blommaert, J., Chipman, A. D., Chittka, L., Fouks, B., Liu, J., O'Neill, M. P., Sumner, S., Puiu, D., Qu, J., Salzberg, S. L., Scherer, S. E., Muzny, D. M., Richards, S., Robinson, G. E., Gibbs, R. A., Schmid-Hempel, P., & Worley, K. C. (2015). The genomes of two key bumblebee species with primitive eusocial organization. *Genome Biol. 16*(1), 76. doi:10.1186/s13059-015-0623-3

Sainte-Laudy, J., Boumediene, A., Touraine, F., Orsel, I., Brianchon, C., Bonnaud, F., & Cogné, M. (2007). Use of both CD63 up regulation and IgE down regulation for the flow cytometric analysis of allergen induced basophil activation. Definition of an activation index. *Inflamm. Res. 56*(7), 291–296. doi:10.1007/s00011-007-7014-5

Sainte-Laudy, J., Touraine, F., Cluzan, D., & Belle Moudourou, F. (2016). Follow-Up of Venom Immunotherapy on Flow Cytometry and Definition of a Protective Index. *Int. Arch. Allergy Immunol. 170*(4), 243–250. doi:10.1159/000449162

Sainte-Laudy, Sabbah, Drouet, Lauret, & Loiry. (2000). Diagnosis of venom allergy by flow cytometry. Correlation with clinical history, skin tests, specific IgE, histamine and leukotriene C4 release. *Clin. Exp. Allergy*, *30*(8), 1166–1171. doi:10.1046/j.1365-2222.2000.00863.x

Santos, A. F., Douiri, A., Bécares, N., Wu, S. Y., Stephens, A., Radulovic, S., Chan, S. M. H., Fox, A. T., Du Toit, G., Turcanu, V., & Lack, G. (2014). Basophil activation test discriminates between allergy and tolerance in peanut-sensitized children. *J. Allergy Clin. Immunol. 134*(3), 645–652. doi:10.1016/j.jaci.2014.04.039

Santos, A. F., Du Toit, G., Douiri, A., Radulovic, S., Stephens, A., Turcanu, V., & Lack, G. (2015). Distinct parameters of the basophil activation test reflect the severity and threshold of allergic reactions to peanut. *J. Allergy Clin. Immunol. 135*(1), 179–186. doi:10.1016/j.jaci.2014.09.001

Sayers, E. W., Barrett, T., Benson, D. A., Bryant, S. H., Canese, K., Chetvernin, V., Church, D. M., DiCuccio, M., Edgar, R., Federhen, S., Feolo, M., Geer, L. Y., Helmberg, W., Kapustin, Y., Landsman, D., Lipman, D. J., Madden, T. L., Maglott, D. R., Miller, V., Mizrachi, I., Ostell, J., Pruitt, K. D., Schuler, G. D., Sequeira, E., Sherry, S. T., Shumway, M., Sirotkin, K., Souvorov, A., Starchenko, G., Tatusova, T. A., Wagner, L., Yaschenko, E., & Ye, J. (2009). Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res. 37*(Database), D5–D15. doi:10.1093/nar/gkn741

Scadding, G. W., Shamji, M. H., Jacobson, M. R., Lee, D. I., Wilson, D., Lima, M. T., Pitkin, L., Pilette, C., Nouri-Aria, K., & Durham, S. R. (2010). Sublingual grass pollen immunotherapy is associated with increases in sublingual Foxp3-expressing cells and elevated allergen-specific immunoglobulin G4, immunoglobulin A and serum inhibitory activity for immunoglobulin E-facilitated allergen binding to B. *Clin. Exp. Allergy*, *40*(4), 598–606. doi:10.1111/j.1365-2222.2010.03462.x

Schandené, L., Alonso-Vega, C., Willems, F., Gérard, C., Delvaux, A., Velu, T., Devos, R., de Boer, M., & Goldman, M. (1994). B7/CD28-dependent IL-5 production by human resting T cells is inhibited by IL-10. *J. Immunol. 152*(9), 4368–74. doi:10.4049/jimmunol.180.9.5771

Scherer, K., Weber, J., Jermann, T., Krautheim, A., Tas, E., Ueberschlag, E., Cammarata, M., & Bircher, A. (2008). Cellular in vitro Assays in the Diagnosis of Hymenoptera Venom Allergy. *Int. Arch. Allergy Immunol. 146*(2), 122–132. doi:10.1159/000113515

Schiener, M., Eberlein, B., Moreno-Aguilar, C., Pietsch, G., Serrano, P., Mcintyre, M., Schwarze, L., Russkamp, D., Biedermann, T., Spillner, E., Darsow, U., Ollert, M., Schmidt-Weber,

C. B., & Blank, S. (2017a). Application of recombinant antigen 5 allergens from seven allergy-relevant Hymenoptera species in diagnostics. *Allergy*, *72*, 98–108. doi:10.1111/all.13000

