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1. THE IMMUNE SYSTEM

The immune system is the body’s defense mechanism to fight off pathogens and tumor growth. It comprises both innate and adaptive components. Innate cells are equipped to recognize conserved components shared by many pathogens, whereas the adaptive cellular response is developed to target specific antigens of different pathogens, and matures upon repeated exposure. Innate cells include monocytes, macrophages, dendritic cells (DC), natural killer cells, neutrophils, eosinophils, mast cells and basophils. These cells are equipped with receptors to detect conserved pathogen components. Many of these cells are also highly efficient at antigen uptake and processing for subsequent presentation to cells of the adaptive immune system, in order to initiate an adaptive response to the presented antigen(s) of the pathogen. Eosinophils, neutrophils and basophils, collectively known as granulocytes, respond primarily to extracellular parasites, and react accordingly. For parasites that are too large for single cells to ingest for either presentation to adaptive cells or intracellular destruction, granulocytes secrete specific compounds to attack the parasite directly and recruit more immune cells to the site. These cells are also capable of responding, via their immunoglobulin Fc receptors, to specific parasite antigens coated by antigen-specific immunoglobulin, produced by cells of the adaptive immune system.

Adaptive immune cells include B and T lymphocytes. B cells can recognize whole antigens via surface-bound immunoglobulin molecules (B cell receptors). Antigen uptake, processing and presentation on MHC class II molecules on the surface of antigen presenting cells (APC) allows for T cell recognition of antigen fragments (peptides) via the T Cell Receptor (TCR). B cells, through interaction with T cells, produce more antigen-specific immunoglobulin with improved antigen specificity. Depending on the type of response required to deal with a pathogen, different T helper (Th) cells come into play. A Th1 response, characterized by the production of IFN-γ and IL-2, is generally initiated towards intracellular pathogens including viruses, bacteria or fungi. Similarly, Th17 cells secrete IL-17 and IL-22, which stimulate neutrophils to combat extracellular bacteria or fungi. In contrast, a Th2 response, characterized by production of IL-4, IL-5 and IL-13, is initiated upon infection with larger parasites, such as helminths. This response is required for recruitment of specific cells equipped to deal with the parasite, such as eosinophils and basophils. In addition, a regulatory T cell response (Treg), marked by Foxp3 expression or IL-10 and TGF-β production, exists to dampen any excessive Th1 or Th2 responses that may be harmful to the body. Th1 responses can result in B cell production of antigen-specific IgG1, IgG2, or IgG3, whereas Th2 responses typically induce IgE, and IgG4 production. Each of these antibody isotypes assists in the development of a targeted immune response, through either neutralizing the pathogen directly or marking it for attack by other cells. In a Treg environment (especially with IL-10), the production of IgG4 is induced, an antibody isotype with many anti-inflammatory attributes. The adaptive cells of the immune system are primed to respond to specific antigens and, upon second exposure, can rapidly expand from a pool of memory cells in order to quickly and efficiently eradicate a recognized pathogen. This is the basis of developing immunity to a pathogen, and the fundamental mechanism involved in effective vaccination. Just as the development of the adaptive immune system is a milestone in our evolution, understanding and exploiting its mechanism of action is a milestone in the development of modern day medicine.

2. ALLERGY

Although adaptive immunity is imperative to our survival, misdirection of this sophisticated mechanism can lead to a wide range of diseases. Central tolerance, originating in the bone marrow and thymus, prevents the immune system from recognizing ‘self’ antigens, and thereby the development of T cell and/or B cell mediated autoimmune disorders. Peripheral tolerance occurs in the lymph nodes and is vital for restraining the adaptive immune system from attacking harmless environmental antigens. Failure to do so can result in allergic disease, characterized by an exaggerated Th2-skewed immune response to harmless components derived from food, pollens, dust mites, molds and other commonly encountered substances. Symptoms of allergic disease may range from mild itching to airway inflammation and even life-threatening anaphylaxis. An anaphylactic reaction is systemic and primarily observed in drug, insect venom and food allergies, although the mechanism behind the severity of symptoms associated with these particular allergies has yet to be determined.

2.1 ALLERGY PREVALENCE

Allergic disease can present in many different forms, including asthma, allergic rhinitis, atopic dermatitis, and food allergies. Progression from early-life atopic dermatitis to later allergic rhinitis, food allergy, and asthma has repeatedly been observed, and is now termed the allergic or atopic march. The actual prevalence of allergies is difficult to assess. Prevalence studies face challenges in several areas, including willingness to participate, which may bias recruitment to include those more likely to be affected; timing, as allergies may resolve or develop at different ages; and the method of diagnosis, from self reported to provocation challenges with the actual allergens. In addition, difficulties arise in the distinction between IgE and non-IgE mediated reactions. Currently, allergic disease is estimated to affect approximately 20% of individuals in developed regions, with the prevalence having increased substantially in the past two decades. There is comparatively little information available on the prevalence of allergies in developing regions of the world. Although the prevalence of allergies and asthma is estimated to be lower than in westernized countries, increased prevalence has been reported in urban areas of developing countries, where a more westernized lifestyle has gradually been adopted.

Food allergies, most commonly directed towards cow’s milk, egg, wheat, soy, peanut, tree nuts, fish, and shellfish are now estimated to affect more than 1-2%, but no more than 10% of the population, based on a comprehensive review of the literature published between 1988.
and 2009\textsuperscript{15}. Cow’s milk and egg allergies are primarily observed in young children, and often resolve by the age of five. Peanut, fish and shellfish allergy, in contrast, generally persist into adulthood\textsuperscript{14}. As reported for other allergic diseases the prevalence of food allergies is estimated to have more than doubled over the last two decades\textsuperscript{15-19}. Launched in 2005, the EuroPrevall project was initiated with the aim to identify key risk factors for the development of food allergy and generate uniform European databases. The project was originally designed to assess European populations but has now extended to Russia, China and India, encompassing a wide range of lifestyles and socioeconomic status. The data resulting from these studies will provide the most comprehensive assessment of food allergy prevalence to date using gold standard methodology\textsuperscript{20-22}.

Allergic disease places a considerable burden on the health care system and society in general. In addition to the negative effects on the quality of life of those afflicted and their caregivers, significant economic consequences arise from both direct and indirect costs of managing this disease\textsuperscript{23, 24}. Considering the high and increasing prevalence, slow resolution, negative effect on quality of life, and financial burdens of allergies, there is considerable interest in identifying risk factors and developing safe and effective therapeutics for this disease.

