Immunological Approaches for Tolerance Induction in Allergy

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Abstract Allergy is the consequence of an inappropriate inflammatory immune response generated against harmless environmental antigens. In allergic disorders such as asthma and rhinitis, the Th2 mediated phenotype is a result of loss of peripheral tolerance mechanisms. In cases such as these, approaches such as immunotherapy attempt to treat the underlying cause of allergic disease by restoring tolerance. Immunotherapy initiates many complex mechanisms within the immune system that result in initiation of innate immunity, activation of both cellular and humoral B cell immunity, as well as triggering T regulatory subsets which are major players in the establishment of peripheral tolerance. Though studies clearly demonstrate immunotherapy to be efficacious, research to improve this treatment is ongoing. Investigation of allergenicity versus immunogenicity, native versus modified allergens, and the use of adjuvant and modality of dosing are all current strategies for immunotherapy advancement that will be reviewed in this article.

Abbreviations

APC	Antigen presenting cell
Breg	B regulatory cell
CTLA4	Cytotoxic lymphocyte antigen 4

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DC	Dendritic cell
FoxP3	Forkhead box protein 3
NLR	NOD-like receptor
PBMC	Peripheral blood mononuclear cells
PRR	Pattern recognition receptor
SCIT	Subcutaneous immotherapy
SLIT	Sublingual immunotherapy
TCR	T cell receptor
Th	T helper
iTreg	Inducible T regulatory cell
TLR	Toll-like receptor
Treg	T regulatory cell
T_R1	Type 1 T regulatory cell
tTreg	Thymus derived t regulatory cell

Contents

1 Introduction

Allergy is based on a complex dysregulation of the immune system whereby harmless environmental antigens trigger an inappropriate immune reaction. Clinically, an allergy can manifest in many different forms including local reactions such as asthma, rhinitis and skin inflammation, as well as systemic reactions to

food, venom or drugs. These allergic phenotypes are complex and depend on many factors including genetic and environmental influences, the specific organs affected and the type and quantity of allergen (Larche 2006). Complicating matters further, exacerbations of current allergy are not only allergen driven but can also be brought about by infection (Tauro et al. 2008), pollutants (Riedl 2008) or non-specific stimuli (Gelardi et al. 2009).

In general, the development of an allergic response requires first that an individual be sensitized, a reaction involving the priming of specific CD4+ T-helper (Th) 2 cells, the production of the cytokine IL-4, isotype switching in B cells to produce IgE antibodies and binding of IgE antibodies to mast cells. After sensitization, secondary exposure to a specific allergen engages IgE coated mast cells which leads to mast cell degranulation and initiation of the allergic immune response. It is during secondary exposure to allergen that the complex clinical phenotype becomes apparent and it is well known that different mechanisms are responsible for the initiation of particular allergic phenotypes. For instance, patients that suffer from allergic asthma and allergic rhinitis exhibit mainly local Th2 type reactions (Pipet et al. 2009), atopic dermatitis patients exhibit an initial Th2 response that is converted to Th1 during the chronification of the disease (Leung and Bieber 2003; Novak et al. 2003; Werfel et al. 1996) and patients with food, drug and venom allergies often react by systemic anaphylaxis (Sicherer and Leung 2009). Due to the complexity of these different allergic responses, this review will concentrate specifically on two Th2 mediated allergic disorders, asthma and rhinitis (hay fever).

2 The Immune Response to Allergens is Regulated by T cells

Many immune mediators play important roles in the development of allergic disease, initiating pathways that cumulate in the differentiation of particular T cell subsets. In both healthy and diseased individuals, these subsets of effector T cells act to coordinate the entire immune system. Classically, Th cells were divided into two major subsets, Th1 and Th2, determined by cytokine profile and effector function. In the last 13 years however, T regulatory (Treg) cells have emerged as very important mediators of immune homeostasis, and are now at the forefront of research efforts. In recent years, still more T cell subtypes have been discovered such as Th17 (Burgler et al. 2009) and Th9 (Dardalhon et al. 2008a) both of which promote tissue inflammation.

The differentiation of naïve T cells to an effector subset is largely dependent on the cytokine milieu. Production of IL-12 and IFNγ by cells of the innate immune system stimulate the production of the transcription factor T-bet, Fig. 1. This results in the differentiation of Th1 cells that principally secrete IFNγ in response to intracellular pathogens. The Th2 cell lineage is generated in the presence of IL-4 due to activation of the transcription factor GATA3, Fig. 1. Th2 cells mainly utilize IL-4, IL-5 and IL-13 to regulate the clearance of extracellular

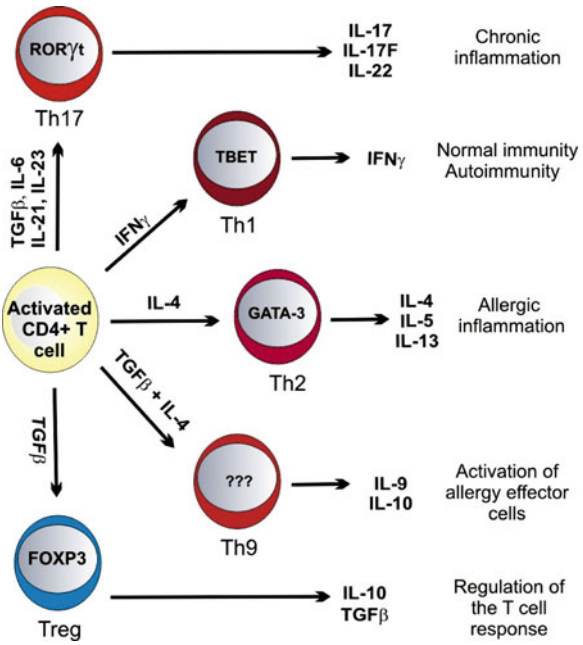

Fig. 1 Differentiation of T effector subsets from an activated CD4+ T cell

pathogens such as parasites. When a Th1 or Th2 response becomes dysregulated it leads to exaggerated inflammatory responses that are the foundation of autoimmunity and allergy, respectively (Dardalhon et al. 2008b).

Relatively new on the scene, Th17 cells are generated in the presence of both IL-6 and TGFβ; these cytokines initiate Th17 cell production by activation of the transcription factor RORγt, shown in Fig. 1. Th17 cells promote neutrophilic inflammation and appear to be responsible for eliminating both intra and extracellular pathogens through the secretion of cytokines IL-6, IL-8, IL-17A, IL-17F, IL-22, IL-26 and TNFα. Similarly to Th1 cells, an overabundance of the TH17 response leads to autoimmune disease and chronic inflammation (Schmidt-Weber et al. 2007). Finally, one of the most recent additions to the T cell subtype family is Th9. During an allergic response, the presence of IL-4 coupled with TGF-β leads to the differentiation of the Th9 cell which produces IL-9 and IL-10. Despite the abundant production of IL-10, Th9 cells do not have regulatory properties and instead act to promote tissue inflammation (Akdis and Akdis 2009; Dardalhon et al. 2008a, b; Veldhoen et al. 2008). While Th1, Th2, Th17 and Th9 subsets all generate inflammatory responses to various mediators, Treg cells act as a safeguard against unnecessary inflammation through immunosuppressive means. It is the complex interplay between these subsets that determines the health status of an individual.

Treg cells are indispensable for the maintenance of immune homeostasis and different subsets of these cells are defined by where they originate and what cytokines they secrete. Thymus derived Treg cells (tTreg, "natural" Treg), which were among the first lineage identified, are produced in the thymus, are

Table 1 Characteristics of T regulatory cell subtypes

	Thymus derived T regs	Induced T regs	
	"Natural"	TR1	Th3
Development			
Region	Thymus	Periphery	Periphery
Precursor	CD4+ precursor	CD4+CD25−	CD4+CD25−
Differentiation factors	?	IL-10, IFNα	TGFβ, IL-4
Markers			
CD4	+	+	+
CD25	+	+	+
CTLA4	+	+	+
FoxP3	+	/	+
Main mode of action	Cell contact suppression	Cytokine secretion	Cytokine secretion
Cytokines secreted			
IL-10	+	+++	+
TGF-β	+	+	+++

+ indicates expression or amount of secretion, /indicates not expressed

CD4+CD25+ and express the transcription factor forkhead box protein 3 (FoxP3), shown in Fig. 1 and Table 1 (Blaser 2008; Feuerer et al. 2009). The importance of this cell type is best exemplified by the Scurfy mouse, in which animals that fail to develop Treg cells acquire a rapidly fatal lymphoproliferative disease (Appleby and Ramsdell 2008; Brunkow et al. 2001; Khattri et al. 2001). Two additional Treg subsets, type 1 regulatory T cells (T_R1) and Th3 cells, have also been identified that can be induced in the periphery by IL-10 and TGFβ exposure, respectively, Table 1. Both tTreg cells and T_R1 cells secrete large amounts of IL-10 and TGFβ, whereas Th3 cells secrete primarily TGFβ (Workman et al. 2009).

Evidence for the regulatory capabilities of Treg cells is demonstrated by functional studies showing the suppression of both autoimmune and allergic responses by this cell type (Ozdemir et al. 2009; Walters et al. 2009). Treg cells have a major influence on both innate and adaptive immune cell types and are capable of suppressing the proliferation and differentiation of T cells as well as limiting the effector functions of B cells, NK and NK T cells, macrophages and dendritic cells (DC), shown in Fig. 2 (Ghiringhelli et al. 2005; Letourneau et al. 2009; Lim et al. 2005; Piccirillo and Shevach 2001). The role of Treg cells in the active suppression of inappropriate or excessive inflammatory responses is known as peripheral tolerance induction.