Schiener, M., Graessel, A., Ollert, M., Schmidt-Weber, C. B., & Blank, S. (2017b). Allergen-specific immunotherapy of Hymenoptera venom allergy – also a matter of diagnosis. *Hum. Vaccin. Immunother. 13*(10), 2467–2481. doi:10.1080/21645515.2017.1334745

Schiener, M., Hilger, C., Eberlein, B., Pascal, M., Kuehn, A., Revets, D., Planchon, S., Pietsch, G., Serrano, P., Moreno-Aguilar, C., de la Roca, F., Biedermann, T., Darsow, U., Schmidt-Weber, C. B., Ollert, M., & Blank, S. (2018). The high molecular weight dipeptidyl peptidase IV Pol d 3 is a major allergen of Polistes dominula venom. *Sci. Rep. 8*(1), 1318. doi:10.1038/s41598-018-19666-7

Schmid, J. M., Würtzen, P. A., Dahl, R., & Hoffmann, H. J. (2014). Early improvement in basophil sensitivity predicts symptom relief with grass pollen immunotherapy. *J. Allergy Clin. Immunol. 134*(3), 741–744.e5. doi:10.1016/j.jaci.2014.04.029

Schuberth, K. C., Lichtenstein, L. M., Kagey-Sobotka, A., Szklo, M., Kwiterovich, K. A., & Valentine, M. D. (1982). An epidemiologic study of insect allergy in children. I. Characteristics of the disease. *J. Pediatr. 100*(4), 546–551. doi:10.1016/S0022-3476(82)80750-6

Schuerwegh, A. J., De Clerck, L. S., Bridts, C. H., & Stevens, W. J. (2001). Wasp venom immunotherapy induces a shift from IL-4-producing towards interferon-gamma- producing CD4+ and CD8+ T lymphocytes. *Clin. Exp. Allergy*, *31*(5), 740–6. doi:10.1046/j.1365-2222.2001.01066.x

Schumacher, M. J., Schmidt, J. O., Egen, N. B., & Dillon, K. A. (1992). Biochemical variability of venoms from individual European and Africanized honeybees (Apis mellifera). *J. Allergy Clin. Immunol. 90*(1), 59–65. doi:10.1016/S0091-6749(06)80011-4

Schumacher, M., Tveten, M., & Egen, N. (1994). Rate and quantity of delivery of venom from honeybee stings. *J. Allergy Clin. Immunol. 93*(5), 831–835. doi:10.1016/0091-6749(94)90373-5

Schwartz, H. J., Sutheimer, C., Gauerke, M. B., & Yunginger, J. W. (1988). Hymenoptera venom-specific IgE antibodies in post-mortem sera from victims of sudden, unexpected death. *Clin. Allergy*, *18*(5), 461–8. doi:10.1111/j.1365-2222.1988.tb02896.x

Scott-Taylor, T. H., Axinia, S.-C., Amin, S., & Pettengell, R. (2017). Immunoglobulin G; structure and functional implications of different subclass modifications in initiation and resolution of allergy. *Immunity, Inflamm. Dis.* 1–21. doi:10.1002/iid3.192

Seismann, H., Blank, S., Braren, I., Greunke, K., Cifuentes, L., Grunwald, T., Bredehorst, R., Ollert, M., & Spillner, E. (2010a). Dissecting cross-reactivity in hymenoptera venom allergy by circumvention of α-1,3-core fucosylation. *Mol. Immunol. 47*(4), 799–808. doi:10.1016/j.molimm.2009.10.005

Seismann, H., Blank, S., Cifuentes, L., Braren, I., Bredehorst, R., Grunwald, T., Ollert, M., & Spillner, E. (2010b). Recombinant phospholipase A1 (Ves v 1) from yellow jacket venom for improved diagnosis of hymenoptera venom hypersensitivity. *Clin. Mol. Allergy*, *8*, 7. doi:10.1186/1476-7961-8-7

Settipane, G. A., Newstead, G. J., & Boyd, G. K. (1972). Frequency of Hymenoptera allergy in an atopic and normal population. *J. Allergy Clin. Immunol. 50*(3), 146–50. doi:10.1016/0091-6749(72)90045-0

Severino, M. G., Campi, P., MacChia, D., Manfredi, M., Turillazzi, S., Spadolini, I., Bilò, M. B., & Bonifazi, F. (2006). European Polistes venom allergy. *Allergy Eur. J. Allergy Clin. Immunol. 61*(Table 1), 860–863. doi:10.1111/j.1398-9995.2006.01077.x

Severino, M., Bonadonna, P., & Passalacqua, G. (2009). Large local reactions from stinging insects: from epidemiology to management. *Curr. Opin. Allergy Clin. Immunol. 9*(4), 334–337. doi:10.1097/ACI.0b013e32832d0668

