



Associate editor: P. Foster

Allergen-related approaches to immunotherapy

Jennifer M. Rolland^{*}, Leanne M. Gardner, Robyn E. O'Hehir

Department of Immunology, Monash University, Commercial Road, Melbourne Vic 3004, Australia

Department of Allergy, Immunology and Respiratory Medicine, Alfred Hospital, Melbourne, Victoria, Australia

ARTICLE INFO

Keywords:

Allergen
Allergy
Allergen immunotherapy
Regulatory T cells
Dendritic cells

ABSTRACT

Allergic diseases, including asthma, rhinitis and eczema, represent a major health burden worldwide. Mainstay treatments are allergen avoidance where feasible and pharmacotherapy for symptom relief. For selected patients, allergen-specific immunotherapy (SIT) offers the prospect of long lasting clinical efficacy. SIT involves the administration of allergen extract using a standardized regimen, usually subcutaneously or increasingly sublingually. However, application of this potentially curative treatment is restricted, largely due to the risk of serious adverse events, especially in asthmatics and for potent allergens such as peanut, seafood and latex. New insights into immunological mechanisms underlying effective SIT and molecular characterization of allergens and their recognition by the immune system suggest strategies for refinement of SIT. Selective targeting of allergen-specific T cells, especially regulatory T cells, is likely to be pivotal for efficacy. Recombinant allergens lacking IgE reactivity and small T cell epitope-based peptides are being trialled clinically with evidence of efficacy without serious IgE-mediated adverse reactions. Adjuvants, either co-administered or incorporated into a recombinant allergen vaccine to target tolerogenic dendritic cells may also increase efficacy. The safer sublingual route of allergen administration is attracting interest and different allergen forms may be optimal for inducing tolerance by this route. Defined allergen-derived molecules or peptides offer ease of standardization and, coupled with appropriate targeting of immunoregulatory mechanisms, will result in more widespread clinical use of SIT. Adjuvant therapies such as anti-IgE antibody and corticosteroids may minimize the likelihood of adverse reactions in those with severe allergic disease who would most benefit from this treatment.

© 2008 Elsevier Inc. All rights reserved.

Contents

1. Introduction	273
2. Allergen-specific immunotherapy	274
3. Mechanisms of specific immunotherapy	275
4. New approaches to allergen immunotherapy	278
5. Conclusions	281
Acknowledgments	281
References	281

1. Introduction

Allergic diseases including asthma, rhinitis/conjunctivitis and eczema affect up to one quarter of the population in Western countries at some time in their lives and the prevalence is rising

(Asher et al., 2006; Floistrup et al., 2006). The socioeconomic impact of these diseases on the community is large, including costs of health care and lost work and school hours (Masoli et al., 2004). Allergic diseases mainly affect atopic individuals, who have an inherited genetic predisposition to synthesize specific immunoglobulin E (IgE)

Abbreviations: APC, antigen presenting cell; DC, dendritic cell; HDM, house dust mite; IgE, immunoglobulin E; IL, interleukin; MHC, major histocompatibility complex; PBMC, peripheral blood mononuclear cells; PRR, pattern recognition receptors; SCIT, subcutaneous allergen-specific immunotherapy; SIT, allergen-specific immunotherapy; SLIT, sublingual allergen-specific immunotherapy; TLR, Toll-like receptor; Tregs, regulatory T cells.

^{*} Corresponding author. Department of Immunology, Monash University, Commercial Road, Melbourne Vic 3004, Australia. Tel.: +61 3 9903 0480; fax: +61 3 9903 0038.

E-mail address: jennifer.rolland@med.monash.edu.au (J.M. Rolland).

antibodies to common environmental allergens. Major perennial allergens are from house dust mites (HDM) and domestic pets (cats, dogs) while the main sources of seasonal allergens are grass, tree and weed pollens. A variety of other substances can be allergenic, including foods and occupational materials such as natural rubber latex. In contrast, bee venom or wasp venom allergy occurs equally in atopic and non-atopic individuals.