3 Tolerance

In order for the immune system to function properly there must be systems in place that allow for the discrimination of self versus non-self as well as harmless versus dangerous foreign molecules. There are several sophisticated mechanisms in the

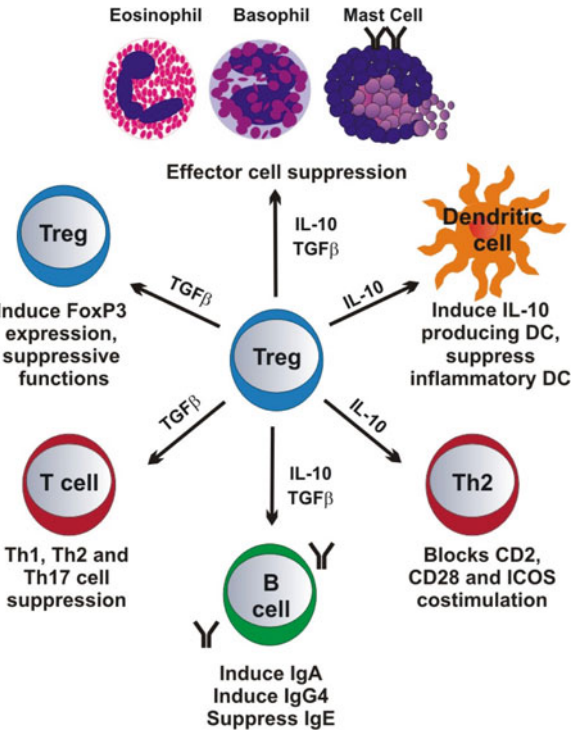

Fig. 2 The effects of Treg generated IL-10 and TGFβ on cells of the immune system

immune system that allow this interplay to occur including clonal deletion, anergy and immunoregulation. Regarding self versus non-self determination, clonal deletion (central tolerance) acts to delete self-reactive lymphocytes during development in the bone marrow and thymus before they mature into competent immune cells (Hogquist et al. 2005; McCaughtry and Hogquist 2008). In the periphery, tolerance mechanisms such as anergy and immunoregulation allow lymphocytes to distinguish between benign and harmful antigens after T cell development (peripheral tolerance). Anergy is a state of unresponsiveness that occurs when lymphocytes recognize an antigen in the absence of a secondary, co-stimulatory signal. Continual recognition of antigen in the absence of co-stimulation eventually results in the elimination of anergic lymphocytes via activation induced cell death (Wells 2009). Immunoregulation is a second system in the maintenance of peripheral tolerance in which Treg cells play an essential role in the control of immune homeostasis. In the context of allergy, dysregulated inflammatory responses are due to a failure of the immunoregulatory mechanisms that suppress reactions to harmless substances in the periphery. Hence, inducing peripheral tolerance to the offending allergen, possibly through the alteration of the Treg response, is the ultimate goal of allergy treatment.

4 Current Treatments for Allergy

Anti-inflammatory therapy and specific immunotherapy are the two present methods used to treat allergy that work in different ways. Anti-inflammatory therapy is an allergen unspecific treatment that involves using medications such as antihistamines, anti-IgE, epinephrine or corticosteroids, alone or in combination, to block the action of allergic mediators or generally suppress the immune system (Bjermer 2008; DuBuske and Kowal 2009; Pelaia et al. 2008). Though the use of medication is presently crucial for the control of allergy, this treatment provides only temporary results. Treatments involving more permanent methods that affect the underlying cause of the allergic disorder are highly desirable.

Immunotherapy differs from anti-inflammatory therapy by attempting to affect the cause of the allergy directly by inducing peripheral immune tolerance to a particular allergen. The earliest attempts to perform immunotherapy for allergy were initiated approximately 100 years ago when, in 1911, Leonard Noon and John Freeman immunized hay fever patients with subcutaneous injections of pollen extract. Though the underlying mechanisms of allergy and immunotherapy would not begin to be discovered for many years and the strategy was erroneously based on the idea that grass pollen was toxic, successful outcomes were seen to last up to one year after cessation of treatment. (Cohen et al. 2003; Noon 1955).

In the present day, allergen specific immunotherapy is recognized as a highly specific and effective method to treat certain types of allergy. Though not generally recommended for food or drug allergies, this therapy is proving particularly effective for patients with allergic rhinitis and allergic asthma (Abramson et al. 2003; Calderon et al. 2007; Pipet et al. 2009). Immunotherapy treatments are beneficial as they target the underlying cause of the disorder with long term results; disease remission is reported to last for 3–5 years after cessation of treatment (Durham 2008). In addition to this, immunotherapy is also extremely important in preventing the progression of allergic disease, for instance from rhinitis to asthma, and stopping the further development of new sensitizations against other allergens (Des Roches et al. 1997; Jacobsen et al. 2007). Though there are many protocol variations, the general treatment method consists of multiple administrations of an allergen vaccine with concentrations that increase in a step-wise manner until the maintenance dose is reached. The maintenance dose is then continued for a minimum of three years (Didier et al. 2007; Srivastava et al. 2009). The most common type of immunotherapy in use today, subcutaneous immunotherapy (SCIT), acts by inducing peripheral tolerance to the allergen vaccine administered.

5 Tolerance Induction

In the process of becoming tolerant, the immune system is modified in many different ways. Innate immune mechanisms are activated which, in turn, trigger the generation of specific T cell subsets. The subsequent cytokine secretion from these

activated T cells has wide ranging effects from reducing proinflammatory cell recruitment and activation, to modulating B cell antibody and cellular responses. Demonstrating this, many types of immune modulation have been documented after successful immunotherapy including direct suppression of antigen presenting cells (APC), induction of Treg subsets, altered IL-10 and TGFβ cytokine levels, suppression of mast cells and basophils and altered allergen specific antibody titres (Akdis et al. 1998, 2007; Francis et al. 2003).

5.1 The T Cell Response and Cytokine Secretion

Of the many T cell effector subsets, Treg cells have been acknowledged as critical players in allergy in both humans and mice. Lessons from mouse models have established the relevance of Treg cells in experimental asthma. Adoptive transfer of Treg cells into cockroach allergen sensitized and challenged mice resulted in improvement of airway reactivity and airway inflammation (McGee and Agrawal 2009). Furthermore, accumulation of Treg cells in the draining lymph nodes of mice is associated with the spontaneous resolution of chronic asthma (Carson et al. 2008). In patients with allergies such as asthma and rhinitis, accumulated evidence also suggests a strong association between allergy and a disruption of Treg cell function. Rhinitis patients have a decreased nasal FoxP3 expression compared with control subjects (Van Bruaene et al. 2008) and Treg cells from allergic subjects have a decreased ability to suppress cytokine responses in vitro (Ling et al. 2004). Dysregulated Treg cell function is associated strongly with allergy in both mouse models and human patients.

Considering immunotherapy, the induction of Treg cells is essential for the generation of immune tolerance. In adult patients who received successful immunotherapy, the frequency of FoxP3 Treg cells was increased in the nasal mucosa following SCIT with grass pollen allergen (Radulovic et al. 2008) and in PBMCs following sublingual immunotherapy (SLIT) with birch pollen allergen (Bohle et al. 2007). Upregulated Treg responses were also correlated with an increase in IL-10 expression. Interestingly, a recent pediatric study measuring the outcome of SLIT for patients treated with tree pollen allergens found correlations between FoxP3 mRNA expression and tolerance induction as well as IL-17 mRNA expression and poor therapeutic outcome (Nieminen et al. 2009). The fact that particular T cell subtypes can be associated with therapeutic outcome highlights the importance of ascertaining the mechanisms that generate T cell subtypes in successful immunotherapy.

Treg cells contribute to tolerance induction in immunotherapy both by cell–cell interactions and cytokine secretion. Concentrating for the moment on direct cellular interactions, cell contact suppression by Tregs is mediated by the constitutive expression of the cytotoxic lymphocyte antigen 4 (CTLA4). CTLA4 is a powerful suppressor of the immune response as evidenced by the lethal multi-organ inflammation observed CTLA4 knockout mice (Waterhouse et al. 1995). In the induction of peripheral tolerance, CTLA4 on allergen-activated Treg cells

interacts with APCs to down modulate their expression. Illustrating this in vitro, CTLA4+FoxP3+ Treg cells out-competed DC interactions with other T cell subsets by forming aggregates around the DC. In addition to this, Treg cells were shown to downregulate APCs both in vitro and in vivo through CTLA4 dependent binding to CD80 and CD86 (Onishi et al. 2008; Wing et al. 2008).

In addition to cellular interactions, certain Treg cell subsets are known for the production of large quantities of IL-10 and TGFβ which play an important role in tolerance induction, Table 1 (Blaser and Akdis 2004). In the normal state, healthy individuals maintain subsets of allergen specific IL-10 secreting T cells at a higher frequency than both IL-4 (Th1) and IFNγ (Th2) secreting T cells (Akdis et al. 2004). In allergy, a marked reduction in IL-10 is noted in comparison with healthy subjects in both mouse models and humans. Patients with asthma have lower concentrations of IL-10 in the bronchoalveolar lavage (BAL) fluid than healthy controls (Borish et al. 1996) and a more severe asthma phenotype is associated with promoter polymorphisms in the IL-10 gene (Lim et al. 1998). Demonstrating the suppressive effects IL-10 and TGFβ, secretion of these cytokines from allergen specific T cells in non-allergic patients acted to suppress both Th1 and Th2 cytokine responses to dust mite and birch pollen allergens. In allergic patients undergoing specific immunotherapy, similar immunosuppressive effects from the CD4+CD25+ T cells were observed (Jutel et al. 2003).