Shamji, M. H., Francis, J. N., Würtzen, P. A., Lund, K., Durham, S. R., & Till, S. J. (2013). Cell-free detection of allergen-IgE cross-linking with immobilized phase CD23: Inhibition by blocking antibody responses after immunotherapy. *J. Allergy Clin. Immunol. 132*(4), 1003–1005. doi:10.1016/j.jaci.2013.05.025

Shamji, M. H., Wilcock, L. K., Wachholz, P. A., Dearman, R. J., Kimber, I., Wurtzen, P. A., Larché, M., Durham, S. R., & Francis, J. N. (2006). The IgE-facilitated allergen binding (FAB) assay: Validation of a novel flow-cytometric based method for the detection of in-

hibitory antibody responses. *J. Immunol. Methods*, *317*(1-2), 71–79. doi:10.1016/j.jim.2006.09.004

Shimoda, K., van Deursent, J., Sangster, M. Y., Sarawar, S. R., Carson, R. T., Tripp, R. A., Chu, C., Quelle, F. W., Nosaka, T., Vignali, D. A. A., Doherty, P. C., Grosveld, G., Paul, W. E., & Ihle, J. N. (1996). Lack of IL-4-induced Th2 response and IgE class switching in mice with disrupted State6 gene. *Nature*, *380*(6575), 630–633. doi:10.1038/380630a0

Shore, P. A., Burkhalter, A., & Cohn, V. H. (1959). A method for the fluorometric assay of histamine in tissues. *J. Pharmacol. Exp. Ther. 127*(3), 182–6.

Siegmund, R., Vogelsang, H., Machnik, A., & Herrmann, D. (2000). Surface membrane antigen alteration on blood basophils in patients with Hymenoptera venom allergy under immunotherapy. *J. Allergy Clin. Immunol. 106*(6), 1190–1195. doi:10.1067/mai.2000.110928

Skov, L. K., Seppälä, U., Coen, J. J. F., Crickmore, N., King, T. P., Monsalve, R., Kastrup, J. S., Spangfort, M. D., & Gajhede, M. (2006). Structure of recombinant Ves v 2 at 2.0 Å resolution: structural analysis of an allergenic hyaluronidase from wasp venom. *Acta Crystallogr. Sect. D Biol. Crystallogr. 62*(6), 595–604. doi:10.1107/S0907444906010687

Soldatova, L., Kochoumian, L., & King, T. P. (1993). Sequence similarity of a hornet (D. maculata) venom allergen phospholipase A 1 with mammalian lipases. *FEBS Lett. 320*(2), 145–149. doi:10.1016/0014-5793(93)80080-E

Standage, D. S., Berens, A. J., Glastad, K. M., Severin, A. J., Brendel, V. P., & Toth, A. L. (2016). Genome, transcriptome and methylome sequencing of a primitively eusocial wasp reveal a greatly reduced DNA methylation system in a social insect. *Mol. Ecol. 25*(8), 1769–1784. doi:10.1111/mec.13578

Steigelman, D. A. & Freeman, T. M. (2013). Imported fire ant allergy: case presentation and review of incidence, prevalence, diagnosis, and current treatment. *Ann. Allergy, Asthma Immunol. 111*(4), 242–245. doi:10.1016/j.anai.2013.07.006

Stevens, R. L. & Adachi, R. (2007). Protease-proteoglycan complexes of mouse and human mast cells and importance of their beta-tryptase-heparin complexes in inflammation and innate immunity. *Immunol. Rev. 217*(1), 155–67. doi:10.1111/j.1600-065X.2007.00525.x

Strom, G. B., Boswell, R. N., & Jacobs, R. L. (1983). In vivo and in vitro comparison of fire ant venom and fire ant whole body extract. *J. Allergy Clin. Immunol. 72*(1), 46–53. doi:10.1016/0091-6749(83)90051-9

Sturm, G. J., Böhm, E., Trummer, M., Weiglhofer, I., Heinemann, A., & Aberer, W. (2004). The CD63 basophil activation test in Hymenoptera venom allergy: A prospective study. *Allergy*, *59*(10), 1110–1117. doi:10.1111/j.1398-9995.2004.00400.x

Sturm, G. J., Hemmer, W., Hawranek, T., Lang, R., Ollert, M., Spillner, E., Blank, S., Bokanovic, D., & Aberer, W. (2011). Detection of IgE to recombinant Api m 1 and rVes v 5 is valuable but not sufficient to distinguish bee from wasp venom allergy. *J. Allergy Clin. Immunol. 128*(1), 247–248. doi:10.1016/j.jaci.2011.02.021

Sturm, G. J., Kranzelbinder, B., Schuster, C., Sturm, E. M., Bokanovic, D., Vollmann, J., Crailsheim, K., Hemmer, W., & Aberer, W. (2014). Sensitization to Hymenoptera venoms is common, but systemic sting reactions are rare. *J. Allergy Clin. Immunol. 133*(6), 1635–1643.e1. doi:10.1016/j.jaci.2013.10.046