2.2. RISK FACTORS FOR THE DEVELOPMENT OF ALLERGIES

2.2.1 Genetic component

The development of allergy is multi-factorial, however, many studies have conclusively shown that an underlying genetic component is involved\textsuperscript{25, 26}. This implies that specific environmental factors can trigger allergy development in those who are genetically susceptible. Variants in numerous genes have been associated with allergic disease many of which can be assigned to groups of genes involved in the immune response to environmental exposure, epithelial barrier integrity, Th1/Th2 response regulation or tissue responses to chronic inflammation\textsuperscript{26}. In addition to variation in DNA sequence, epigenetic modifications may mediate genetic susceptibility to allergy. Epigenetic modifications include DNA methylation and histone modifications, each altering the expression of a gene. For example, sequence variants in the HLA-DRB1 and HLA-DQB1 loci have been associated with peanut allergy in children of European descent\textsuperscript{27, 28} and accounted for peanut allergy in 20% of the study population\textsuperscript{28}. The identified variants were linked to differential DNA methylation patterns in the HLA-DQB1 and HLA-DRB1 genes\textsuperscript{28}. Epigenetic modifications in these HLA class II loci may determine which peptides are presented to T cells by APC, thereby having a direct effect on the response mounted to an antigen. This avenue of research in allergy is in the early stages, and further research is needed to determine which, possibly manageable, environmental factors influence epigenetic modifications.

2.2.2 Hygiene Hypothesis

Although genetic factors contribute to the development of allergic disease, they cannot account for the observed increase in prevalence during the past two decades. Allergy predominates in developed countries, which has led to the hypothesis that improved hygiene conditions and decreased exposure to infectious and microbial agents in childhood are key components in the development of the disease. This is termed the ‘hygiene hypothesis’ and was introduced in 1989 by David P. Strachan\textsuperscript{29}. The hypothesis is based on the notion that reduced pathogenic microbial and viral exposure, which would induce a Th1 response, leads to a disturbed Th1/Th2 balance, in which the Th2 response dominates. More recently this hypothesis has been modified and expanded and now suggests that decreased exposure to common environmental microbes including bacteria, fungi and parasites, leads to a general excessive inflammatory state\textsuperscript{30, 31}. This ‘old friends’ hypothesis, in contrast to the hygiene hypothesis, would explain the recent increase in both allergy and autoimmune disorders, as it does not imply a Th1/Th2 imbalance, but rather an overall hyper-inflammatory response\textsuperscript{31}. Furthermore, this theory would also support the reported correlation between helmint infection, which induces a strong Th2 type response, and decreased allergy prevalence, observed in helmint-endemic areas\textsuperscript{32-34}. In essence, due to early, repeated exposure to diverse environmental microbes, including those that inhabit the skin, gut, and respiratory tract, an immune-regulatory network develops, suppressing hyper-inflammatory and potentially chronic disorders. This hypothesis provides a general explanation for the differences in prevalence of allergy throughout various regions of the world, as well as other protective factors that have been identified, including natural as opposed to cesarean birth\textsuperscript{35-37}, siblings or large families\textsuperscript{29, 38-40}, communal childcare\textsuperscript{40, 41}, and farming lifestyle or animal exposure\textsuperscript{42-44}. However, additional factors have been identified that may contribute to the development of allergy, and food allergy in particular.

2.2.3 Diet

A recently identified protective factor for the development of allergy is high dietary fiber intake. Fiber and other undigested carbohydrates in the colon are fermented by specific strains of anaerobic bacteria, resulting in the production of short chain fatty acids (SCFA), predominantly acetate, butyrate and propionate\textsuperscript{45}. These SCFA affect immune cell gene expression, leading to differentiation of Foxp3\textsuperscript{+} or IL-10\textsuperscript{+} T regulatory cells\textsuperscript{46-48}, and bind to G-protein coupled receptors on APC, inducing a more tolerogenic cell phenotype. These effects suppress inflammation in the gut. The level of fiber intake also affects the composition of the gut microbiota by promoting the growth of certain strains of bacteria required for fiber fermentation, such as Clostridia. In addition, Clostridia induce IL-22 production by lymphocytes in the colon lamina propria\textsuperscript{49}, which increases intestinal epithelial barrier integrity, thereby reducing permeability to (potentially allergenic) dietary proteins\textsuperscript{50-52}. Indeed, impaired intestinal permeability has been associated with food allergy or food hypersensitivity when compared to non-atopic controls. Furthermore, the degree of intestinal barrier permeability correlated with the severity of allergic symptoms experienced upon ingestion of the allergen\textsuperscript{53}. It is becoming clear that the typical ‘Western
diet” (high in fat and sugar and low in fiber) may negatively affect the composition of intestinal bacterial stains, resulting in decreased immune tolerance and increased epithelial barrier permeability, both contributing factors to the development of allergic disease.

2.2.4 Impaired epithelial barrier

Another identified risk factor for the development of food allergy is impaired epidermal barrier function due to eczema68 (atopic dermatitis) or filaggrin gene (FLG) loss of function mutations69–75. Filaggrin is an epidermal structural protein essential for skin integrity, and it is estimated that approximately 50% of moderate to severe cases of atopic dermatitis and about 15% of mild to moderate cases are associated with a FLG variant76–78. Disruptions to the epidermal barrier, as occurs with eczema, cause epithelial cells to release pro-inflammatory chemokines and cytokines, including those mediating Th2-cell polarization79. Murine studies have shown that epicutaneous application of ovalbumin (OVA) and house dust mite (HDM) allergens to a disrupted epidermal barrier induces a local and systemic Th2 dominated response80–84. One study showed the development of peanut and OVA allergy in a mouse model after application of the allergens to adhesive tape-disrupted skin. The investigators reported IL-4 secretion from T cells in the draining lymph node and high levels of antigen-specific IgE and IgG1. Furthermore, increased expression of MHC Class II and costimulatory molecules CD80 and CD86, along with CD54 and CD11c, were also observed on Langerhans cells in the disrupted epidermis; however, migration of these cells occurred only after the introduction of an antigen. In contrast, subcutaneous injection of the antigen into the dermal layer without prior disruption of the skin led to a predominantly Th1 response85. Several other murine studies have shown that cutaneous exposure to an allergen, along with a danger signal through either barrier disruption or an adjuvant, results in allergen sensitization and an allergic response upon subsequent oral ingestion of the allergens86. This highlights the importance of barrier dysfunction in the development of a Th2-skewed response to an allergen. Furthermore, observational studies have suggested that the treatment of atopic skin with emollients containing food allergens manifests a higher risk of developing a corresponding food allergy87–90. This possible route of sensitization also brings into question the role of oral tolerance, and whether early introduction of allergen via the oral route may prevent the development of sensitization91. This avenue of research further emphasizes the role of oral exposure in food allergy (in particular food allergy), but also elucidates the immunological mechanisms involved.