IL-10 and TGFβ have established roles in the generation of peripheral tolerance, shown in Fig. 2. Originally described as a mouse Th2 factor that inhibited Th1 cytokine secretion (Fiorentino et al. 1989), IL-10 is now known to be produced by numerous cell types such as Treg cells, Th1 and Th2 lymphocytes, B lymphocytes, DCs, monocytes, macrophages, mast cells and natural killer cells (Ogawa et al. 2008). IL-10 is recognized for its powerful inhibitory effects on many cell types and the literature provides numerous examples of this in both humans and mice (Moore et al. 2001). In a mouse model of experimental asthma IL-10 administration suppressed both IL-5 production and eosinophil recruitment (Zuany-Amorim et al. 1995). In humans cells in vitro, IL-10 was shown to suppress T cell cytokine synthesis from PBMC by inhibition of CD28/B7.1 interaction (Schandene et al. 1994) and modulate antibody production by inhibiting IgE and enhancing IgG4 (Punnonen et al. 1993; Satoguina et al. 2005). These inhibitory effects coupled with the strong association of IL-10 secretion with allergen tolerance induced by immunotherapy illustrates the importance of this cytokine. TGFβ is an essential cytokine in the maintenance of the Treg population. In mice it is required for the proliferation and suppressive actions of Treg cells (Huber et al. 2004), as well as for the peripheral induction of CD4+CD25− cells to a FoxP3+CD4+CD25+ subtype, shown in Table 1 (Chen et al. 2003). As a suppressive cytokine in human immunotherapy, TGFβ affects T cell proliferation, differentiation and apoptosis. Additionally, both IL-10 and TGFβ have the ability to down regulate MHC II expression and suppress costimulatory molecules on APCs (Jutel et al. 2003). Secretion of IL-10 and TGFβ from Treg cells as well as direct cell–cell interactions are essential for peripheral tolerance and are heavily involved in successful immunotherapy treatment (Workman et al. 2009).

5.2 Histamine and Histamine Receptors in Tolerance Induction

Histamine is a pharmacologically active mediator secreted from effector cells during allergic inflammation. Of the four different histamine receptors (HR1, HR2, HR3 and HR4), HR1 and HR2 are expressed on activated effector T cells (Jutel et al. 2001, 2002). Human CD4+Th1 cells predominantly express the HR1 whereas Th2 cells predominantly display the HR2. This differential expression of HR1 and HR2 results in a differential regulation of the allergic response by histamine. (Jutel et al. 2001, 2002). In this regulatory process, histamine induces the production of IL-10 by DCs (Mazzoni et al. 2001) and Th2 cells (Osna et al. 2001) as well as enhancing the suppressive activity of TGFβ secreted by T cells (Kunzmann et al. 2003). These regulatory effects which suppress IL-4 and IL-13 production as well as T cell proliferation, are mediated via the HR2 mostly expressed on Th2 cells. (Jutel et al. 2001). Thus, HR2 up-regulation on allergen specific Th2 cells increases IL-10 and TGFβ production and regulatory activity and suppresses the allergen-stimulated Th2 response. Accordingly, histamine and its HR2, participates in feedback- and fine-regulation of the allergic immune response and of peripheral tolerance induction.

5.3 B Cells in Tolerance Induction

Though normally thought of as pathogenic when referring to allergy, B cells also play a positive role in immunotherapy. Accordingly, the production of allergen specific IgG4 during successful immunotherapy is proposed to play a major role in tolerance induction. In certain patients, levels of IgG4 in patients reflect allergen exposure, increase in a dose dependent manner, are highly stable during the long immunotherapy time period and appear to protect against allergy (Peng et al. 1992; Rossi et al. 2007). The protective phenotype associated with the emergence of allergen specific IgG4 antibodies includes a reduction in mast cells, basophils and inflammatory mediators, as well as prevention of IgE mediated allergen presentation to T cells (Mobs et al. 2008; Strait et al. 2006). It has been proposed that IgG4 acts as either a blocking antibody that competes for IgE binding sites (Flicker et al. 2002; Jackola et al. 2002; van Neerven et al. 1999) or acts via the binding of IgG4-allergen complexes to the FcγRIIB receptors on mast cells to induce a deactivation signal (Daeron et al. 1995; Pipet et al. 2009).

In some patients treated successfully with immunotherapy, marked changes are observed in the allergen specific antibody composition. After an initial, transient increase in serum specific IgE, levels of this antibody decrease over months and years of treatment (Fennerty et al. 1988; Van Ree et al. 1997). Together with decreases in IgE levels, allergen specific IgG4 antibodies are observed to increase throughout the treatment period (Mobs et al. 2008; Wachholz et al. 2003). Demonstrating the clinical importance of allergen specific antibody isotypes, IgG4

concentrations or ratios of IgG4:IgG_{total} and IgE:IgG4 may be used as an indicator of treatment efficacy and tolerance induction (Aalberse et al. 2009; Nouri-Aria et al. 2004). However, due to large variances in antibody production in individual patients, IgG4 antibody levels cannot be used as a predictable marker for successful immunotherapy in the individual patient (Jeannin et al. 1994). Future research into individual IgG4 antibody production may shed light into how this isotype plays a role in tolerance induction and help to identify traits in individuals that are likely to activate IgG4 production.

In addition to antibody production, B cells may also play an antibody-independent role in the induction of peripheral tolerance. Evidence for this is seen in a mouse model of tolerance to aeroallergens in which the presence of allergen specific B cells in BCR transgenic mice resulted in enhancement of CD4+ T cell tolerance to intranasally applied allergen. This regulation by B cells occurred in an antibody-independent manner. In contrast, tolerance was not achievable in B cell deficient mice (Tsitoura et al. 2002). Due to the antibody-independent nature of this response, it is hypothesized that different B cell subsets may play an immunoregulatory role through either cytokine secretion or cell contact mechanisms. B cells with suppressive capabilities, dubbed B regulatory cells (Breg), are specifically induced under inflammatory conditions and are capable of contributing to tolerance mechanisms (Mizoguchi and Bhan 2006).

B cells with regulatory capabilities were first recognized in 1974 with the demonstration of B cell mediated suppression of delayed type sensitivity reactions in guinea pigs (Katz et al. 1974; Neta and Salvin 1974). Though the majority of Breg cell studies are currently investigating autoimmunity (Manjarrez-Orduno et al. 2009) recent studies in allergy associate B cell secretion of IL-10 and TGFβ with suppressive capabilities (Mizoguchi and Bhan 2006). When stimulated in vitro by immunotherapy extracts, human B cells secrete high amounts of IL-10, which act to suppress IL-4 mediated IgE expression (Milovanovic et al. 2009). In a mouse model of ovalbumin induced experimental asthma, adoptive transfer of B cells into sensitized mice attenuated the resulting allergic airway disease through migration to local inflammatory sites and TGFβ mediated conversion of CD4+CD45− effector T cells to CD4+CD25+FoxP3+ Treg cells (Singh et al. 2008). The involvement of Breg cells in immunotherapy provides an interesting new avenue for exploration in allergy and immunotherapy as an additional system that contributes to tolerance induction.

5.4 Innate Immunity and Tolerance Induction

As natural allergen extracts applied in immunotherapy may also contain components such as lipopolysaccharide, saccharides and nucleic acids, immunotherapy treatments can also have a non-allergen specific effect. These non-allergenic molecules contain pathogen associated molecular patterns (PAMPs) that activate innate immune cells through pattern recognition receptors (PRR) in the Toll-like

receptor (TLR) or NOD-like receptor (NLR) families. Initiation of the immune system by PAMPS generates particular antigen presenting cell subtypes which subsequently results in co-stimulation and production of beneficial T cell subsets such as Treg cells. The generation of Treg cells thus contributes to the suppression of the allergic phenotype (Pipet et al. 2009). Examples of innate immune stimulation in patients undergoing SCIT include IL-10 production by monocytes and macrophages (Nouri-Aria et al. 2004), and a mouse model of oral tolerance has identified TGFβ expressing DC subsets that induce functional FoxP3+ Treg cells from FoxP3− cells in the periphery (Yamazaki and Steinman 2009).

6 Improving Current Immunotherapy Strategies

The goal of immunotherapy is to induce peripheral immune tolerance to specific allergens while maintaining safety and tolerability for the patient (Schmidt-Weber and Blaser 2005). While clinical studies indicate that with strict adherence to guidelines, SCIT is relatively well tolerated, alternative methods to induce tolerance while reducing the risk of adverse events and simplifying the protocol for patient compliance, continue to be investigated (Pipet et al. 2009). A number of considerations such as the type of allergen, the modality and the use of adjuvant all represent potential targets for the advancement of immunotherapy.

6.1 Allergen: Immunogenicity and Allergenicity

Focusing on allergens, tolerance induction may be dependent on both the immunogenicity and allergenicity of the vaccine used for immunotherapy. Immunogenicity is defined as the capacity of a vaccine to induce a beneficial immune response, whereas allergenicity is the potential to cause an allergic reaction. The immunogenicity and allergenicity of a molecule are defined by specific epitopes present on the macromolecule. B cell epitopes, which are specifically recognized by antibodies, are located on the three dimensional structure of the antigen molecule whereas T cell epitopes must be first phagocytosed by an APC and presented in the context of an MHC class II molecule on the cell surface. T cell epitopes usually consist of 8–11 linearly arranged amino acids and are specifically recognized by the T cell receptor. During an immune response, activation through a B cell epitope results in antibody class switching, whereas activation of a T cell epitope stimulates the production of T cell subsets that act to regulate the immune response. While it is not known exactly how the expression of particular epitopes shapes the immune response, it is hypothesized that the epitope profile, and thus the particular type of allergen used in vaccination, is of great importance in the induction of peripheral tolerance (Pomes 2008; Szalai et al. 2008).

Table 2 Summary of the different types of allergen used for specific immunotherapy

Allergen class	Treatment	Result
Natural	Extract from natural sources	Multiple allergens and naturally occurring substances
Chemical	Chemical—formaldehyde/ gluteraldehyde	High molecular weight allergen polymer—linked through lysine residues
	Chemical—carbamylation	Low molecular weight allergen—lysines modified to ureido groups
Recombinant	Peptide production	Generation of specific T cell epitopes only
	Intact major allergen	Recombinant whole protein allergen
	Intact hypoallergenic allergen	Recombinant whole protein allergen that mimics naturally occurring allergen isoforms with low IgE binding capacity
	Fragmentation	Fragmentation of allergen tertiary structure results in loss of B cell epitopes
	Amalgamation	Creation of allergen dimers and trimers using cDNA and expression plasmids

6.2 Type of Allergen

In an attempt to improve immunotherapy, many investigations have been aimed at modifying the allergen vaccine to reduce allergenicity while maintaining the immunogenicity and, thus, allow for successful treatment. Of the major classifications of allergens for use in immunotherapy, native, chemically modified and recombinant allergens, summarized in Table 2, will be discussed further in this section.