Sturm, G. J., Varga, E.-M., Roberts, G., Mosbech, H., Bilò, M. B., Akdis, C. A., Antolín-Amérigo, D., Cichocka-Jarosz, E., Gawlik, R., Jakob, T., Kosnik, M., Lange, J., Mingomataj, E., Mitsias, D. I., Ollert, M., Oude Elberink, J. N., Pfaar, O., Pitsios, C., Pravettoni, V., Ruëff, F., Sin, B. A., Agache, I., Angier, E., Arasi, S., Calderón, M. A., Fernandez-Rivas, M., Halken, S., Jutel, M., Lau, S., Pajno, G. B., van Ree, R., Ryan, D., Spranger, O., van Wijk, R. G., Dhami, S., Zaman, H., Sheikh, A., & Muraro, A. (2017). EAACI Guidelines on Allergen Immunotherapy: Hymenoptera venom allergy. *Allergy*, *12*(10), 3218–3221. doi:10.1111/all.13262

Sturm, G., Kränke, B., Rudolph, C., & Aberer, W. (2002). Rush Hymenoptera venom immunotherapy: A safe and practical protocol for high-risk patients. *J. Allergy Clin. Immunol. 110*(6), 928–933. doi:10.1067/mai.2002.129124

Tourlas, K. & Burman, D. (2016). Allergy Testing. *Prim. Care Clin. Off. Pract. 43*(3), 363–374. doi:10.1016/j.pop.2016.04.001

Trabado, A. R., Hijón, C. C., Cantariño, A. R., Romero-Chala, S., García-Trujillo, J. A., & Fernández Pereira, L. M. (2016). Short-, intermediate-, and long-term changes in basophil

reactivity induced by venom immunotherapy. *Allergy, Asthma Immunol. Res. 8*(5), 412–420. doi:10.4168/aair.2016.8.5.412

Urbanek, R., Kemeny, D. M., & Richards, D. (1986). Sub-class of IgG anti-bee venom antibody produced during bee venom immunotherapy and its relationship to long-term protection from bee stings and following termination of venom immunotherapy. *Clin. Allergy*, *16*(4), 317–22. doi:10.1111/j.1365-2222.1986.tb01963.x

Vachová, M., Panzner, P., Malkusová, I., Hanzlíková, J., & Vlas, T. (2016). Utility of laboratory testing for the diagnosis of Hymenoptera venom allergy. *Allergy Asthma Proc. 37*(3), 248–255. doi:10.2500/aap.2016.37.3934

Valenta, R., Lidholm, J., Niederberger, V., Hayek, B., Kraft, D., & Grönlund, H. (1999). The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). *Clin. Exp. Allergy*, *29*(7), 896–904. doi:10.1046/j.1365-2222.1999.00653.x

Valentine, M. D., Schuberth, K. C., Kagey-Sobotka, A., Graft, D. F., Kwiterovich, K. A., Szklo, M., & Lichtenstein, L. M. (1990). The Value of Immunotherapy with Venom in Children with Allergy to Insect Stings. *N. Engl. J. Med. 323*(23), 1601–1603. doi:10.1056/NEJM199012063232305

van de Veen, W., Stanic, B., Wirz, O. F., Jansen, K., Globinska, A., & Akdis, M. (2016). Role of regulatory B cells in immune tolerance to allergens and beyond. *J. Allergy Clin. Immunol. 138*(3), 654–665. doi:10.1016/j.jaci.2016.07.006

van de Veen, W., Stanic, B., Yaman, G., Wawrzyniak, M., Söllner, S., Akdis, D. G., Rückert, B., Akdis, C. A., & Akdis, M. (2013). IgG4 production is confined to human IL-10–producing regulatory B cells that suppress antigen-specific immune responses. *J. Allergy Clin. Immunol. 131*(4), 1204–1212. doi:10.1016/j.jaci.2013.01.014

Van Halteren, H. K., Van Der Linden, P. W. G., Burgers, J. A., & Bartelink, A. K. (1997). Discontinuation of yellow jacket venom immunotherapy: Follow-up of 75 patients by means of deliberate sting challenge. *J. Allergy Clin. Immunol. 100*(6 I), 767–770. doi:10.1016/S0091-6749(97)70271-9

Van Vaerenbergh, M., Cardoen, D., Formesyn, E. M., Brunain, M., Van Driessche, G., Blank, S., Spillner, E., Verleyen, P., Wenseleers, T., Schoofs, L., Devreese, B., & de Graaf, D. C. (2013). Extending the honey bee venome with the antimicrobial peptide apidaecin and a protein resembling wasp antigen 5. *Insect Mol. Biol. 22*(2), 199–210. doi:10.1111/imb.12013

Van Vaerenbergh, M., De Smet, L., Rafei-Shamsabadi, D., Blank, S., Spillner, E., Ebo, D. G., Devreese, B., Jakob, T., & de Graaf, D. C. (2015a). IgE recognition of chimeric isoforms of the honeybee (Apis mellifera) venom allergen Api m 10 evaluated by protein array technology. *Mol. Immunol. 63*(2), 449–455. doi:10.1016/j.molimm.2014.09.018