3. MECHANISM OF AN ALLERGIC REACTION

The mechanism behind an allergic reaction consists of two phases (Figure 1). The first phase involves the initial sensitization, where an antigen is encountered by an APC and processed for presentation to naïve T cells. The antigen can be introduced through various routes, including the gastrointestinal tract (food allergens) and lung epithelial cells (aeroallergens) and, as mentioned previously, the skin. Accumulating evidence suggests that the state of the epithelial barrier (healthy versus damaged due to for example smoke or pollutant inhalation in the lung or eczema of the skin) at the antigen contact site can determine the type of response that is induced72. Furthermore, many allergens contain proteolytic properties enabling penetration of the epithelial barrier93. Barrier disruption and introduction of allergens accompanied by danger signals can lead to activation of epithelial cells with secretion of Th2 skewing cytokines IL-25, IL-33 and TSLP94.76,79.

In the next line of defense, immature DC respond to the encountered allergen both directly, through innate immune receptors such as Toll Like Receptors (TLR), Protease Activated Receptors (PAR) or C-type Lectin Receptors (CLR) and, indirectly, through cytokines produced by other cells, including epithelial cells72. Upon activation and allergen uptake, maturation of the cells occurs with up-regulation of MHC Class II and costimulatory molecules CD80 or CD86, in preparation for subsequent antigen presentation to naive T cells in the lymph node87. In order to present antigen to T cells, APC must internalize and process the antigen, then load the resulting peptides into the grooves of MHC Class II molecules for transport to the cell surface. Various components are required for successful peptide-MHC Class II complex formation and presentation. The MHC Class II peptide-binding groove is blocked by the invariant chain (CD74) during synthesis in the endoplasmic reticulum, to prevent binding of cellular or endogenous peptides before encountering peptides of the endocytosed digested proteins87. Upon fusion of the endosomes containing newly synthesized MHC class II with the late endosomes containing exogenous peptides, cathepsin proteases facilitate the removal of the invariant chain leaving only a small portion in the groove, termed CLIP95,96. Finally, CLIP is removed from the groove by HLA-DM87, allowing for exogenous peptide loading and presentation of the newly formed MHC Class II-peptide complex on the cell surface. This complex interacts with the TCR of the naïve CD4+ T cells, providing the first of three signals required for T cell activation and differentiation. Costimulatory molecules expressed by the APC, including CD80 and CD86, interact with CD28 present on the T cell, providing the second signal84,85. The third signal determines the resulting T cell phenotype. In order to induce a Th1 phenotype, DC produce IL-12, which increases T cell IFN-γ expression resulting in the induction of the master Th1 transcription regulator T-bet, resulting in further expression of IFN-γ and suppression of IL-497. The mechanism through which DC induce Th2 differentiation of naïve T cells, regulated by the master transcription factor GATA398, is less clear. Various cells, including basophils, mast cells, and innate lymphoid type 2 cells (ILC2), do produce significant amounts of this cytokine99. In some models of Th2-associated disease, including allergy, IL-4 is a necessity for the development of Th2 immunity; therefore accessory cells capable of IL-4 production may be required in the process of antigen presentation. Alternatively, it has been suggested that IL-4 producing cells, specifically
basophils, are able to function as APC inducing Th2 responses entirely independent of DC\textsuperscript{95-97}.

In the development of an allergic response, Th2 differentiated CD4\textsuperscript{+} T cells, capable of producing IL-4, IL-5 and IL-13, interact with B cells to induce isotype class switching resulting in antigen-specific IgE production. For this to occur, B cell receptor activation by the antigen, the presence of a Th2 cytokine environment, and signaling through CD40 is required. Although these signals are provided by T cells, T cell-independent class switching has also been described, where other cells such as basophils take on this role\textsuperscript{99}. The resulting antigen-specific IgE is a key player in the second phase of allergic inflammation, and therefore also a therapeutic target. Secreted locally in tissue and systemically in peripheral blood, IgE molecules are captured by IgE receptors on a variety of cells. Two types of IgE receptors exist. Low affinity receptors (CD23) are present on B cells, T cells, Langerhans cells, monocytes, macrophages, platelets, follicular DC and eosinophils\textsuperscript{99,100}, and play an important role in IgE production regulation\textsuperscript{101,102}. Binding of IgE or IgE-immune complexes induces a negative feedback signal preventing further IgE synthesis\textsuperscript{103}.

In contrast, soluble forms of CD23 up-regulate IgE production by B cells\textsuperscript{104}. High affinity IgE receptors (FcεRI) are present on basophils, mast cells, monocytes and DC, and are a key component of the immediate hypersensitivity that is observed in the second phase of allergic inflammation. The second phase of allergic inflammation is induced upon secondary encounter of the antigen (challenge). Conformational epitopes of the allergen will bind and cross-link allergen-specific IgE bound to FcεRI molecules on basophils and mast cells (effector cells). This induces cell activation through a cascade of intracellular protein phosphorylation and calcium influx, eventually resulting in granule release of pre-formed inflammatory mediators. These mediators include histamine, proteases (e.g. tryptase), proteoglycans (e.g. heparin) and cytokines (IL-4 in basophils and TNF-α in mast cells). Activation of mast cells and basophils also results in
4. THE BASOPHIL- EFFECTOR CELL AND VALUABLE TOOL