6.2.1 Native Allergens

Traditionally in immunotherapy, the allergen used for treatment is obtained from 'native' extracts purified from natural sources. One drawback of native allergen use is the difficulty of standardizing production. Indeed, recent studies have found significant concentration variances in birch pollen allergen preparations (Focke et al. 2009) as well as the presence of unrelated allergens (van der Veen et al. 1996) which can be problematic due to the possibility of patients developing new IgE reactivities (Moverare et al. 2002; Pauli et al. 2008). Other studies investigating native allergen extracts have found contamination in the form of endotoxin (Trivedi et al. 2003) and beta glucans (Finkelman et al. 2006), though the possible adjuvant effects of these 'contaminating' molecules are not fully understood and must be researched further. Though addressing the aforementioned concerns and

developing a global standard for native allergen extract preparation are essential, further research into modified and/or recombinant allergens will provide alternative concepts to improve the allergen vaccines used in immunotherapy.

6.2.2 'Allergoid': Chemically Modified Allergens

One of the first attempts to modify allergen for use in immunotherapy was to chemically modify naturally extracted allergens by treatment with dilute formaldehyde. Today it is known that chemical modification of an allergen with formaldehyde or glutaraldehyde acts to create a high molecular weight allergen polymer by linking the amine groups from exposed lysines. Creation of allergen polymers by this method maintains immunogenicity while strongly reducing the IgE binding capability (Kahlert et al. 2000). In addition to high molecular weight allergen polymers produced by formaldehyde/glutaraldehyde treatment, treatment of allergens by carbamylation produces low molecular weight allergens that can, for example, be easily absorbed through mucosal surfaces. Carbamylation acts by transforming the N-terminus of lysine residues to a ureido group which functionally reduces allergenicity of the protein molecule (Mistrello et al. 1996; Velickovic and Jankov 2008). Successful use of chemical modification of an allergen vaccine was first performed by Marsh et al. in 1970 and allergoid vaccines have since been successfully tested in immunotherapy in both adults and children (Keskin et al. 2006; La Grutta et al. 2007; Palma-Carlos et al. 2006; Williams et al. 2007).

6.2.3 Recombinant Allergens

Yet another approach for the creation of safer immunotherapy vaccines is the production of recombinant allergens such as peptides, recombinant protein, recombinant hypoallergenic protein, allergen fragments and oligomers. Before discussing these alternatives, however, it must be considered whether single recombinant allergens can indeed mimic native allergen extracts. The fact that native allergen extracts contain a large compliment of different molecules and that there exists a virtually limitless range of potential allergens in the environment, engenders the question of whether a limited number of representative recombinant allergens can induce tolerance across a spectrum (Vrtala 2008). Studies investigating the diagnostic capabilities of recombinant allergens have found, for example, that of the six main grass pollen allergens, a panel of four was able to diagnose grass pollen allergy in all patients tested (Laffer et al. 1996; Valenta et al. 1998; Vrtala et al. 1993). Consequently, it appears that of all allergenic possibilities, a few major allergens can determine the susceptibility of many.

Using recombinant technology it is possible to generate peptides containing short, linear, allergen-derived T cell epitopes for use in immunotherapy to

specifically target CD4+ T cells. The process involves screening for T cell activity after stimulation with overlapping synthetic peptides that span the known allergen molecule sequence (Akdis and Blaser 2000). Due to their small size and lack of secondary and tertiary structures, peptide sequences have a reduced ability to bind IgE and thus have greatly reduced allergenicity. Murine studies have demonstrated the effectiveness of peptide immunotherapy in inducing tolerance to a mite allergen *Der p 1* (Hoyne et al. 1993) and a cat allergen *Fel d 1* (Briner et al. 1993). Clinically, the allergens *Fel d 1*, bee venom *Api m 1* and ragweed *Amb a 1* have been heavily researched in humans. Although initial clinical trials using *Fel d 1* peptide immunotherapy demonstrated positive effects in patients with asthma, treatment also induced numerous adverse effects that were later attributed to the peptide length and high dosage (Larche 2007). More recent trials with *Fel d 1* have managed to achieve tolerance induction with greatly reduced adverse events through the use of shorter peptides and reduced doses (Alexander et al. 2005; Verhoef et al. 2005), however much research is still required to determine the optimal dosing, timing and route for peptide immunotherapy.

The use of recombinant whole protein and fragmented allergens are also being investigated for immunotherapy improvement. Considering for the moment whole protein, initial clinical trials have found recombinant major grass pollen (Jutel et al. 2005) and birch pollen allergens (Pauli et al. 2008) to be effective for immunotherapy treatment. Moreover, comparison of clinical studies using either recombinant allergen or native allergen extract revealed similar efficacy in both of these treatments (Kahler et al. 1999; Pauli et al. 2008). In addition to recombinant major allergens, there has also been recent interest in the generation of hypoallergenic allergens. Hypoallergenic allergens are naturally occurring isoforms of a particular allergen that have reduced IgE reactivity while maintaining T cell epitope recognition. It is hypothesized that the use of recombinant hypoallergenic isoforms can further reduce adverse events generated by immunotherapy due to the greatly reduced allergenicity of the protein. Current research has revealed hypoallergenic isoforms of birch pollen *Bet v 1* (Wagner et al. 2008) mite *Blo t 12* (Zakzuk et al. 2009) and *Der p 1* (Walgraffe et al. 2009) that may be beneficial for use in future allergen vaccines.

Recombinant technology offers many techniques for the generation of new allergen vaccines. While peptides and whole protein allergens continue to be investigated, engineered fragments of allergen also provide a promising avenue for exploration. Fragmentation reduces the allergenicity of a protein molecule since during the process one or more major B cell epitopes are removed from the tertiary structure. Illustrating this concept, *Bet v 1* birch pollen allergen fragments have been engineered that promote tolerogenic responses in both mice and rabbits (Vrtala et al. 2000). Finally, just as fragments of allergen have reduced allergenicity, allergen multimers also contain fewer B cell epitopes. Allergen dimers and trimers that are genetically engineered using cDNA subunits and expression plasmids are also being studied for use in immunotherapy. Demonstrating the functionality of multimeric allergen molecules, *Bet v 1* dimers and trimers

stimulate T cell activity in PBMCs from allergic individuals, and have strongly reduced anaphylactic activity as observed by basophil histamine release and skin prick testing (van Hage-Hamsten et al. 1999; Vrtala et al. 1999).

The use of recombinant allergens has a great deal of potential for the improvement of allergen vaccines. Due to the many types of recombinant technologies available, allergens can be modified in numerous ways to potentially reduce allergenicity while inducing tolerance. Though many advancements have been made in the creation of novel allergen vaccines, much research is still required to reach the full potential of recombinant allergens in the clinical setting. Through rigorous testing and understanding of the mechanisms of tolerance induction it is possible to improve immunotherapy by the use of recombinant allergens.

6.3 Modality: Route of Dosing

Since the discovery of immunotherapy, the subcutaneous route of application has been the gold standard. In an attempt to counter some of the adverse reactions that accompany SCIT, SLIT (consisting of absorption of an allergen solution or tablet through the mucosal layers under the tongue) was developed as an alternative. Considering that pediatric patients are a major group of candidates for allergy therapy, SLIT is an appealing alternative due to the simpler dosing route and the possibility of fewer clinic visits for treatment (Halken et al. 2008). Meta-analysis of SLIT in adults revealed safety and efficacy mainly for allergic rhinitis (Compalati et al. 2009; Wilson et al. 2005), however, due to current controversy in the literature over the efficacy of SLIT for children, the results of more studies must be published before conclusive decisions can be made (Campbell 2009; Halken et al. 2008; Larenas-Linnemann 2009; van Wijk 2008). SLIT is now accepted to be significantly safer than SCIT as there have been only few case reports of anaphylactic reactions (Moingeon et al. 2006) however, there is cause for caution when treating patients with SLIT that have previously discontinued SCIT due to adverse reactions (Cochard and Eigenmann 2009).

Though SLIT is an effective alternative to SCIT under these considerations, generating further knowledge regarding its mechanism of action will provide opportunities for improved second generation vaccines. Comparison of successful SLIT and SCIT treatments reveals that though both treatments generate peripheral tolerance via the induction of regulatory T cells, the manner of tolerance induction is highly dependent on the draining lymph nodes at the site of antigen application. At sites of oral immunization for SLIT, allergen is captured by Langerhans-like DCs which subsequently upregulate the expression of adhesion and trafficking receptors such as CCR7 and migrate to the mucosal draining lymph nodes (internal jugular, superficial cervical and submaxillary) in the mucosa-associated axis (Kraal et al. 2006; Moingeon et al. 2006).

While SCIT relies on introduction of allergen to sites under the skin, SLIT takes advantage of already existing oral tolerance mechanisms that are present to ensure immune tolerance to food and commensal bacteria. Lymph nodes near mucosal sites maintain a particular microenvironment that is favorable for tolerance induction. Illustrating this, murine studies show a preferential generation of "blocking" IgG2b antibodies and higher antibody responses in mucosal lymph nodes than lymph nodes near subcutaneous sites of injection (Aoyama-Kondo et al. 1992; van Helvoort et al. 2004). Though there is still much work to be done to elucidate the mechanism of SLIT, it is hypothesized to involve B cell dependent generation of Treg cells and subsequent T effector cell suppression, as well as a B cell independent development of Foxp3-LAP+TGFβ+Tregs (Sun et al. 2008).