Van Vaerenbergh, M., Debyser, G., Devreese, B., & de Graaf, D. C. (2014). Exploring the hidden honeybee (Apis mellifera) venom proteome by integrating a combinatorial peptide ligand library approach with FTMS. *J. Proteomics*, *99*, 169–78. doi:10.1016/j.jprot.2013.04.039

Van Vaerenbergh, M., Debyser, G., Smagghe, G., Devreese, B., & de Graaf, D. C. (2015b). Unraveling the venom proteome of the bumblebee (Bombus terrestris) by integrating a combinatorial peptide ligand library approach with FT-ICR MS. *Toxicon*, *102*, 81–88. doi:10.1016/j.toxicon.2013.10.002

van Halteren, H. K., van der Linden, P.-W. G., Burgers, S. A., & Bartelink, A. K. (1996). Hymenoptera sting challenge of 348 patients: Relation to subsequent field stings. *J. Allergy Clin. Immunol. 97*(5), 1058–1063. doi:10.1016/S0091-6749(96)70258-0

Varga, E.-M., Francis, J. N., Zach, M. S., Klunker, S., Aberer, W., & Durham, S. R. (2009). Time course of serum inhibitory activity for facilitated allergen-IgE binding during bee venom immunotherapy in children. *Clin. Exp. Allergy*, *39*(9), 1353–1357. doi:10.1111/j.1365-2222.2009.03303.x

Vercelli, D. (2008). Discovering susceptibility genes for asthma and allergy. *Nat. Rev. Immunol. 8*(3), 169–182. doi:10.1038/nri2257

Vinzón, S. E., Marino-Buslje, C., Rivera, E., & Biscoglio de Jiménez Bonino, M. (2012). A Naturally Occurring Hypoallergenic Variant of Vespid Antigen 5 from Polybia scutellaris Venom as a Candidate for Allergen-Specific Immunotherapy. *PLoS One*, *7*(7), e41351. doi:10.1371/journal.pone.0041351

von Pirquet, C. (1906). Allergie. *Münchner medizinische Wochenschrift*, *53*, 1457–58.

Vos, B., Köhler, J., Müller, S., Stretz, E., Ruëff, F., & Jakob, T. (2013). Spiking venom with rVes v 5 improves sensitivity of IgE detection in patients with allergy to Vespula venom. *J. Allergy Clin. Immunol. 131*(4), 1225–1227.e1. doi:10.1016/j.jaci.2012.07.041

Wachholz, P. A. & Durham, S. R. (2004). Mechanisms of immunotherapy: IgG revisited. *Curr. Opin. Allergy Clin. Immunol. 4*(4), 313–318. doi:10.1097/01.all.0000136753.35948.c0

Wambre, E., DeLong, J. H., James, E. A., LaFond, R. E., Robinson, D., & Kwok, W. W. (2012). Differentiation stage determines pathologic and protective allergen-specific CD4+ T-cell outcomes during specific immunotherapy. *J. Allergy Clin. Immunol. 129*(2), 544–551.e7. doi:10.1016/j.jaci.2011.08.034

Warrington, R., Watson, W., Kim, H. L., & Antonetti, F. (2011). An introduction to immunology and immunopathology. *Allergy, Asthma Clin. Immunol. 7*(Suppl 1), S1. doi:10.1186/1710-1492-7-S1-S1

Wedemeyer, J. & Galli, S. J. (2000). Mast cells and basophils in acquired immunity. *Br. Med. Bull. 56*(4), 936–955. doi:10.1258/0007142001903616