Basophils and mast cells are key players in immediate hypersensitivity reactions through their ability to degranulate and release inflammatory mediators, including histamine, upon exposure to allergen. Basophils enter the circulation from the bone marrow as fully mature cells and have a relatively short lifespan of only several days \(^{106, 107}\). Mast cells, in contrast, can survive for several months and reside in the tissue \(^{108}\). Due to their unique attributes, basophils can serve as an excellent tool in allergy research and clinical diagnosis. They are easily accessible, only requiring a blood sample equivalent to that used to measure serum IgE, and their relatively short lifespan allows for a clear representation of the current serum IgE repertoire. Following identification of the degranulation marker CD63 in the early 1990s \(^{110}\), the in vitro basophil activation test (BAT) was developed \(^{110}\). CD63 is a tetraspanin protein located within the secretory granules that contain histamine. Upon activation and degranulation, this membrane along with the CD63 protein is transported to the cell surface to fuse with the outer membrane, allowing the CD63 molecules on live cells to become accessible to flow cytometry detection antibodies (Figure 2A,B). A range of allergen concentrations is included in the BAT to create a dose-response curve, from which both basophil reactivity (maximal percentage of CD63\(^{+}\) basophils detected) and basophil sensitivity (concentration of allergen required to induce 50% of maximal reactivity) can be measured \(^{111}\) (Figure 2C). Within this test basophils can be detected with a number of different markers. Most commonly used markers include IgE\(^{++}\), CD123\(^{+}\)/HLA-DR\(^{-}\), CCR3\(^{+}\) and CD203c\(^{+}\), often in combination with flow cytometry side scatter profile and CD3 to distinguish these cells from eosinophils and T cells, respectively \(^{112}\). CD203c is a basophil lineage specific marker expressed at low levels on resting basophils, which is then upregulated upon activation. The marker is used both to identify basophils and as an activation marker, although its pathway of expression differs slightly from that of CD63\(^{113}\). Positive controls of the BAT include anti-IgE or anti-FceRI antibodies that induce the IgE pathway of activation of the cells, and N-formylmethionine-leucyl-phenylalanine (fMLP), a bacterial peptide that induces basophil activation in an IgE-independent manner. These controls aid in confirming that the test procedure was followed correctly, and in identifying “non-responder” subjects. Despite the presence of cell surface IgE, the basophils of “non-responders” fail to activate upon IgE cross-linking, presumably due to a Syk protein deficiency in the IgE intracellular signaling pathway \(^{114, 115}\). This occurs in approximately 10% of individuals \(^{114, 116}\).

Both CD63 and CD203c are robust basophil activation markers and have been shown to correlate well with histamine release and clinical symptoms. Basophil activation has proven to be sensitive and specific in the diagnosis of various IgE-mediated allergies, including hymenoptera venom \(^{110, 117}\), food \(^{122, 124}\) and drug allergies \(^{125, 126}\). The diagnosis of allergy currently relies on clinical history, skin prick testing and the detection of serum allergen-specific IgE, however, these methods can provide conflicting results \(^{127}\). The gold standard in diagnosis is an allergen provocation test, which in some cases carries the risk of inducing a severe reaction. Furthermore, although serum allergen-specific IgE is a key component of IgE mediated allergy diagnosis, the presence of this molecule alone does not indicate its functional contribution to effector cell activation. Due to its close correlation with clinical symptoms and the gold standard allergen challenge \(^{124}\), the BAT is applicable in not only allergy diagnosis but also in monitoring treatment efficacy and predicting the safety of novel allergy therapeutics \(^{128}\). The BAT is currently applied as a research tool, however, future standardization and diagnostic laboratory implementation of this technique will greatly enhance its utility in difficult to diagnose allergies and allergies with high risk of anaphylaxis upon in vivo allergen challenge.
CHAPTER 1

5. THERAPEUTICS - ALLERGEN IMMUNOTHERAPY (AIT)

Allergen Immunotherapy (AIT) has been used in clinical practice since the early 20th century and is currently the only therapy to deal with the underlying cause of allergy, by inducing immune desensitization or tolerance to the allergen with long term or permanent resolution of the disease and associated symptoms. Typically this is achieved by repeatedly administering increasing doses of the allergen to the patient. After the initial phase of increasing dose delivery, a maintenance phase follows, during which a consistent amount of allergen is administered on a regular basis for up to several years. Desensitization is achieved when an allergic subject can be exposed to or ingests an amount of allergen without the occurrence of an adverse reaction; however, the subject must encounter the allergen on a regular basis, as occurs during the maintenance phase of immunotherapy, in order to maintain desensitization. Tolerance or sustained unresponsiveness, on the other hand, is achieved when a subject who is no longer receiving an AIT maintenance dose, can be exposed to the allergen sporadically without adverse effects. Sustained unresponsiveness is the ultimate goal of AIT and the success in achieving this may be dependent on various factors, including the duration of AIT, severity of the allergy as suggested by clinical history, baseline skin prick test results, and serum antigen-specific IgE levels.

5.1 ROUTES OF AIT DELIVERY

AIT is routinely delivered through either subcutaneous (SCIT) injection or sublingual (SLIT) drops or tablets, but may also be administered by intradermal injection or orally. Recently, novel routes of delivery have been investigated including epicutaneous application, whereby the allergen is applied to the skin, and intralymphatic injection, which involves injection of the allergen directly into the lymph node. Both SCIT and SLIT have been shown to be effective in placebo-controlled trials, although it is not yet clear whether effects are comparable between the two delivery methods. There is some evidence that SCIT may be clinically more effective, while SLIT can be self-administered at home and is associated with fewer adverse events. Oral immunotherapy has been used in clinical trials of immunotherapy for food allergies due to the severe side effects encountered after subcutaneous administration. Although clinical efficacy has been achieved using this route of delivery, adverse events are still frequent and, depending on the allergen involved, in some cases severe.

The efficacy of immunotherapy relies on adequate delivery of the allergen to APC, preferably without the encounter of highly vascularized areas or large numbers of mast cells to limit (systemic) adverse events. The skin contains several distinct subsets of DC, Langerhans cells (LC) in the epidermis and CD1c+ DC, CD14+ DC and CD141+ DC in the dermis (termed dermal DC). Langerhans cells are positioned as the first line of defense, surveying the area for foreign antigen and migrating to the skin draining lymph nodes. They have been shown to induce a Th2 type response in vitro. CD141+ dermal DC, on the other hand, are major producers of IL-10 and capable of inducing a Treg response, thereby playing an important role in maintaining skin homeostasis. Subcutaneous delivery targets the connective tissue and adipose layer under the dermis, which also contains a dispersed population of DC. This route of delivery requires high doses of allergen to achieve tolerance. Intradermal delivery targets the relatively abundant population of dermal DC, with the added benefit of direct access to the lymphatic system. This may explain the relatively low dose of allergen required to achieve desensitization in intradermal delivery compared to subcutaneous delivery. Intralymphatic delivery deposits the allergen to the highly populated area of DC in the lymph node, where presentation of the allergen to T cells occurs. Although this route has recently been shown to be safe and effective for grass pollen and cat allergy, further research is required to confirm its safety and applicability for other allergies, particularly those associated with anaphylaxis.