In comparison with SCIT, one immediate drawback of SLIT is the need for 50–100 times higher allergen concentration which over time can greatly add to the cost of treatment. One of the possible explanations for this is that SCIT protocols routinely use an adjuvant as part of the vaccination regimen whereas SLIT treatment protocols do not. The use of adjuvants for immunotherapy is important for protocol improvement and many studies are testing adjuvants currently in use as well as new candidate mucosal adjuvants for future immunotherapy trials (Moingeon et al. 2006).

6.4 Adjuvant

As mentioned previously, SCIT treatment often utilizes the adjuvant aluminum hydroxide (alum) as an adsorbant and immunostimulant. Consequently, the emergence of novel immunotherapy techniques such as use of recombinant allergens (which are less potent immune stimulators than allergen extracts) and sublingual allergen application has generated a parallel search for mucosal adjuvants that can enhance the peripheral tolerance mechanisms induced during SLIT (Goldman 2008). In a murine model of SLIT using the allergen ovalbumin in conjunction with the adjuvant cholera toxin B (CTB), Sun et al. found an increased expression of FoxP3+CD25+CD4+ Treg cells in the draining lymph nodes; CTB application also greatly reduced the amount of allergen needed to elicit this effect (Sun et al. 2006). TLR ligands are also being studied as potential adjuvants due to their ability to activiate IL-10 secreting DCs that are likely to promote the emergence of Tregs. Studies by Lombardi et al. demonstrate that the application of the TLR2 ligand, Pam3CSK4 promotes the differentiation of IFNγ and IL-10 secreting CD4+ T cells both in vitro in cell culture and in vivo in a murine experimental asthma model in which mice were treated sublingually with both allergen and Pam3CSK4 (Lombardi et al. 2008). Due to the specialized microenvironments of the mucosal lymph nodes and the efficacy of SLIT, the generation of mucosal adjuvants provides a promising area for the creation of second generation SLIT vaccines.

7 Future Perspectives

Immunotherapy is a highly effective method of treating allergies such as asthma and rhinitis by inducing allergen specific tolerance in the periphery. Notwithstanding, the subject of allergy prevention by non-specific tolerance induction is also a topic of intense interest. Epidemiological findings demonstrate that certain environmental exposures such as unhygienic contact with older siblings and growing up in a farming environment may confer protection against certain allergies (Strachan 1989, 2000; von Mutius and Radon 2008). Observations from epidemiological studies lead to the formation of the hygiene hypothesis by Strachan in 1989 which basically states that exposure to microbes either prenatally or early in life may reduce the risk of developing allergies.

In addition to the great deal of epidemiological evidence supporting the hygiene hypothesis (Douwes et al. 2007; Kiechl-Kohlendorfer et al. 2007; Matheson et al. 2009; Seiskari et al. 2007), proof of concept has also been shown in animal models. In a prevention model, protective effects against murine experimental asthma were seen with both *Lactococcus* and *Acinetobacter* bacterial strains isolated from farm sites (Debarry et al. 2007), lipopolysaccharide (Lundy et al. 2003) and *Mycobacterium vaccae* (Yazi et al. 2007). Furthermore, microbes applied prenatally were also shown to have asthma preventative effects in offspring; the mechanism of which was demonstrated to be fully dependent on functional maternal TLRs (Blümer et al. 2007; Conrad et al. 2009). Though research into the mechanisms of non-specific tolerance induction and the hygiene hypothesis is still in its infancy, this subject will surely garner much interest in the years to come.

8 Conclusion

Allergy is the consequence of an inappropriate inflammatory immune response generated against harmless environmental antigens. Characterized by Th2 cytokine secretion and the production of allergen specific IgE antibodies, this type of immune reaction is the result of an imbalance between different T cell subsets, namely a reduced presence of Treg cells. Of the different methods to treat allergy, immunotherapy is a specific, highly effective means of treating allergic asthma and rhinitis that is shown to have long lasting effects after cessation of the therapy. Immunotherapy acts by initiating peripheral tolerance mechanisms and thus functions to correct the underlying pathomechanisms of allergic disease.

Though allergen immunotherapy is an effective method to treat certain allergies, there is currently much interest in improving the allergen vaccines used for treatment. Experimentation with chemical allergen modification as well as the generation of recombinant allergen proteins and peptides represent exciting new methods to improve treatment. Furthermore, testing of the route of allergen vaccine application and stimulation of the immune system by specific adjuvants may

also reveal novel methods for allergen application. As more is learned about the mechanisms of tolerance induction and the improvement of allergen vaccines, we approach a better understanding of treating the underlying causes of allergy.

References

Aalberse RC, Stapel SO, Schuurman J, Rispens T (2009) Immunoglobulin G4: an odd antibody. Clin Exp Allergy 39:469–477

Abramson MJ, Puy RM, Weiner JM (2003) Allergen immunotherapy for asthma. Cochrane Database Syst Rev 4:CD001186

Akdis M, Akdis CA (2007) Mechanisms of allergen-specific immunotherapy. J Allergy Clin Immunol 119:780–791

Akdis CA, Akdis M (2009) Mechanisms and treatment of allergic disease in the big picture of regulatory T cells. J Allergy Clin Immunol 123:735–748

Akdis CA, Blaser K (2000) Regulation of specific immune responses by chemical and structural modifications of allergens. Int Arch Allergy Immunol 121:261–269

Akdis CA, Blesken T, Akdis M, Wuthrich B, Blaser K (1998) Role of interleukin 10 in specific immunotherapy. J Clin Invest 102:98–106

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 CB, Blaser K, Akdis CA (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 199:1567–1575

Alexander C, Tarzi M, Larche M, Kay AB (2005) The effect of Fel d 1-derived T-cell peptides on upper and lower airway outcome measurements in cat-allergic subjects. Allergy 60: 1269–1274

Aoyama-Kondo T, Yoshida T, Tsobe K, Nakayama A, Asai J, Oka T, Nakashima I (1992) Characterization of antibody responses of local lymph nodes to antigen given under the oral submucosa. Immunobiology 184:372–383

Appleby MW, Ramsdell F (2008) Scurfy, the Foxp3 locus, and the molecular basis of peripheral tolerance. Curr Top Microbiol Immunol 321:151–168

Bjermer L (2008) Evaluating combination therapies for asthma: pros, cons, and comparative benefits. Ther Adv Respir Dis 2:149–161

Blaser K (2008) Role of T-cell subtypes in allergic inflammations. Chem Immunol Allergy 94: xi–xv

Blaser K, Akdis CA (2004) Interleukin-10, T regulatory cells and specific allergy treatment. Clin Exp Allergy 34:328–331

Blümer N, Sel S, Virna S, Patrascan CC, Zimmermann S, Herz U, Renz H, Garn H (2007) Perinatal maternal application of *Lactobacillus rhamnosus* GG suppresses allergic airway inflammation in mouse offspring. Clin Exp Allergy 37:348–357

Bohle B, Kinaciyan T, Gerstmayr M, Radakovics A, Jahn-Schmid B, Ebner C (2007) Sublingual immunotherapy induces IL-10-producing T regulatory cells, allergen-specific T-cell tolerance, and immune deviation. J Allergy Clin Immunol 120:707–713

Borish L, Aarons A, Rumbyrt J, Cvietusa P, Negri J, Wenzel S (1996) Interleukin-10 regulation in normal subjects and patients with asthma. J Allergy Clin Immunol 97:1288–1296

Briner TJ, Kuo MC, Keating KM, Rogers BL, Greenstein JL (1993) Peripheral T-cell tolerance induced in naive and primed mice by subcutaneous injection of peptides from the major cat allergen Fel d I. Proc Natl Acad Sci USA 90:7608–7612

Brunkow ME, Jeffery EW, Hjerrild KA, Paeper B, Clark LB, Yasayko SA, Wilkinson JE, Galas D, Ziegler SF, Ramsdell F (2001) Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. Nat Genet 27:68–73

Burgler S, Ouaked N, Bassin C, Basinski TM, Mantel PY, Siegmund K, Meyer N, Akdis CA, Schmidt-Weber CB (2009) Differentiation and functional analysis of human T(H)17 cells. J Allergy Clin Immunol 123:588–595, 595 e581–587

Calderon MA, Alves B, Jacobson M, Hurwitz B, Sheikh A, Durham S (2007) Allergen injection immunotherapy for seasonal allergic rhinitis. Cochrane Database Syst Rev 1:CD001936

Campbell DE (2009) Sublingual immunotherapy for children: are we there yet? Defining its role in clinical practice. Paediatr Respir Rev 10:69–74 quiz 74

Carson WF IV, Guernsey LA, Singh A, Vella AT, Schramm CM, Thrall RS (2008) Accumulation of regulatory T cells in local draining lymph nodes of the lung correlates with spontaneous resolution of chronic asthma in a murine model. Int Arch Allergy Immunol 145:231–243

Chen W, Jin W, Hardegen N, Lei KJ, Li L, Marinos N, McGrady G, Wahl SM (2003) Conversion of peripheral CD4+CD25− naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. J Exp Med 198:1875–1886

Cochard MM, Eigenmann PA (2009) Sublingual immunotherapy is not always a safe alternative to subcutaneous immunotherapy. J Allergy Clin Immunol 124:378–379

Cohen SG, Frankland AW, Dworetzky M (2003) Noon and Freeman on prophylactic inoculation against hay fever. J Allergy Clin Immunol 111:1142–1150

Compalati E, Penagos M, Tarantini F, Passalacqua G, Canonica GW (2009) Specific immunotherapy for respiratory allergy: state of the art according to current meta-analyses. Ann Allergy Asthma Immunol 102:22–28

Conrad ML, Ferstl R, Teich R, Brand S, Blümer N, Yildirim AO, Patrascan CC, Hanuszkiewicz A, Akira S, Wagner H, Holst O, von Mutius E, Pfefferle PI, Kirschning CJ, Garn H, Renz H (2009) Maternal TLR signaling is required for prenatal asthma protection by the nonpathogenic microbe Acinetobacter lwoffii F78. J Exp Med 206:2869–2877