Following initial exposure to allergen in susceptible individuals, IgE antibodies are generated and bind to high affinity IgE Fc receptors (FcεRI) on blood basophils and skin and mucosal tissue mast cells. On re-exposure to the relevant allergen, a biphasic response may be elicited with an immediate hypersensitivity and a delayed inflammatory component (Fig. 1). The early phase reaction is a consequence of allergen molecules cross-linking mast cell-bound specific IgE and activating release of inflammatory mediators including histamine, serine proteases, leukotrienes, prostaglandins, platelet activating factor and cytokines. The late phase response results from a further wave of inflammatory mediators released from the recruited cellular infiltrate of eosinophils, neutrophils, basophils, macrophages and CD4⁺ T cells (see review, Kay, 2001).

At sites of exposure such as the respiratory tract, allergen is initially taken up by antigen presenting cells (APC) especially dendritic cells (DC). In allergic individuals, specific IgE bound to low affinity IgE Fc receptors (CD23) on DC may play a role in facilitating allergen uptake (van der Heijden et al., 1993). Allergen-loaded DC traffic to draining lymph nodes where they present processed allergen on their surface as peptides in the context of major histocompatibility complex (MHC) class II molecules to T cells with allergen-specific receptors. Following engagement of antigen-specific receptors and appropriate co-stimulatory molecules, activated T cells release cytokines which facilitate allergen-specific B cell activation and differentiation to antibody producing plasma cells. Activated T cells also traffic to sites of allergen encounter and elaborate local reactions.

CD4⁺ T cells play a central role in orchestrating the immune response to allergen. The peripheral CD4⁺ T cell repertoire of both atopic and non-atopic individuals includes those with allergen specificity, but their functional response differs between these groups (O'Hehir et al., 1991a; Kapsenberg et al., 1992; Romagnani, 1994; Turcanu et al., 2003). In atopic individuals the cytokine profile of activated allergen-specific T cells is Th2-polarised with production of interleukin (IL)-4, IL-5 and IL-13 which drive B cell immunoglobulin class switching to IgE, recruit and activate allergic inflammatory cells (mast cells, basophils, eosinophils) and increase mucus production by mucosal goblet cells. In contrast, activated allergen-specific T cells from non-atopic subjects are Th1-polarised with dominant IFN-γ secretion driving production of non-pathogenic specific IgG antibodies. Factors which influence the type of immune response which results from allergen encounter include the genotype of the individual, antigen form and concentration, the antigen presenting cell type and activation status, and the local cytokine milieu (Murphy & Reiner, 2002; Gardner et al., 2003).

Mainstay treatment for allergic diseases is allergen avoidance, where feasible, and the use of non-specific pharmacotherapy including antihistamines, beta-2 agonists and corticosteroids for symptomatic relief. Severe, life-threatening anaphylactic reactions require treatment with adrenaline. Although effective at controlling symptoms, administration of anti-inflammatory treatments is not without side effects especially with prolonged use. In selected patients where there is a clear demonstration of symptoms on exposure to the offending allergen and documentation of allergen-specific IgE, allergen-specific immunotherapy (SIT) to target the underlying disease process may be instigated. This treatment is attractive as it selectively modulates the allergen-specific immune response and is potentially curative. This review will describe current use of SIT for treatment of allergic diseases and consider potential allergen formulations for improving efficacy and safety of treatment based

on insights into immunoregulatory mechanisms and the underlying immune mechanisms in successful SIT.

2. Allergen-specific immunotherapy

In retrospect, the rationale for using allergen to treat an allergen-induced disease as pioneered by Noon and Freeman in 1911 (Noon, 1911) seems counter-intuitive. However, inspired by other vaccination successes of the day, these workers sought to vaccinate allergic individuals to induce “anti-toxins” which would prevent disease. Indeed they found that appropriate doses of allergen could induce a state of clinical and immune tolerance to that allergen. Since this time, subcutaneous allergen-specific immunotherapy (SCIT) has developed as a routine therapy and best practice protocols have been defined (Bousquet et al., 1998; Joint Task Force on Practice Parameters, 2007). Conventional SCIT involves injection of increasing quantities of allergen extract during an up dosing phase followed by repeated injection of allergen at a maximum tolerated dose over three to five years in a maintenance phase (Rolland et al., 2000). Controlled trials have demonstrated efficacy in appropriately selected patients with venom hypersensitivity (Lerch & Muller, 1998), seasonal allergic rhinitis/conjunctivitis (Varney et al., 1991; Calderon et al., 2007) and mild asthma (Abramson et al., 2003). Importantly, SCIT can prevent the development of new sensitivities in monosensitized patients (Des Roches et al., 1997; Purrello-D'Ambrosio et al., 2001) as well as the progression to asthma in patients with allergic rhinitis (Moller et al., 2002; Jacobsen et al., 2007). However, despite advances in standardization of allergen extracts and treatment protocols, SCIT is not recommended for patients with moderate to severe asthma, and immunotherapy with unfractionated extracts of potent allergens such as natural rubber latex, peanuts and shellfish is not currently available due to the high risk of systemic adverse events (Rolland et al., 2000). Modification of SIT regimens, especially allergen preparations, is required to permit more widespread use of this therapy in clinical practice.