The sublingual and oral routes involve APC within the oral cavity and intestinal mucosa, including tolerogenic LC capable of inducing a highly desirable Treg response to the allergen. This tolerogenic phenotype is a necessity in areas exposed to high loads of harmless foreign (food-derived) antigens and commensal bacteria encountered in the gut. As evidence for this, TLR4 ligation on oral LC results in up-regulation of co-inhibitory molecules, down regulation of costimulatory molecules and increased IL-10 production. Subsequently, these cells are able to induce T cell production of regulatory and type 1 cytokines. Furthermore, allergen (Phl p 5) binding to oral LC not only induces IL-10 and TGF-β production, but also attenuates their maturation, which has been shown to induce tolerance. In addition to these tolerogenic features, the sublingual and oral routes of delivery can induce local allergen-specific IgA, providing an additional first level of protection from an allergic response to ingested food allergens. Finally, the chosen route of delivery may also affect compliance of the patient, considering that a less invasive, home-administered therapeutic regimen may be easier to maintain than one that involves repeated hospital/clinic-administered injections.

5.2 MECHANISM OF CONVENTIONAL AIT

The mechanism behind conventional immunotherapy for allergy consists of several immune-modulatory events. In the early phase, basophil reactivity, and thereby mediator release, is hampered by rapid up-regulation of the histamine receptor HR2. The following phase consists of the emergence of natural and/or induced allergen-specific regulatory T and B cells and the suppression of allergen-specific effector T cells. Induced regulatory T and B cells are characterized by production of the anti-inflammatory cytokines IL-10 (Tr1 and Br1 cells) or TGF-β (Tr3 and Br3 cells). TGF-β inhibits B cell IgE production while inducing anti-inflammatory mucosal allergen-specific IgA. Further effects of TGF-β include suppression of Th1 and Th2 lymphocyte differentiation and the induction of Treg development through the transcription factor Foxp3. IL-10 is a key component of the induction of tolerance and is initially produced by...
Treg, however, in the following phases of AIT, monocytes and regulatory B cells also produce IL-10. The suppressive effects of IL-10 result in further induction of regulatory cells and a decrease in allergen-induced proliferation and cytokine production by effector cells. Further suppression is achieved with the up-regulation of allergen-specific IgA, IgG, and IgG₄, although it has been shown that functional IgG₄, which competes directly with allergen-specific IgE molecules as opposed to merely immunoreactive IgG₄, is required for this effect. An additional effect of allergen-specific IgG is the prevention of IgE-facilitated antigen presentation through CD23-bound IgE on professional APC, which would otherwise boost the Th₂ response in a positive-feedback loop. Recently, it was shown that IL-10 producing B regulatory cells were responsible for bee venom allergen-specific IgG₄ production in tolerant beekeepers. Finally, upon nasal or cutaneous allergen challenge, a reduction in recruitment of eosinophils, mast cells and basophils and IgE-dependent mediator release occurs. Allergen-specific IgE generally increases early after AIT induction followed by a gradual decrease. The complete course of immunotherapy often lasts three to five years, however, the nadir of the allergen-specific IgE levels may not be reached for several years after completion of the AIT. With successful AIT, a marked improvement in allergic symptoms is observed, with the additional advantage of a reduced risk of developing new allergic sensitizations.

Although many aspects of the mechanism of AIT have been revealed, the precise mechanism behind the down-regulated Th2 cell response is not fully understood. There is evidence for a combination of Treg induction, T cell anergy and T cell deletion. T cell anergy describes the induction of non-responsiveness of T cells to their corresponding ligand. Anergy to any antigen can be induced in a number of different ways, primarily involving aspects of TCR and CD28 engagement with their respective ligand during antigen presentation. IL-10 can induce T cell anergy through inhibition of the CD28 signaling pathway. During low TCR triggering, CD28 engagement is necessary for T cell activation, a lack of which results in anergy. Furthermore, IL-10 inhibits DC maturation leading to reduced MHC Class II and costimulatory ligand expression (CD80/CD86); antigen presentation by these immature DC also results in anergy of the T cells. In addition to the effects of IL-10, anergy can also be achieved in T cells through exposure to high concentrations of specific antigen, coupled with insufficient costimulation, or through altered peptide ligands capable of only partial TCR binding. Another aspect of T cell anergy is that it can be reversed under specific circumstances. After bee venom immunotherapy, PBMC of treated patients displayed reduced antigen-specific T cell proliferation and Th1 (IL-2, IFN-γ) and Th2 (IL-4, IL-5, IL-13) cytokine production. Through in vitro culture of the PBMC with antigen and IL-2, IL-15 or IL-4, proliferation and the production of Th1 and Th2 cytokines could be restored. This suggests that the cytokines present in the microenvironment of the cell can influence the phenotype of the restored responsiveness. Should the environment remain predominantly Th2, the restored T cells could potentially regain their Th2 phenotype, which may result in relapse in allergen sensitivity and render the AIT unsuccessful.

Recently, evidence for allergen-specific T cell deletion in AIT has also emerged. Using a MHC Class II/peptide tetramer approach, Wambre et al. were able to track the fate of allergen-specific T cells during timothy grass AIT and found no change in allergen-specific Th1/Th1 cell frequency, but rather a decrease in allergen-specific Th2 cell frequency. Along with the observed low level of the survival protein Bcl-2 in these Th2 cells compared to the Th1/Th1 cells, targeted deletion of these cells seems the likely cause. This supports earlier studies showing induced apoptosis in antigen-specific IL-4+ T cells after HDM or grass pollen immunotherapy in atopic individuals, or through high dose allergen stimulation in vitro.