Daeron M, Malbec O, Latour S, Arock M, Fridman WH (1995) Regulation of high-affinity IgE receptor-mediated mast cell activation by murine low-affinity IgG receptors. J Clin Invest 95:577–585

Dardalhon V, Awasthi A, Kwon H, Galileos G, Gao W, Sobel RA, Mitsdoerffer M, Strom TB, Elyaman W, Ho IC, Khoury S, Oukka M, Kuchroo VK (2008a) IL-4 inhibits TGF-beta-induced Foxp3+T cells and, together with TGF-beta, generates IL-9+IL-10+Foxp3(-) effector T cells. Nat Immunol 9:1347–1355

Dardalhon V, Korn T, Kuchroo VK, Anderson AC (2008b) Role of Th1 and Th17 cells in organ-specific autoimmunity. J Autoimmun 31:252–256

Debarry J, Garn H, Hanuszkiewicz A, Dickgreber N, Blümer N, von Mutius E, Bufe A, Gatermann S, Renz H, Holst O, Heine H (2007) *Acinetobacter lwoffii* and *Lactococcus lactis* strains isolated from farm cowsheds possess strong allergy-protective properties. J Allergy Clin Immunol 119:1514–1521

Des Roches A, Paradis L, Menardo JL, Bouges S, Daures JP, Bousquet J (1997) Immunotherapy with a standardized Dermatophagoides pteronyssinus extract. VI. Specific immunotherapy prevents the onset of new sensitizations in children. J Allergy Clin Immunol 99:450–453

Didier A, Malling HJ, Worm M, Horak F, Jager S, Montagut A, Andre C, de Beaumont O, Melac M (2007) Optimal dose, efficacy, and safety of once-daily sublingual immunotherapy with a 5-grass pollen tablet for seasonal allergic rhinitis. J Allergy Clin Immunol 120:1338–1345

Douwes J, Travier N, Huang K, Cheng S, McKenzie J, Le Gros G, von Mutius E, Pearce N (2007) Lifelong farm exposure may strongly reduce the risk of asthma in adults. Allergy 62: 1158–1165

DuBuske L, Kowal K (2009) Update on prescription and over-the-counter histamine inverse agonists in rhinitis therapy. Curr Allergy Asthma Rep 9:140–148

Durham SR (2008) Sublingual immunotherapy: what have we learnt from the 'big trials'? Curr Opin Allergy Clin Immunol 8:577–584

Fennerty AG, Jones KP, Davies BH, Fifield R, Edwards J (1988) Immunological changes associated with a successful outcome of pollen immunotherapy. Allergy 43:415–419

Feuerer M, Hill JA, Mathis D, Benoist C (2009) Foxp3+ regulatory T cells: differentiation, specification, subphenotypes. Nat Immunol 10:689–695

Finkelman MA, Lempitski SJ, Slater JE (2006) beta-Glucans in standardized allergen extracts. J Endotoxin Res 12:241–245

Fiorentino DF, Bond MW, Mosmann TR (1989) Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. J Exp Med 170: 2081–2095

Flicker S, Steinberger P, Norderhaug L, Sperr WR, Majlesi Y, Valent P, Kraft D, Valenta R (2002) Conversion of grass pollen allergen-specific human IgE into a protective IgG(1) antibody. Eur J Immunol 32:2156–2162

Focke M, Marth K, Valenta R (2009) Molecular composition and biological activity of commercial birch pollen allergen extracts. Eur J Clin Invest 39:429–436

Francis JN, Till SJ, Durham SR (2003) Induction of IL-10+CD4+CD25+ T cells by grass pollen immunotherapy. J Allergy Clin Immunol 111:1255–1261

Gelardi M, Russo C, Fiorella ML, Fiorella R, Canonica GW, Passalacqua G (2009) When allergic rhinitis is not only allergic. Am J Rhinol Allergy 23:312–315

Ghiringhelli F, Menard C, Terme M, Flament C, Taieb J, Chaput N, Puig PE, Novault S, Escudier B, Vivier E, Lecesne A, Robert C, Blay JY, Bernard J, Caillat-Zucman S, Freitas A, Tursz T, Wagner-Ballon O, Capron C, Vainchencker W, Martin F, Zitvogel L (2005) CD4+CD25+ regulatory T cells inhibit natural killer cell functions in a transforming growth factor-beta-dependent manner. J Exp Med 202:1075–1085

Goldman M (2008) Sublingual immunotherapy: the quest for innovative adjuvants. Clin Exp Allergy 38:1705–1706

Halken S, Lau S, Valovirta E (2008) New visions in specific immunotherapy in children: an iPAC summary and future trends. Pediatr Allergy Immunol 19(Suppl 19):60–70

Hogquist KA, Baldwin TA, Jameson SC (2005) Central tolerance: learning self-control in the thymus. Nat Rev Immunol 5:772–782

Hoyne GF, O'Hehir RE, Wraith DC, Thomas WR, Lamb JR (1993) Inhibition of T cell and antibody responses to house dust mite allergen by inhalation of the dominant T cell epitope in naive and sensitized mice. J Exp Med 178:1783–1788

Huber S, Schramm C, Lehr HA, Mann A, Schmitt S, Becker C, Protschka M, Galle PR, Neurath MF, Blessing M (2004) Cutting edge: TGF-beta signaling is required for the in vivo expansion and immunosuppressive capacity of regulatory CD4+CD25+ T cells. J Immunol 173: 6526–6531

Jackola DR, Pierson-Mullany LK, Liebeler CL, Blumenthal MN, Rosenberg A (2002) Variable binding affinities for allergen suggest a 'selective competition' among immunoglobulins in atopic and non-atopic humans. Mol Immunol 39:367–377

Jacobsen L, Niggemann B, Dreborg S, Ferdousi HA, Halken S, Host A, Koivikko A, Norberg LA, Valovirta E, Wahn U, Moller C (2007) Specific immunotherapy has long-term preventive effect of seasonal and perennial asthma: 10-year follow-up on the PAT study. Allergy 62: 943–948

Jeannin P, Delneste Y, Tillie-Leblond I, Wallaert B, carlier A, Pestel J, Tonnel AB (1994) Abnormal IgG4 antibody response to aeroallergens in allergic patients. Int Arch Allergy Immunol 104:191–198

Jutel M, Watanabe T, Klunker S, Akdis M, Thomet OA, Malolepszy J, Zak-Nejmark T, Koga R, Kobayashi T, Blaser K, Akdis CA (2001) Histamine regulates T-cell and antibody responses by differential expression of H1 and H2 receptors. Nature 413:420–425

Jutel M, Watanabe T, Akdis M, Blaser K, Akdis CA (2002) Immune regulation by histamine. Curr Opin Immunol 14:735–740

Jutel M, Akdis M, Budak F, Aebischer-Casaulta C, Wrzyszcz M, Blaser K, Akdis CA (2003) IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. Eur J Immunol 33:1205–1214

Jutel M, Jaeger L, Suck R, Meyer H, Fiebig H, Cromwell O (2005) Allergen-specific immunotherapy with recombinant grass pollen allergens. J Allergy Clin Immunol 116: 608–613

Kahler H, Stuwe H, Cromwell O, Fiebig H (1999) Reactivity of T cells with grass pollen allergen extract and allergoid. Int Arch Allergy Immunol 120:146–157

Kahlert H, Grage-Griebenow E, Stuwe HT, Cromwell O, Fiebig H (2000) T cell reactivity with allergoids: influence of the type of APC. J Immunol 165:1807–1815

Katz SI, Parker D, Turk JL (1974) B-cell suppression of delayed hypersensitivity reactions. Nature 251:550–551

Keskin O, Tuncer A, Adalioglu G, Sekerel BE, Sackesen C, Kalayci O (2006) The effects of grass pollen allergoid immunotherapy on clinical and immunological parameters in children with allergic rhinitis. Pediatr Allergy Immunol 17:396–407

Khattri R, Kasprowicz D, Cox T, Mortrud M, Appleby MW, Brunkow ME, Ziegler SF, Ramsdell F (2001) The amount of scurfin protein determines peripheral T cell number and responsiveness. J Immunol 167:6312–6320

Kiechl-Kohlendorfer U, Horak E, Mueller W, Strobl R, Haberland C, Fink FM, Schwaiger M, Gutenberger KH, Reich H, Meraner D, Kiechl S (2007) Neonatal characteristics and risk of atopic asthma in schoolchildren: results from a large prospective birth-cohort study. Acta Paediatr 96:1606–1610

Kraal G, Samsom JN, Mebius RE (2006) The importance of regional lymph nodes for mucosal tolerance. Immunol Rev 213:119–130

Kunzmann S, Mantel PY, Wohlfahrt JG, Akdis M, Blaser K, Schmidt-Weber CB (2003) Histamine enhances TGF-beta1-mediated suppression of Th2 responses. Faseb J 17: 1089–1095

La Grutta S, Arena A, D'Anneo WR, Gammeri E, Leonardi S, Trimarchi A, Platania D, La Rosa M (2007) Evaluation of the antiinflammatory and clinical effects of sublingual immunotherapy with carbamylated allergoid in allergic asthma with or without rhinitis. A 12-month perspective randomized, controlled, trial. Eur Ann Allergy Clin Immunol 39:40–44

Laffer S, Spitzauer S, Susani M, Pairleitner H, Schweiger C, Gronlund H, Menz G, Pauli G, Ishii T, Nolte H, Ebner C, Sehon AH, Kraft D, Eichler HG, Valenta R (1996) Comparison of recombinant timothy grass pollen allergens with natural extract for diagnosis of grass pollen allergy in different populations. J Allergy Clin Immunol 98:652–658

Larche M (2006) Immunoregulation by targeting T cells in the treatment of allergy and asthma. Curr Opin Immunol 18:745–750

Larche M (2007) Update on the current status of peptide immunotherapy. J Allergy Clin Immunol 119:906–909