2.1. Current immunotherapy schedules

Conventional SCIT is administered by subcutaneous injection of unfractionated extract in either alum-precipitated or aqueous form. Aqueous allergen preparations allow more rapid allergen absorption but are often highly potent. To minimize adverse reactions due to inappropriate dosage, allergen extracts are standardized as “Bioequivalent Allergy Units” or by quantitation of the dominant allergen (Joint Task Force on Practice Parameters, 2007). Although attractive for treatment of patients with multiple sensitivities, use of mixtures of unrelated allergens may be less efficacious and only proven mixtures containing known amounts of each component should be used (see World Health Organisation position paper, Bousquet et al., 1998). During the up dosing phase, a small dose of allergen extract is given first followed by slowly increasing increments to minimize adverse allergic reactions, particularly anaphylaxis. The standard SCIT dosage schedule involves injections given once to twice weekly during the induction phase followed by maintenance injections at increasing intervals as tolerated up to monthly. Regimens are usually continued for three to five years (Lerch & Muller, 1998; Durham et al., 1999). More rapid dose escalations may be required for urgent treatment of anaphylaxis and venom allergy. For these regimens, the induction phase is completed over a period of about five days (rush) or even one day (ultra-rush) with typically injection intervals of 1 h and treatment is usually performed in a hospital setting due to the higher incidence of systemic reactions (Hejjaoui et al., 1990; Nelson, 2007; Cox, 2008). Notwithstanding the regimen, all immunotherapy injections require close medical supervision with adrenaline and adequate resuscitation facilities available. Immunotherapy is rarely started in children before the age of five years.