### 5.3 DEVELOPMENT OF CONVENTIONAL AIT FOR FOOD ALLERGIES

Although AIT is successful in treating allergic rhinitis, HDM allergy and animal dander allergy, its application is limited in treating food allergy, due to the risk of anaphylaxis during treatment. Subcutaneous administration of peanut immunotherapy has been performed in two clinical trials. Although signs of efficacy were observed in both, systemic adverse reaction rates were unacceptably high, with one trial ending prematurely due to fatal anaphylaxis in a placebo group patient who erroneously received an active dose intended for the treatment group. Since these early trials, subcutaneous administration of food allergens for immunotherapy has largely been abandoned, however, numerous clinical trials of oral immunotherapy (OIT) for milk, egg, and also peanut allergy have since been conducted and shown success in achieving allergen desensitization. Open studies, case series and controlled trials of OIT for peanut allergy have shown some evidence of allergen desensitization, through increased thresholds of allergen ingestion (several grams, the equivalent of 10 peanuts) without symptoms in oral food challenges. In the first randomized placebo controlled trial of oral peanut immunotherapy in 19 children, investigators reported successful desensitization in 84% of the cohort after 1 year, with subjects passing a final oral challenge of 20 peanuts in the active group, compared to only 1 peanut in the placebo group. Desensitization is accompanied by immunological changes previously shown to correlate with AIT efficacy, including increased allergen-specific IgG, decreased Th2 cytokine production and increased Treg populations. Unfortunately, adverse events including upper respiratory symptoms, nausea, abdominal pain and diarrhea during the initial escalation phase are frequent and, in some cases, severe. Importantly, anaphylaxis was reported in three separate studies and included patients with no prior history of anaphylaxis. In addition, subjects with a history of severe anaphylaxis are generally excluded from AIT, which is a significant limitation as these individuals have the most to gain from peanut immunotherapy and may therefore be more inclined to seek treatment. The results of these trials clearly indicate the potential for immunotherapy to treat food allergy, but also highlight the need for a safer alternative to conventional AIT.
CHAPTER 1

6. PEPTIDE IMMUNOTHERAPY (PIT)

Several alternative approaches to AIT are under investigation in order to achieve desensitization and tolerance through allergen administration without inducing an IgE-mediated allergic reaction. One is the use of modified hypoallergenic recombinant proteins, which lack either key components required for protein folding or IgE-epitope sequences, eliminating the conformational epitope required for recognition by IgE. This has been demonstrated with Bet v 1, in which folding of the protein was disrupted by reduction and alkylation\(^1^98, 199\). These non-allergenic proteins can also be developed through the introduction of point mutations to disrupt disulfide bonds of the protein, partial sequence deletion, or fusion with variant proteins\(^200\). Many of these modifications have yet to be used in patient therapy, however, in a recent phase 1 clinical trial the safety of recombinant major allergens of peanut, modified by amino acid substitution at major IgE-binding epitopes, was assessed in peanut allergic patients. The vaccine, delivered rectally in an E. coli-encapsulated form, led to frequent and sometimes severe allergic reactions, indicating that despite best efforts, it may be challenging to remove the heterogeneous range of IgE-binding epitopes recognized by an allergic population\(^201\).

Another approach to reducing the allergenicity of the therapeutic for AIT is the use of short T cell targeted peptides derived from the allergen, which lack the ability to cause IgE crosslinking required for effector cell activation and an allergic response, as opposed to whole proteins. Although many aspects of the initiation of an allergic response are unclear, it is widely recognized that T cells play a fundamental role. Allergen-specific Th2 cells are necessary for the production of Th2 cytokines, which recruit effector cells, and initiate Ig class switching to IgE isotype in B cells. Antigen-specific Th1 cells and Treg predominate in non-allergic or desensitized subjects\(^193, 202\), and tolerance through allergen administration without inducing an IgE-mediated allergic response, as opposed to whole proteins. T cell targeted peptides derived from the allergen, which lack the ability to cause IgE crosslinking required for effector cell activation and an allergic response, as opposed to whole proteins.

6.1. EFFICACY OF PIT

This approach was first tested for safety and efficacy in clinical trial using immune-dominant T cell epitopes of major allergens involved in cat allergy (Fel d 1) and bee venom (PLA2) allergy. In the first study of bee venom PIT, five patients were treated with a mixture of three short (18 amino acids or less) PLA2-derived peptides\(^202\). Clinical efficacy was observed in this study, with two patients experiencing only mild symptoms to bee sting challenge after PIT, and three being non-symptomatic. Furthermore, no local or systemic reactions were observed during PIT. Concurrent immunological changes included suppression of T cell proliferative responses and both Th2 and Th1 cytokine production. Antigen-specific IgG, was induced upon challenge with the whole bee venom allergen after completion of PIT, but not during the treatment. Following on from this study, a mixture of three long synthetic overlapping peptides (between 45-60 amino acids) of PLA2 were administered to patients in a placebo controlled trial. Immunological changes observed in this trial resembled those of conventional AIT, with decreased T cell responsiveness, increased IL-10 and IFN-γ production, along with the emergence of allergen-specific IgG\(^\). During the course of PIT, mild late adverse reactions were observed in two patients\(^204\). In the most recent study of PIT for bee venom allergy, twelve subjects received nine intradermal injections with a mixture of 18-mer PLA2 peptides. The treatment was received without any adverse events and, along with a reduction in the late phase response to bee venom challenge, reduced production of IL-13 and IFN-γ and increased IL-10 from PLA2 stimulated PBMC was observed. A transient, but modest increase in PLA2-specific IgG\(_2\) was also observed\(^205\).

Using a slightly different approach, a birch pollen specific immunotherapy was designed based on eight long (over 48 residues) contiguous overlapping peptides (COPs) spanning the entire sequence of the major allergen Bet v 1. Based on their inability to induce basophil activation or skin prick reactivity in allergic subjects\(^215\), three of these peptides (collectively named AllerT) were tested for efficacy in a phase IIa clinical trial\(^211\). Five injections of increasing doses of AllerT or placebo were administered to twenty randomized patients over two months. No immediate reactions or episodes of anaphylaxis occurred during treatment and the frequency and severity of local adverse events were comparable between the treatment and placebo groups. Two of the fifteen AllerT-treated patients experienced systemic adverse events several hours after injection, possibly caused by T cell cytokine production upon activation by the peptides. In vitro AllerT, Bet v 1 or birch pollen stimulated PBMC showed increased proliferative responses and increased production of IL-10, TH1 and Th2 cytokines in AllerT-treated but not placebo-treated patients. Although the nasal allergen challenge results were inconclusive, a trend towards improved quality of life was reported in the AllerT-treated group. Furthermore there was an increase in Bet v 1-specific IgG\(_4\) level that was maintained for up to 3 years post treatment\(^211\).