Larenas-Linnemann D (2009) Sublingual immunotherapy in children: complete and updated review supporting evidence of effect. Curr Opin Allergy Clin Immunol 9:168–176

Letourneau S, Krieg C, Pantaleo G, Boyman O (2009) IL-2- and CD25-dependent immunoregulatory mechanisms in the homeostasis of T-cell subsets. J Allergy Clin Immunol 123:758–762

Leung DY, Bieber T (2003) Atopic dermatitis. Lancet 361:151–160

Lim S, Crawley E, Woo P, Barnes PJ (1998) Haplotype associated with low interleukin-10 production in patients with severe asthma. Lancet 352:113

Lim HW, Hillsamer P, Banham AH, Kim CH (2005) Cutting edge: direct suppression of B cells by CD4+CD25+ regulatory T cells. J Immunol 175:4180–4183

Ling EM, Smith T, Nguyen XD, Pridgeon C, Dallman M, Arbery J, Carr VA, Robinson DS (2004) Relation of CD4+CD25+ regulatory T-cell suppression of allergen-driven T-cell activation to atopic status and expression of allergic disease. Lancet 363:608–615

Lombardi V, Van Overtvelt L, Horiot S, Moussu H, Chabre H, Louise A, Balazuc AM, Mascarell L, Moingeon P (2008) Toll-like receptor 2 agonist Pam3CSK4 enhances the induction of antigen-specific tolerance via the sublingual route. Clin Exp Allergy 38:1819–1829

Lundy SK, Berlin AA, Lukacs NW (2003) Interleukin-12-independent down-modulation of cockroach antigen-induced asthma in mice by intranasal exposure to bacterial lipopolysaccharide. Am J Pathol 163:1961–1968

Manjarrez-Orduno N, Quach TD, Sanz I (2009) B cells and immunological tolerance. J Invest Dermatol 129:278–288

Marsh DG, Lichtenstein LM, Campbell DH (1970) Studies on "allergoids" prepared from naturally occurring allergens. I. Assay of allergenicity and antigenicity of formalinized rye group I component. Immunology 18:705–722

Matheson MC, Walters EH, Simpson JA, Wharton CL, Ponsonby AL, Johns DP, Jenkins MA, Giles GG, Hopper JL, Abramson MJ, Dharmage SC (2009) Relevance of the hygiene hypothesis to early vs. late onset allergic rhinitis. Clin Exp Allergy 39:370–378

Mazzoni A, Young HA, Spitzer JH, Visintin A, Segal DM (2001) Histamine regulates cytokine production in maturing dendritic cells, resulting in altered T cell polarization. J Clin Invest 108:1865–1873

McCaughtry TM, Hogquist KA (2008) Central tolerance: what have we learned from mice? Semin Immunopathol 30:399–409

McGee HS, Agrawal DK (2009) Naturally occurring and inducible T-regulatory cells modulating immune response in allergic asthma. Am J Respir Crit Care Med

Milovanovic M, Heine G, Zuberbier T, Worm M (2009) Allergen extract-induced interleukin-10 in human memory B cells inhibits immunoglobulin E production. Clin Exp Allergy 39: 671–678

Mistrello G, Brenna O, Roncarolo D, Zanoni D, Gentili M, Falagiani P (1996) Monomeric chemically modified allergens: immunologic and physicochemical characterization. Allergy 51:8–15

Mizoguchi A, Bhan AK (2006) A case for regulatory B cells. J Immunol 176:705–710

Mobs C, Slotosch C, Loffler H, Pfutzner W, Hertl M (2008) Cellular and humoral mechanisms of immune tolerance in immediate-type allergy induced by specific immunotherapy. Int Arch Allergy Immunol 147:171–178

Moingeon P, Batard T, Fadel R, Frati F, Sieber J, Van Overtvelt L (2006) Immune mechanisms of allergen-specific sublingual immunotherapy. Allergy 61:151–165

Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A (2001) Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 19:683–765

Moverare R, Elfman L, Vesterinen E, Metso T, Haahtela T (2002) Development of new IgE specificities to allergenic components in birch pollen extract during specific immunotherapy studied with immunoblotting and Pharmacia CAP System. Allergy 57:423–430

Neta R, Salvin SB (1974) Specific suppression of delayed hypersensitivity: the possible presence of a suppressor B cell in the regulation of delayed hypersensitivity. J Immunol 113:1716–1725

Nieminen K, Valovirta E, Savolainen J (2009) Clinical outcome and IL-17, IL-23, IL-27 and FOXP3 expression in peripheral blood mononuclear cells of pollen-allergic children during sublingual immunotherapy. Pediatr Allergy Immunol 21:e174–e84

Noon L (1955) Prophylactic inoculation against hay fever (historical document). Ann Allergy 13:713–716 passim

Nouri-Aria KT, Wachholz PA, Francis JN, Jacobson MR, Walker SM, Wilcock LK, Staple SQ, Aalberse RC, Till SJ, Durham SR (2004) Grass pollen immunotherapy induces mucosal and peripheral IL-10 responses and blocking IgG activity. J Immunol 172:3252–3259

Novak N, Bieber T, Leung DY (2003) Immune mechanisms leading to atopic dermatitis. J Allergy Clin Immunol 112:S128–S139

Ogawa Y, Duru EA, Ameredes BT (2008) Role of IL-10 in the resolution of airway inflammation. Curr Mol Med 8:437–445

Onishi Y, Fehervari Z, Yamaguchi T, Sakaguchi S (2008) Foxp3+ natural regulatory T cells preferentially form aggregates on dendritic cells in vitro and actively inhibit their maturation. Proc Natl Acad Sci USA 105:10113–10118

Osna N, Elliott K, Khan MM (2001) Regulation of interleukin-10 secretion by histamine in TH2 cells and splenocytes. Int Immunopharmacol 1:85–96

Ozdemir C, Akdis M, Akdis CA (2009) T regulatory cells and their counterparts: masters of immune regulation. Clin Exp Allergy 39:626–639

Palma-Carlos AG, Santos AS, Branco-Ferreira M, Pregal AL, Palma-Carlos ML, Bruno ME, Falagiani P, Riva G (2006) Clinical efficacy and safety of preseasonal sublingual

immunotherapy with grass pollen carbamylated allergoid in rhinitic patients. A double-blind, placebo-controlled study. Allergol Immunopathol (Madr) 34:194–198
Pauli G, Larsen TH, Rak S, Horak F, Pastorello E, Valenta R, Purohit A, Arvidsson M, Kavina A, Schroeder JW, Mothes N, Spitzauer S, Montagut A, Galvain S, Melac M, Andre C, Poulsen LK, Malling HJ (2008) Efficacy of recombinant birch pollen vaccine for the treatment of birch-allergic rhinoconjunctivitis. J Allergy Clin Immunol 122:951–960
Pelaia G, Renda T, Romeo P, Busceti MT, Maselli R (2008) Omalizumab in the treatment of severe asthma: efficacy and current problems. Ther Adv Respir Dis 2:409–421
Peng ZK, Naclerio RM, Norman PS, Adkinson NF Jr (1992) Quantitative IgE- and IgG-subclass responses during and after long-term ragweed immunotherapy. J Allergy Clin Immunol 89:519–529
Piccirillo CA, Shevach EM (2001) Cutting edge: control of CD8+ T cell activation by CD4+CD 25+ immunoregulatory cells. J Immunol 167:1137–1140
Pipet A, Botturi K, Pinot D, Vervloet D, Magnan A (2009) Allergen-specific immunotherapy in allergic rhinitis and asthma. Mechanisms and proof of efficacy. Respir Med 103:800–812
Pomes A (2008) Allergen structures and biologic functions: the cutting edge of allergy research. Curr Allergy Asthma Rep 8:425–432
Punnonen J, de Waal Malefyt R, van Vlasselaer P, Gauchat JF, de Vries JE (1993) IL-10 and viral IL-10 prevent IL-4-induced IgE synthesis by inhibiting the accessory cell function of monocytes. J Immunol 151:1280–1289
Radulovic S, Jacobson MR, Durham SR, Nouri-Aria KT (2008) Grass pollen immunotherapy induces Foxp3-expressi1472 e1461ng CD4+ CD25+ cells in the nasal mucosa. J Allergy Clin Immunol 121:1467–1472, 1472 e1461
Riedl MA (2008) The effect of air pollution on asthma and allergy. Curr Allergy Asthma Rep 8:139–146
Rossi RE, Monasterolo G, Coco G, Silvestro L, Operti D (2007) Evaluation of serum IgG4 antibodies specific to grass pollen allergen components in the follow up of allergic patients undergoing subcutaneous and sublingual immunotherapy. Vaccine 25:957–964
Satoguina JS, Weyand E, Larbi J, Hoerauf A (2005) T regulatory-1 cells induce IgG4 production by B cells: role of IL-10. J Immunol 174:4718–4726
Schandene L, Alonso-Vega C, Willems F, Gerard 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 152:4368–4374
Schmidt-Weber CB, Blaser K (2005) New insights into the mechanisms of allergen-specific immunotherapy. Curr Opin Allergy Clin Immunol 5:525–530
Schmidt-Weber CB, Akdis M, Akdis CA (2007) TH17 cells in the big picture of immunology. J Allergy Clin Immunol 120:247–254
Seiskari T, Kondrashova A, Viskari H, Kaila M, Haapala AM, Aittoniemi J, Virta M, Hurme M, Uibo R, Knip M, Hyoty H (2007) Allergic sensitization and microbial load–a comparison between Finland and Russian Karelia. Clin Exp Immunol 148:47–52
Sicherer SH, Leung DY (2009) Advances in allergic skin disease, anaphylaxis, and hypersensitivity reactions to foods, drugs, and insects in 2008. J Allergy Clin Immunol 123:319–327
Singh A, Carson WF IV, Secor ER Jr, Guernsey LA, Flavell RA, Clark RB, Thrall RS, Schramm CM (2008) Regulatory role of B cells in a murine model of allergic airway disease. J Immunol 180:7318–7326
Srivastava D, Arora N, Singh BP (2009) Current immunological approaches for management of allergic rhinitis and bronchial asthma. Inflamm Res 58:523–536
Strachan DP (1989) Hay fever, hygiene, and household size. BMJ 299:1259–1260
Strachan DP (2000) Family size, infection and atopy: the first decade of the "hygiene hypothesis". Thorax 55(Suppl 1):S2–S10
Strait RT, Morris SC, Finkelman FD (2006) IgG-blocking antibodies inhibit IgE-mediated anaphylaxis in vivo through both antigen interception and Fc gamma RIIb cross-linking. J Clin Invest 116:833–841