Weinstock, G. M., Robinson, G. E., Gibbs, R. A., Weinstock, G. M., Weinstock, G. M., Robinson, G. E., Worley, K. C., Evans, J. D., Maleszka, R., Robertson, H. M., Weaver, D. B., Beye, M., Bork, P., Elsik, C. G., Evans, J. D., Hartfelder, K., Hunt, G. J., Robertson, H. M., Robinson, G. E., Maleszka, R., Weinstock, G. M., Worley, K. C., Zdobnov, E. M., Hartfelder, K., Amdam, G. V., Bitondi, M. M. G., Collins, A. M., Cristino, A. S., Evans, J. D., Michael, H., Lattorff, G., Lobo, C. H., Moritz, R. F. A., Nunes, F. M. F., Page, R. E., Simões, Z. L. P., Wheeler, D., Carninci, P., Fukuda, S., Hayashizaki, Y., Kai, C., Kawai, J., Sakazume, N., Sasaki, D., Tagami, M., Maleszka, R., Amdam, G. V., Albert, S., Baggerman, G., Beggs, K. T., Bloch, G., Cazzamali, G., Cohen, M., Drapeau, M. D., Eisenhardt, D., Emore, C., Ewing, M. A., Fahrbach, S. E., Forêt, S., Grimmelikhuijzen, C. J. P., Hauser, F., Hummon, A. B., Hunt, G. J., Huybrechts, J., Jones, A. K., Kadowaki, T., Kaplan, N., Kucharski, R., Leboulle, G., Linial, M., Littleton, J. T., Mercer, A. R., Page, R. E., Robertson, H. M., Robinson, G. E., Richmond, T. A., RodriguezZas, S. L., Rubin, E. B., Sattelle, D. B., Schlipalius, D., Schoofs, L., Shemesh, Y., Sweedler, J. V., Velarde, R., Verleyen, P., Vierstraete, E., Williamson, M. R., Beye, M., Ament, S. A., Brown, S. J., Corona, M., Dearden, P. K., Dunn, W. A., Elekonich, M. M., Elsik, C. G., Forêt, S., Fujiyuki, T., Gattermeier, I., Gempe, T., Hasselmann, M., Kadowaki, T., Kage, E., Kamikouchi, A., Kubo, T., Kucharski, R., Kunieda, T., Lorenzen, M., Maleszka, R., Milshina, N. V., Morioka, M., Ohashi, K., Overbeek, R., Page, R. E., Robertson, H. M., Robinson, G. E., Ross, C. A., Schioett, M., Shippy, T., Takeuchi, H., Toth, A. L., Willis, J. H., Wilson, M. J., Robertson, H. M., Zdobnov, E. M., Bork, P., Elsik, C. G., Gordon, K. H. J., Letunic, I., Hackett, K., Peterson, J., Felsenfeld, A., Guyer, M., Solignac, M., Agarwala, R., Cornuet, J. M., Elsik, C. G., Emore, C., Hunt, G. J., Monnerot, M., Mougel, F., Reese, J. T., Schlipalius, D., Vautrin, D., Weaver, D. B., Gillespie, J. J., Cannone, J. J., Gutell, R. R., Johnston, J. S., Elsik, C. G., Cazzamali, G., Eisen, M. B., Grimmelikhuijzen, C. J. P., Hauser, F., Hummon, A. B., Iyer, V. N., Iyer, V., Kosarev, P., Mackey, A. J., Maleszka, R., Reese, J. T., Richmond, T. A., Robertson, H. M., Solovyev, V., Souvorov, A., Sweedler, J. V., Weinstock, G. M., Williamson, M. R., Zdobnov, E. M., Evans, J. D., Aronstein, K. A., Bilikova, K., Chen, Y. P., Clark, A. G., Decanini, L. I., Gelbart, W. M., Hetru, C., Hultmark, D., Imler, J.-L., Jiang, H., Kanost, M., Kimura, K., Lazzaro, B. P., Lopez, D. L., Simuth, J., Thompson, G. J., Zou, Z., De Jong, P., Sodergren, E., Csűrös, M., Milosavljevic, A., Johnston, J. S., Osoegawa, K., Richards, S., Shu, C.-L., Weinstock, G. M., Elsik, C. G., Duret, L., Elhaik, E., Graur, D., Reese, J. T., Robertson, H. M., Robertson, H. M., Elsik, C. G., Maleszka, R., Weaver, D. B., Amdam, G. V., Anzola, J. M., Campbell, K. S., Childs, K. L., Collinge, D., Crosby, M. A., Dickens, C. M., Elsik, C. G., Gordon, K. H. J., Gramates, L. S., Grozinger, C. M., Jones, P. L., Jorda, M., Ling, X., Matthews, B. B., Miller, J., Milshina, N. V., Mizzen, C., Peinado, M. A., Reese, J. T., Reid, J. G., Robertson, H. M., Robinson, G. E., Russo, S. M., Schroeder, A. J., St Pierre, S. E., Wang, Y., Zhou, P., Robertson, H. M., Agarwala, R., Elsik, C. G., Milshina, N. V., Reese, J. T., Weaver, D. B., Worley, K. C., Childs, K. L., Dickens, C. M., Elsik, C. G., Gelbart, W. M., Jiang, H., Kitts, P., Milshina, N. V., Reese, J. T., Ruef, B., Russo, S. M., Venkatraman, A., Weinstock, G. M., Zhang, L., Zhou, P., Johnston, J. S., Aquino-Perez, G., Cornuet, J. M., Monnerot, M., Solignac, M., Vautrin, D., Whitfield, C. W., Behura, S. K., Berlocher, S. H., Clark, A. G., Gibbs, R. A., Johnston, J. S., Sheppard, W. S., Smith, D. R., Suarez, A. V., Tsutsui, N. D., Weaver,