The PIT designed to treat cat allergy, termed Cat-PAD, originated from studies performed by Larche and Kay in the late 1990s. It is the first in its class to reach phase III clinical trials, comprising seven short synthetic T cell epitope peptides derived from Fel d 1\(^212\). Earlier trials of the peptide immunotherapy Allervax CAT, demonstrated some clinical efficacy at high doses (750 µg) but also reported both immediate and late phase adverse events, some requiring adrenaline\(^212-214\). The Allervax CAT PIT consisted of two long peptides derived from Fel d 1, each 27 amino acids in length, and was administered subcutaneously. The length of the peptides, possibly allowing for formation of IgE reactive conformational epitopes, was thought to have contributed to the immediate adverse events. A subsequent Fel d 1-based PIT consisted of 12 short (16-17 residues) peptides. Clinical efficacy was assessed in several studies, revealing decreased airway hyper-responsiveness and a reduction in late phase cutaneous reactions and late asthmatic reactions to whole cat allergen, although not all patients responded to the
therapy\textsuperscript{216-219}. Adverse reactions were minimal, consisting mainly of mild late asthmatic reactions that were readily reversed with inhaled bronchodilator. Decreased antigen-specific CD4\textsuperscript{+} T cell proliferation and cytokine Th2 production (IL-4, IL-5 or IL-13) were generally observed in PBMC cultures after PIT\textsuperscript{218, 220, 221}. Finally, Cat-PAD was designed comprising seven of the original twelve Fel d 1 peptides selected for their binding affinity to commonly expressed HLA-DR molecules, their ability to induce PBMC proliferation and cytokine production in vitro (IFN-\(\gamma\), IL-10 and IL-13), and their inability to cause histamine release from basophils\textsuperscript{222}. In addition, agents were included to prevent peptide dimer formation through disulfide bridging between cysteine residues. The selected peptides have since been tested clinically for efficacy and safety, with highly encouraging results\textsuperscript{223, 224}. Investigators reported a reduction in rhinoconjunctivitis symptoms upon challenge in an environmental exposure chamber (EEC), persisting for up to 2 years after a short course of treatment with 4 intradermal injections of 6 nmol Cat-PAD IT\textsuperscript{223}. Many details of the peptide characteristics, optimal dosing and schedule\textsuperscript{224} as well as the route of delivery have been thoroughly investigated in the course of development of the current cat-PAD therapy, providing valuable guidelines for the design of other allergy PIT in the future.

Indeed, several clinical trials assessing the efficacy of similarly designed PIT for HDM\textsuperscript{225}, grass pollen\textsuperscript{226}, and ragweed pollen\textsuperscript{227} allergy are currently underway, showing promising results.

6.2 MECHANISM OF PIT

Although clinical efficacy has been reported for peptide-based allergen immunotherapy, the underlying mechanisms of these effects have yet to be elucidated. Evidence derived from in vitro studies, mouse models, and current PIT trials, indicate that whilst some mechanisms overlap, others may differ to those thought to underlie conventional whole AIT (Figure 3).

6.2.1 T cell regulation and linked epitope suppression

Similarly to reports from conventional AIT, increased production of the immunoregulatory cytokine IL-10 has been observed in bee venom PIT trials, however, the role of IL-10 in immune-suppression by Fel d 1 derived PIT is less clear. There are, however, indications of a role for CD4\textsuperscript{+} T cells with a regulatory function. Allergen-dependent recruitment of CD4\textsuperscript{+}CD25\textsuperscript{+} cells to the skin was observed after Fel d 1 PIT (containing 12 peptides), but this was not related to IL-10 production\textsuperscript{227}. In a related study, increased Treg function, defined by increased suppression of allergen-specific proliferative responses of CD4\textsuperscript{+} cells by CD4\textsuperscript{+} cells after Fel d 1 PIT, was reported. Although no increase in CD4\textsuperscript{+}CD25\textsuperscript{+} cells was observed in PBMC, CD5 levels were increased on both CD4\textsuperscript{+} and CD8\textsuperscript{+} cells. CD5 is a negative regulator of TCR signaling, and elevated levels of this molecule are associated with decreased T cell responses to antigen\textsuperscript{221}. A subsequent placebo-controlled study of the same Fel d 1 PIT reported decreased allergen-specific CD4\textsuperscript{+} T cell responses, but no change in suppression by CD4\textsuperscript{+}CD25\textsuperscript{+} cells or IL-10 production\textsuperscript{226}. In contrast, an earlier trial showed a significant increase in IL-10 production by allergen stimulated PBMC from allergic asthma patients after Fel d 1 PIT treatment compared to pre-treatment levels. It should be noted, however, that the change in IL-10 production did not differ significantly between the treatment and placebo groups\textsuperscript{218}. Further immunological analysis of participants in this trial revealed a role for linked epitope suppression. The patients were treated with 12 Fel d 1 derived peptides, but showed decreased PBMC proliferative and cytokine responses to another 4 peptides of Fel d 1 not included in the PIT mix\textsuperscript{226}. Further evidence of linked suppression was provided in this study using an HLA-DR1 transgenic mouse model sensitized to Fel d 1 to track allergen-specific T cells with Fel d 1 (29-45) DR1 tetramers after treatment with a low dose of the Fel d 1 (29-45) peptide. Suppression of peptide (29-45)-specific T cell responses was observed, with linked suppression to other regions of the Fel d 1 protein. Furthermore, with this model the nature of the suppressive effects could be determined, which was based on immunoregulation through the production of IL-10 by peptide (29-45)-specific T cells as well as bystander T cells. There is also evidence that linked epitope suppression can be induced through cell-to-cell contact. Using a murine model of HDM allergy, it was determined that Delta 1 expression could be transiently upregulated on peripheral T cells after intranasal delivery of high dose Der p 1 (110-131) peptide. T cell responses to both minor (e.g. 81-102) and dominant (110-131) Der p 1 peptides were suppressed by Delta 1 transfected Der p 1 (110-131) reactive T cells. In this case, linked epitope suppression may be induced through Delta-1/Notch-1 interactions between T cells gathering at the cell membrane of APC, presenting both minor and dominant epitopes of the same protein\textsuperscript{229}.