Sun JB, Cuburu N, Blomquist M, Li BL, Czerkinsky C, Holmgren J (2006) Sublingual tolerance induction with antigen conjugated to cholera toxin B subunit induces Foxp3+CD25+CD4+ regulatory T cells and suppresses delayed-type hypersensitivity reactions. Scand J Immunol 64:251–259

Sun JB, Flach CF, Czerkinsky C, Holmgren J (2008) B lymphocytes promote expansion of regulatory T cells in oral tolerance: powerful induction by antigen coupled to cholera toxin B subunit. J Immunol 181:8278–8287

Szalai K, Jensen-Jarolim E, Pali-Scholl I (2008) Vaccination strategies based on the mimotope concept. G Ital Dermatol Venereol 143:95–104

Tauro S, Su YC, Thomas S, Schwarze J, Matthaei KI, Townsend D, Simson L, Tripp RA, Mahalingam S (2008) Molecular and cellular mechanisms in the viral exacerbation of asthma. Microbes Infect 10:1014–1023

Trivedi B, Valerio C, Slater JE (2003) Endotoxin content of standardized allergen vaccines. J Allergy Clin Immunol 111:777–783

Tsitoura DC, Yeung VP, DeKruyff RH, Umetsu DT (2002) Critical role of B cells in the development of T cell tolerance to aeroallergens. Int Immunol 14:659–667

Valenta R, Vrtala S, Laffer S, Spitzauer S, Kraft D (1998) Recombinant allergens. Allergy 53: 552–561

Van Bruaene N, Perez-Novo CA, Basinski TM, Van Zele T, Holtappels G, De Ruyck N, Schmidt-Weber C, Akdis C, Van Cauwenberge P, Bachert C, Gevaert P (2008) T-cell regulation in chronic paranasal sinus disease. J Allergy Clin Immunol 121:1435–1441, 1441 e1431–1433

van der Veen MJ, Mulder M, Witteman AM, van Ree R, Aalberse RC, Jansen HM, van der Zee JS (1996) False-positive skin prick test responses to commercially available dog dander extracts caused by contamination with house dust mite (Dermatophagoides pteronyssinus) allergens. J Allergy Clin Immunol 98:1028–1034

van Hage-Hamsten M, Kronqvist M, Zetterstrom O, Johansson E, Niederberger V, Vrtala S, Gronlund H, Gronneberg R, Valenta R (1999) Skin test evaluation of genetically engineered hypoallergenic derivatives of the major birch pollen allergen, Bet v 1: results obtained with a mix of two recombinant Bet v 1 fragments and recombinant Bet v 1 trimer in a Swedish population before the birch pollen season. J Allergy Clin Immunol 104:969–977

van Helvoort JM, Samsom JN, Chantry D, Jansen W, Schadee-Eestermans I, Thepen T, Mebius RE, Kraal G (2004) Preferential expression of IgG2b in nose draining cervical lymph nodes and its putative role in mucosal tolerance induction. Allergy 59:1211–1218

van Neerven RJ, Wikborg T, Lund G, Jacobsen B, Brinch-Nielsen A, Arnved J, Ipsen H (1999) Blocking antibodies induced by specific allergy vaccination prevent the activation of CD4 + T cells by inhibiting serum-IgE-facilitated allergen presentation. J Immunol 163:2944–2952

Van Ree R, Van Leeuwen WA, Dieges PH, Van Wijk RG, De Jong N, Brewczyski PZ, Kroon AM, Schilte PP, Tan KY, Simon-Licht IF, Roberts AM, Stapel SO, Aalberse RC (1997) Measurement of IgE antibodies against purified grass pollen allergens (Lol p 1, 2, 3 and 5) during immunotherapy. Clin Exp Allergy 27:68–74

van Wijk RG (2008) When to initiate immunotherapy in children with allergic disease? Lessons from the paediatric studies. Curr Opin Allergy Clin Immunol 8:565–570

Veldhoen M, Uyttenhove C, van Snick J, Helmby H, Westendorf A, Buer J, Martin B, Wilhelm C, Stockinger B (2008) Transforming growth factor-beta 'reprograms' the differentiation of T helper 2 cells and promotes an interleukin 9-producing subset. Nat Immunol 9:1341–1346

Velickovic TC, Jankov RM (2008) Design and modifications of allergens for improving specific immunotherapy. Inflamm Allergy Drug Targets 7:270–278

Verhoef A, Alexander C, Kay AB, Larche M (2005) T cell epitope immunotherapy induces a CD4+ T cell population with regulatory activity. PLoS Med 2:e78

von Mutius E, Radon K (2008) Living on a farm: impact on asthma induction and clinical course. Immunol Allergy Clin North Am 28:631–647, ix-x

Vrtala S (2008) From allergen genes to new forms of allergy diagnosis and treatment. Allergy 63:299–309

Vrtala S, Sperr WR, Reimitzer I, van Ree R, Laffer S, Muller WD, Valent P, Lechner K, Rumpold H, Kraft D et al (1993) cDNA cloning of a major allergen from timothy grass (Phleum pratense) pollen; characterization of the recombinant Phl pV allergen. J Immunol 151: 4773–4781

Vrtala S, Hirtenlehner K, Susani M, Hufnagl P, Binder BR, Vangelista L, Pastore A, Sperr WR, Valent P, Ebner C, Kraft D, Valenta R (1999) Genetic engineering of recombinant hypoallergenic oligomers of the major birch pollen allergen, Bet v 1: candidates for specific immunotherapy. Int Arch Allergy Immunol 118:218–219

Vrtala S, Akdis CA, Budak F, Akdis M, Blaser K, Kraft D, Valenta R (2000) T cell epitope-containing hypoallergenic recombinant fragments of the major birch pollen allergen, Bet v 1, induce blocking antibodies. J Immunol 165:6653–6659

Wachholz PA, Soni NK, Till SJ, Durham SR (2003) Inhibition of allergen-IgE binding to B cells by IgG antibodies after grass pollen immunotherapy. J Allergy Clin Immunol 112:915–922

Wagner S, Radauer C, Bublin M, Hoffmann-Sommergruber K, Kopp T, Greisenegger EK, Vogel L, Vieths S, Scheiner O, Breiteneder H (2008) Naturally occurring hypoallergenic Bet v 1 isoforms fail to induce IgE responses in individuals with birch pollen allergy. J Allergy Clin Immunol 121:246–252

Walgraffe D, Matteotti C, El Bakkoury M, Garcia L, Marchand C, Bullens D, Vandenbranden M, Jacquet A (2009) A hypoallergenic variant of Der p 1 as a candidate for mite allergy vaccines. J Allergy Clin Immunol 123:1150–1156

Walters S, Webster KE, Sutherland A, Gardam S, Groom J, Liuwantara D, Marino E, Thaxton J, Weinberg A, Mackay F, Brink R, Sprent J, Grey ST (2009) Increased CD4+Foxp3+ T cells in BAFF-transgenic mice suppress T cell effector responses. J Immunol 182:793–801

Waterhouse P, Penninger JM, Timms E, Wakeham A, Shahinian A, Lee KP, Thompson CB, Griesser H, Mak TW (1995) Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4. Science 270:985–988

Wells AD (2009) New insights into the molecular basis of T cell anergy: anergy factors, avoidance sensors, and epigenetic imprinting. J Immunol 182:7331–7341

Werfel T, Morita A, Grewe M, Renz H, Wahn U, Krutmann J, Kapp A (1996) Allergen specificity of skin-infiltrating T cells is not restricted to a type-2 cytokine pattern in chronic skin lesions of atopic dermatitis. J Invest Dermatol 107:871–876

Williams A, Henzgen M, Rajakulasingam K (2007) Additional benefit of a third year of specific grass pollen allergoid immunotherapy in patients with seasonal allergic rhinitis. Eur Ann Allergy Clin Immunol 39:123–126

Wilson DR, Lima MT, Durham SR (2005) Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. Allergy 60:4–12

Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z, Nomura T, Sakaguchi S (2008) CTLA-4 control over Foxp3+ regulatory T cell function. Science 322:271–275

Workman CJ, Szymczak-Workman AL, Collison LW, Pillai MR, Vignali DA (2009) The development and function of regulatory T cells. Cell Mol Life Sci

Yamazaki S, Steinman RM (2009) Dendritic cells as controllers of antigen-specific Foxp3+ regulatory T cells. J Dermatol Sci 54:69–75

Yazi D, Akkoc T, Ozdemir C, Yesil O, Aydogan M, Sancak R, Bahceciler NN, Barlan IB (2007) Long-term modulatory effect of Mycobacterium vaccae treatment on histopathologic changes in a murine model of asthma. Ann Allergy Asthma Immunol 98:573–579

Zakzuk J, Jimenez S, Cheong N, Puerta L, Lee BW, Chua KY, Caraballo L (2009) Immunological characterization of a Blo t 12 isoallergen: identification of immunoglobulin E epitopes. Clin Exp Allergy 39:608–616

Zuany-Amorim C, Haile S, Leduc D, Dumarey C, Huerre M, Vargaftig BB, Pretolani M (1995) Interleukin-10 inhibits antigen-induced cellular recruitment into the airways of sensitized mice. J Clin Invest 95:2644–2651