D. B., Wei, X., Wheeler, D., Weinstock, G. M., Worley, K. C., Havlak, P., Li, B., Liu, Y., Sodergren, E., Zhang, L., Beye, M., Hasselmann, M., Jolivet, A., Lee, S., Nazareth, L. V., Pu, L.-L., Thorn, R., Weinstock, G. M., Stolc, V., Robinson, G. E., Maleszka, R., Newman, T., Samanta, M., Tongprasit, W. A., Aronstein, K. A., Claudianos, C., Berenbaum, M. R., Biswas, S., de Graaf, D. C., Feyereisen, R., Johnson, R. M., Oakeshott, J. G., Ranson, H., Schuler, M. A., Muzny, D., Gibbs, R. A., Weinstock, G. M., Chacko, J., Davis, C., Dinh, H., Gill, R., Hernandez, J., Hines, S., Hume, J., Jackson, L., Kovar, C., Lewis, L., Miner, G., Morgan, M., Nazareth, L. V., Nguyen, N., Okwuonu, G., Paul, H., Richards, S., Santibanez, J., Savery, G., Sodergren, E., Svatek, A., Villasana, D., & Wright, R. (2006). Insights into social insects from the genome of the honeybee Apis mellifera. *Nature*, *443*(7114), 931–949. doi:10.1038/nature05260

Wide, L., Bennich, H., & Johansson, S. (1967). Diagnosis of Allergy by an in-vitro Test for Allergen Antibodies. *Lancet*, *290*(7526), 1105–1107. doi:10.1016/S0140-6736(67)90615-0

Wills-Karp, M. (1999). Immunologic basis of antigen-induced airway hyperresponsiveness. *Annu. Rev. Immunol. 17*(1), 255–81. doi:10.1146/annurev.immunol.17.1.255

Winningham, K. M., Fitch, C. D., Schmidt, M., & Hoffman, D. R. (2004). Hymenoptera venom protease allergens. *J. Allergy Clin. Immunol. 114*(4), 928–933. doi:10.1016/j.jaci.2004.07.043

Worm, M., Moneret-Vautrin, A., Scherer, K., Lang, R., Fernandez-Rivas, M., Cardona, V., Kowalski, M. L., Jutel, M., Poziomkowska-Gesicka, I., Papadopoulos, N. G., Beyer, K., Mustakov, T., Christoff, G., Bilò, M. B., Muraro, A., Hourihane, J. O. B., & Grabenhenrich, L. B. (2014). First European data from the network of severe allergic reactions (NORA). *Allergy*, *69*(10), 1397–1404. doi:10.1111/all.12475

Wright, D. N. & Lockey, R. F. (1990). Local Reactions to Stinging Insects (Hymenoptera). *Allergy Asthma Proc. 11*(1), 23–28. doi:10.2500/108854190778999474

Wurm, Y., Wang, J., Riba-Grognuz, O., Corona, M., Nygaard, S., Hunt, B. G., Ingram, K. K., Falquet, L., Nipitwattanaphon, M., Gotzek, D., Dijkstra, M. B., Oettler, J., Comtesse, F., Shih, C.-J., Wu, W.-J., Yang, C.-C., Thomas, J., Beaudoing, E., Pradervand, S., Flegel, V., Cook, E. D., Fabbretti, R., Stockinger, H., Long, L., Farmerie, W. G., Oakey, J., Boomsma, J. J., Pamilo, P., Yi, S. V., Heinze, J., Goodisman, M. A. D., Farinelli, L., Harshman, K., Hulo, N., Cerutti, L., Xenarios, I., Shoemaker, D., & Keller, L. (2011). The genome of the fire ant Solenopsis invicta. *Proc. Natl. Acad. Sci. 108*(14), 5679–5684. doi:10.1073/pnas.1009690108

Yazdanbakhsh, M. & Matricardi, P. M. (2004). Parasites and the Hygiene Hypothesis: Regulating the Immune System? *Clin. Rev. Allergy Immunol. 26*(1), 15–24. doi:10.1385/CRIAI:26:1:15

Žitnik, S. E. K., Vesel, T., Avčin, T., Šilar, M., Košnik, M., & Korošec, P. (2012). Monitoring honeybee venom immunotherapy in children with the basophil activation test. *Pediatr. Allergy Immunol. 23*(2), 167–172. doi:10.1111/j.1399-3038.2011.01233.x

List of Figures

Figure 1 Peptides Presented to Naive T Cells and T Cell Subsets Relevant for Insect Venom Allergy. 5

Figure 2 Structure of an Antibody Molecule. 7

Figure 3 Mechanisms of Allergic Sensitization. 9

Figure 4 Mast Cell and Basophil Activation by Cross-Linked IgE on FcϵRI. 10

Figure 5 Taxonomy of Allergy-Relevant Species of the Order of Hymenoptera. Apis mellifera (https://tinyurl.com/yagc8m3w) by Carnat Joel (https://www.flickr.com/photos/21708387@N02/), Buff-tailed bumblebee (https://tinyurl.com/ydf7abql) by Jonas Myrenås (https://www.flickr.com/photos/primavera133/), Common Wasp (Vespula (Paravespula) vulgaris) (https://tinyurl.com/ycqztdx5) by Martin Cooper (https://www.flickr.com/photos/m-a-r-t-i-n/), licensed under CC BY 2.0 (https://creativecommons.org/licenses/by/2.0/). Polistes dominula (https://tinyurl.com/y88w8bt7) by Ferran Turmo Gort (https://www.flickr.com/photos/fturmog/) licensed under CC BY-NC-SA 2.0 (https://creativecommons.org/licenses/by-nc-sa/2.0/), Red Imported Fire Ant (Solenopsis invicta) (https://tinyurl.com/yaf7xnzu) by stevenw12339 (https://www.flickr.com/photos/100620478@N03/) licensed under CC BY-NC 2.0 (https://creativecommons.org/licenses/by-nc/2.0/). 16