6.2.2 T cell anergy

In early in vitro studies, T cell anergy to HDM allergen was achieved through stimulation with supraoptimal levels of specific peptide, in which an initial increase in IL-4 production was followed by decreased T cell proliferation and IL-4 and IL-5, but not IFN-\(\gamma\) cytokine production upon subsequent antigen exposure\textsuperscript{220, 221}. This anergic response has been shown to occur with both HLA-DR and HLA-DP restricted peptides, in the presence or absence of APC, and is associated with downregulation of TCR expression and upregulation of CD2 and CD25\textsuperscript{230-233}. Furthermore, significant CD28 downregulation at both the cytoplasmic mRNA and protein level is involved in this process\textsuperscript{234}. Induction of anergy has also been shown in cloned human CD4\textsuperscript{+} bee venom (PLA)-specific Th2 cells upon incubation with PLA peptide and APC. An increase in T cell CD25 expression was observed, and although CD3 and CD28 levels remained unaltered, defective membrane TCR signaling through abrogation of pS6ck or ZAP-70 tyrosine phosphorylation was detected\textsuperscript{235}. An additional aspect of PIT that may contribute to the induction of anergy is the introduction of the T cell epitope peptides in the absence of an accompanying danger signal. Danger signals can originate from various pathogenic components, but may also result from administration of whole allergen as used in conventional subcutaneous AIT. A murine study showed the induction of T cell tolerance to OVA peptide when delivered alone, but not OVA peptide combined with LPS. The mechanism behind this is thought to be dependent on peptide...
presentation to T cells by DC in the absence of TLR activation, which would otherwise induce DC costimulatory molecule upregulation and T cell activation\textsuperscript{236}. Murine studies also provide in vivo evidence of T cell anergy following transient T cell activation after high dose PIT\textsuperscript{237, 238}. In line with the previously mentioned in vitro studies, the anergic state of the allergen-specific T cells correlated with decreased TCR expression\textsuperscript{237}. Furthermore, the induction of anergy was shown to be reversible but long-lasting, with anergic T cells surviving for up to several months in the lymphoid tissue\textsuperscript{238}.

6.2.3 T cell deletion

To date, direct evidence of T cell deletion during PIT is limited, however, it has been noted that the dose of peptide administered may influence the mechanism of tolerance in PIT, with high doses associated with deletion of peptide-specific T cells. In transgenic mice expressing TCR specific for a peptide derived from the influenza virus hemagglutinin, intravenous injection of high dose (750 mg), but not low dose (75 mg), of the peptide resulted in both thymic and peripheral T cell apoptosis, in addition to T cell anergy\textsuperscript{238}. Evidence of T cell deletion following conventional AIT has been reported\textsuperscript{239} and by applying the knowledge of the epitopes involved, PIT could enable a targeted approach to inducing specific T cell deletion.

It is clear that PIT can be clinically effective and associated with important immunological changes, consisting of decreased allergen-specific T cell responsiveness at both the proliferative and cytokine production level. The specific mechanism involved is not entirely clear, but may depend on both the peptide dose and the dosing regimen employed. Induction of allergen-specific IgG\textsubscript{4} production, although a prominent component of conventional AIT, so far has not proven to play a comparable role in PIT containing short peptides\textsuperscript{207, 209}.

Figure 3. Mechanisms involved in T-cell epitope peptide mediated tolerance.

Based on murine and human studies suppression of the allergic response by PIT can be achieved through induced anergy of allergen-specific naïve CD4\textsuperscript{+} T cells (nT) and Th2 cells, deletion of allergen-specific Th2 cells, and the emergence of allergen-specific Treg cells, capable of inhibiting of Th1, Th2 and effector cell function. In contrast to conventional allergen immunotherapy, the role of allergen-specific IgG\textsubscript{4} in PIT is unclear. [adapted from Prickett, Clin & Exp Allergy 2015]

7. BIOLOGICALS

Based on increased knowledge of the mechanisms behind an allergic reaction, several targeted therapeutics have been developed. Many of these consist of humanized antibodies specific for various components of the Th2 pathway, including TSLP, IL-4, IL-5, IL-13, CD23 or IgE\textsuperscript{240-242}. Omalizumab, targeting human IgE, was designed to capture free IgE before it attaches to its corresponding high affinity receptor (FcεRI) on effector cells, including basophils, mast cells and DC. The antibody specifically targets the CH3 region of IgE that is necessary for binding to the alpha-chain of FcεRI. Therefore, it not only prevents free IgE from binding to empty receptors but also lacks the ability to bind to and crosslink FcεRI bound–IgE, which would otherwise result in effector cell activation, as occurs in an allergic reaction\textsuperscript{243}. In addition, due to the high turnover of FcεRI molecules, newly produced FcεRI molecules will not encounter IgE upon administration of omalizumab, and remain empty. Empty FcεRI molecules are rapidly internalized, and the
overall expression of FcεRI is then down regulated in response to the decreased levels of IgE in the environment of the cell. It is known that FcεRI expression levels are directly related to the level of IgE encountered by the cell244, 245.

Omalizumab was developed by Roche/Genentech and Novartis, and has been available for clinical use since 2002246. The drug is currently indicated for severe allergic asthma, and has been shown to be effective in reducing exacerbation frequency247, 248. More recent results have even shown that this treatment can lead to an improvement in lung function249 and reduction in the use of systemic corticosteroids250, however, it is becoming clear that its efficacy may vary, depending on subtle differences in the disease endotype. Although originally intended for allergic asthma, omalizumab may also be effective in treating other (IgE-related) diseases251 including mastocytosis252, allergic rhinitis253, chronic urticaria254-256, and allergic bronchopulmonary aspergillosis (ABA)257-259. This has been indicated in various case studies and case series, however, for conclusive evidence of efficacy in these disorders, placebo-controlled clinical trials are essential.

8. SCOPE OF THIS THESIS

The first aim of this thesis is to investigate basic components of the initiation of an allergic response, including the route of sensitization and the cells responsible for the initiation of Th2 skewing in allergic subjects. More specifically, in Chapter 2 both clinical and immunological evidence of food allergy development through cutaneous sensitization are presented in the form of a case study. In Chapter 3 the ability of human basophils to initiate a Th2 response through direct antigen presentation to T cells is investigated.

The second aim of this thesis is to investigate the use of current therapies and the development of new therapies for difficult-to-treat allergic diseases. In Chapters 4 and 5 dominant T cell epitopes of major peanut allergen proteins are identified and characterized providing the first step in the design of a safe and effective peptide based T cell targeted immunotherapy to treat peanut allergy. The identified dominant T cell epitopes are then further investigated in Chapter 6, assessing their stability and resistance to enzymatic digestion for potential oral administration.

Finally in Chapter 7, the immunological and clinical efficacy of omalizumab for the treatment of allergic bronchopulmonary aspergillosis is assessed, for the first time, in a randomized placebo controlled clinical trial.

In Chapter 8 the findings of this thesis are summarized and discussed, focusing on the contributions they have made to the discipline of allergy and future directions in the field. Throughout these studies, basophils play an important role as either the object of investigation or as a tool in diagnosis, allowing determination of the efficacy of existing therapeutics, or assessment of the safety of novel therapies.

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CHAPTER 1

GENERAL INTRODUCTION


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CHAPTER 1

GENERAL INTRODUCTION

CHAPTER 1

GENERAL INTRODUCTION


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