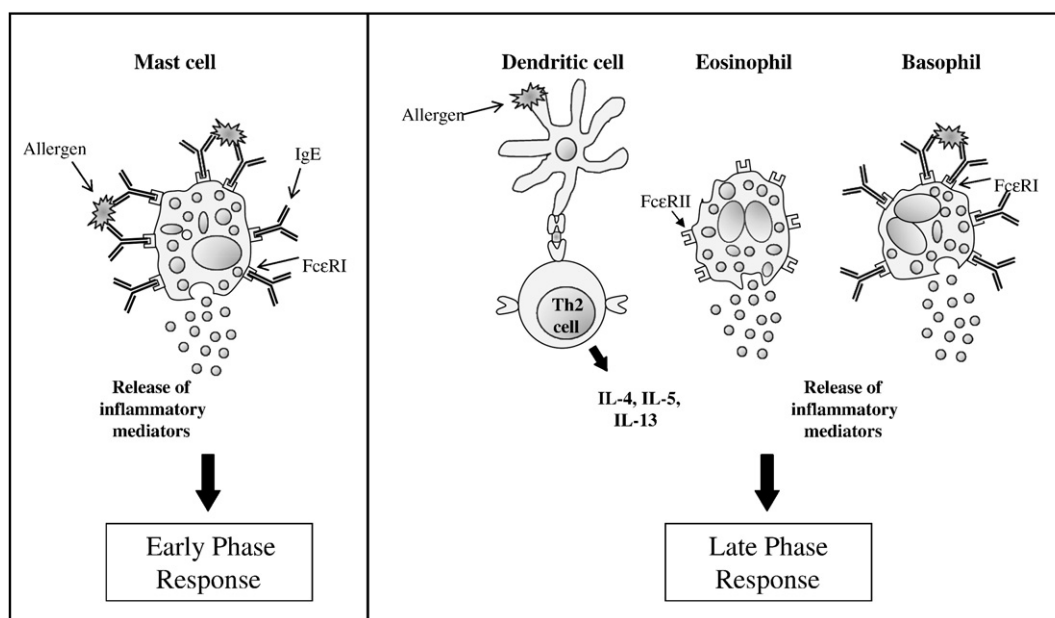


Fig. 1. Early and late phase response to allergen exposure. In a previously sensitized individual, re-exposure to allergen results in cross-linking of allergen-specific IgE bound to mast cells and release of inflammatory mediators including histamine, serine proteases, leukotrienes, prostaglandins and cytokines. This occurs within 5 to 15 min of exposure to allergen and is termed the early phase response. The late phase response results from a second wave of inflammatory mediators released from activated T cells, eosinophils and basophils attracted to the site during the early phase.

In an attempt to enhance patient acceptance and minimize the risk of anaphylactic reactions, alternative routes for local administration of allergen immunotherapy including nasal insufflation, bronchial inhalation and sublingual absorption have been explored (see reviews, [Malling et al., 1998](#); [Canonica & Passalacqua, 2003](#); [O'Hehir et al., 2007](#)). The most promising of these has been the sublingual route, with several clinical trials demonstrating significant symptomatic improvement (see meta-analysis by [Wilson et al., 2005](#)). Sublingual/swallow immunotherapy (SLIT) involves holding the allergen solution under the tongue for a period of 2 min before swallowing. Despite requiring doses of allergen at least 50–100 times higher than for the subcutaneous route, there is evidence that SLIT is safer than SCIT, including for asthma ([Gidaro et al., 2005](#); [Rienzo et al., 2005](#); [Calamita et al., 2006](#); [Nelson, 2007](#)). Treatment is usually continued for at least two years although an ultra-rush protocol for SLIT has also been described ([Vervloet et al., 2007](#)). The relative clinical efficacy of SLIT compared with SCIT is not yet clear and more well-controlled comparative studies are required ([Cox et al., 2006](#)). The use of a tablet formulation instead of liquid should improve reliability of dosage ([Kleine-Tebbe et al., 2006](#); [Malling et al., 2006](#)). SLIT is now very popular in Europe and increasingly under evaluation in North America ([Frew, 2008](#)).

2.2. Limitations and adverse reactions with current SIT

Safe SCIT is not currently available for IgE-mediated food or latex allergy due to the high risk of anaphylaxis. Even for routinely used aeroallergens, local reactions occur in up to 25% of patients, and large reactions may require a reduction in dose. Systemic adverse events are estimated at a frequency of 1/500 injections ([Frew, 1993](#); [Nelson, 2007](#)). Alum precipitation of allergen extract greatly improves the safety of immunotherapy. Deaths are fortunately rare and in most cases are associated with dosage errors, new bottles of extract, concomitant medication use (for example beta blockers) and delayed treatment of anaphylaxis. Symptomatic asthmatic patients are at greatest risk of adverse reactions.

The improved safety and convenience of administration of allergen by the sublingual route as mentioned previously is increasingly attracting the attention of clinicians. Although not yet clear, suggested

reasons for the increased safety of SLIT are the lack of systemic absorption of allergen from the oral mucosa ([Bagnasco et al., 1997](#)) and a relative paucity of mast cells in the oral mucosa ([Moingeon et al., 2006](#)). In general, adverse reactions to SLIT are only mild and self-resolving mouth itching and swelling, and some clinical trials of SLIT for grass pollen and HDM allergy reported no episodes of anaphylaxis or severe systemic reactions ([Gidaro et al., 2005](#); [Passalacqua et al., 2007](#)). However, other studies report episodes of severe reactions especially with more potent allergens such as latex including a case of anaphylaxis during rush SLIT for latex allergy ([Antico et al., 2006](#)). Clearly the development of refined allergen preparations for SIT should not only take into account rational targeting of immunoregulatory mechanisms but also consider the causes of adverse reactions.