Figure 6 Schematic Overview of the Aim of the Study. 32

Figure 7 Schematic Overview of the Results of this Study Together with the Titles of Published Articles. 37

List of Tables

Table 1 Overview of Hymenoptera venom allergens currently listed in the WHO/IUIS Allergen Database. 22

Table 1 (Continued) Overview of Hymenoptera venom allergens currently listed in the WHO/IUIS Allergen Database. 23

Table 2 Overview of Hymenoptera venom allergens currently listed in the WHO/IUIS Allergen Database of species, for which single allergens are available for routine diagnosis. 25

Acknowledgement

First of all, I would like to thank my supervisor PD Dr. rer. nat. Simon Blank. Thank you Simon for giving me the possibility to do my PhD thesis in the AG Blank and made a smooth start in the lab possible. I always liked the freedom to develop my own ideas in the lab. I surely appreciate your great support not only scientifically but also personally. Thank you for giving me the opportunity to attend national and international conferences as well as PhD schools.

I also want to thank the head of the Institute for Allergy Research and Center for Allergy and Environment, Prof. Dr. rer. nat. Carsten Schmidt-Weber. Thank you for giving me the opportunity to work in an institute situated perfectly between the Department of Dermatology and Allergy at the clinic and a basic research department. Thank you for your support as supervisor and mentor as well as for your time and ideas during thesis committee meetings.

Thank you Prof. Dr. phil. nat. Michael Sattler for your agreement to be the second supervisor of this PhD thesis. I greatly appreciate your time and input in my thesis committee meetings.

I would also like to thank all my collaborators Christiane Hilger, Annette Kuehn and Dominique Revets for excellent mass spectrometry data and other collaborative projects. Thank you Prof. Dr. med. Bernadette Eberlein and Dr. Mariona Pascal for the great basophil activation data and Dr. Carmen Moreno-Aguilar, Prof. Dr. med. Ulf Darsow for organizing the serum samples as well a thank you to all patients that participate in the studies.

Furthermore, I would like to give a special thank to all past and present members of the AG Blank for the great atmosphere in our small and well organized lab. Dennis, it was fun spending various hours at scientific meetings, in the lab as well as various nights out in countless pubs often ending in hotel bars. I am glad we became friends and hope we will finally manage some trips to the mountains. Anke, I am very happy we met and became close friends. Thank you for all your support and your daily motivation. Thank you for teaching me so many things in the lab. Sitting next to you and looking forward to a sunny lunchtime made it worth going to work even on unpleasant days. Thank you for occasionally sharing your hotel room during conferences and the time we spent in all the different cities we visited after the conferences. Thank you Steffi for your (technical) support in the lab. I appreciate your organizational skills and your endless motivation for all kinds of lab work. Thank you for all your fruitful scientific discussions (also during centrifugation times) and all the time we were able to spend together. I also want to thank Alex and Michael for taking over my projects. Thank you Sabrina and Johannes for your great help in the lab during your lab internships.

Additionally, I would like to thank all members of the IAF, Mary, Tanja, Steffi, Maria,

Johanna, Benjamin, Evi, Francesca, Stefan and Caspar for all the support and motivation as well as a great work atmosphere. Thank you to everyone I met at ZAUM, especially to Anne for your support, help and Granada, Linda for answering statistic questions and Marta for our great collaboration.

Thank you Renske, Manu, Pati, Yvonne, Carlos and Nicole for the fun in the lab during my time at the Institute for Cardiovascular Prevention and thank you Larisa for all your support and showing me the fun of cloning. Thank you all members of the IPEK for showing me the good and the bad of science.

A very special thank goes to my friends I already met during the time of my studies. Ramona, Ana, Sarah, Sarah, Nina, Meli, Sandra and Julia, it is of inestimable value for me to have gone this journey since almost twelve years together with each and every single one of you. I really appreciate your endless support and motivation and especially all the time we spend together. It is wonderful to know that we manage to keep up our friendship after such a long time and even though we are spread all over Germany. Bang Boom Bonn!

Thank you from the bottom of my heart to my family and Leon. Mama and Papa, without your support of all thinkable kinds, all this would have not been possible. Thank you Leon for continuously calming me down and going this journey together with me. Thank you for all your support and time, listening to all problems and your effort to bring back my motivation.