3. Mechanisms of specific immunotherapy

Successful SIT reduces symptoms of allergic disease and need for medication. Late phase responses to local allergen challenge in the skin and nasal mucosa are decreased ([Varney et al., 1993](#); [Durham et al., 1996](#)) and in asthmatics, non-specific airway hyper-reactivity and bronchial response to inhaled allergen challenge are decreased ([Warner et al., 1978](#); [Walker et al., 2001](#)). Local tissue changes include reduced recruitment of eosinophils into challenge sites ([Durham et al., 1996](#)), and reduced release of inflammatory mediators such as eosinophil cationic protein and platelet activating factor ([Hsieh & Ng, 1993](#); [D'Amato et al., 1996](#)). To understand the underlying mechanisms for these clinical and tissue changes and to identify potential reliable monitoring assays, many studies have examined changes in immunological parameters accompanying clinically effective SCIT (see reviews, [Jutel et al., 2006](#); [Larche et al., 2006](#); [Akdis & Akdis, 2007](#); [O'Hehir et al., 2007](#)). Mechanisms underlying SLIT are less well understood but emerging evidence suggests some common immunological changes ([Bohle et al., 2007](#); [O'Hehir et al., 2007](#); [Dahl et al., 2008](#); [James & Durham, 2008](#)).

Allergen-specific IgE antibodies are the hallmark of the immune response to allergens and an important diagnostic tool for allergic disease. Following SCIT, allergen-specific IgE antibody levels usually show an initial increase and then a gradual decrease over several months or years of treatment, correlating poorly with overall clinical efficacy ([Creticos et al., 1984](#); [Gleich et al., 1982](#)). At the same time,

allergen-specific IgG₁ and IgG₄ antibody levels may increase (Muller et al., 1989). Allergen-specific IgG antibodies are suggested to act as “blocking” antibodies, inhibiting IgE-facilitated allergen uptake by DC and preventing IgE-mediated allergen activation of basophils and mast cells and consequent release of inflammatory mediators (van Neerven et al., 1999; Wachholz & Durham, 2003). Specific IgA₂ levels are also reported to increase with SIT (Pilette et al., 2007) and secreted specific IgA is suggested to play a protective role at mucosal surfaces following SLIT (Bottcher et al., 2002).

A unifying mechanism to account for the observed changes in inflammatory cell activity and antibody responses is altered allergen-specific T cell reactivity (Fig. 2). Changes in T cell response to allergen correlate well with clinical improvement, and include decreases in antigen-specific proliferation and production of IL-4, but increased production of IL-10 and TGF- β (Jutel et al., 2006). Elucidation of the precise mechanisms by which allergen administration during SIT elicits these changes in T cell function will inform refined strategies for SIT specifically targeting the T cell response to allergen.

3.1. Anergy and apoptosis

Anergy and/or deletion of allergen-specific T cells during SIT is consistent with the decreased peripheral blood T cell proliferative

response to allergen generally observed in SIT-treated patients (see review, Rolland & O’Hehir, 1998). T cell anergy is defined as antigen-induced specific non-responsiveness to subsequent challenge with immunogenic concentrations of antigen (Lamb et al., 1983). Treatment with high doses of antigen in the form of dominant T cell epitope peptides (Lamb et al., 1983; O’Hehir et al., 1991b; Higgins et al., 1992) or antigen stimulation in the absence of antigen presenting cells (i.e. co-stimulation) (Mueller et al., 1989) can result in T cell anergy. Doses of allergen given in immunotherapy regimens are estimated to be considerably higher than those encountered naturally especially for SLIT, and in vitro studies show that proliferation of allergen-donor peripheral blood T cells is decreased by stimulation with higher concentrations of allergen compared with low concentrations (Gardner et al., 2004a). Evidence to support anergy as a mechanism for decreased T cell proliferative response following SIT is antigen specificity of the decreased response and reversal by added IL-2. This was shown in a study of bee venom immunotherapy where there was a decreased T cell response to the major bee venom allergen phospholipase A₂ (PLA₂) but no parallel change in response to control antigens PPD and tetanus toxoid (Akdis et al., 1996). The allergen-induced proliferative response was recovered by addition of IL-2 or IL-15 to the in vitro cultures. Other studies have shown that IL-10 is a key cytokine mediating the reduced proliferative response by reversing

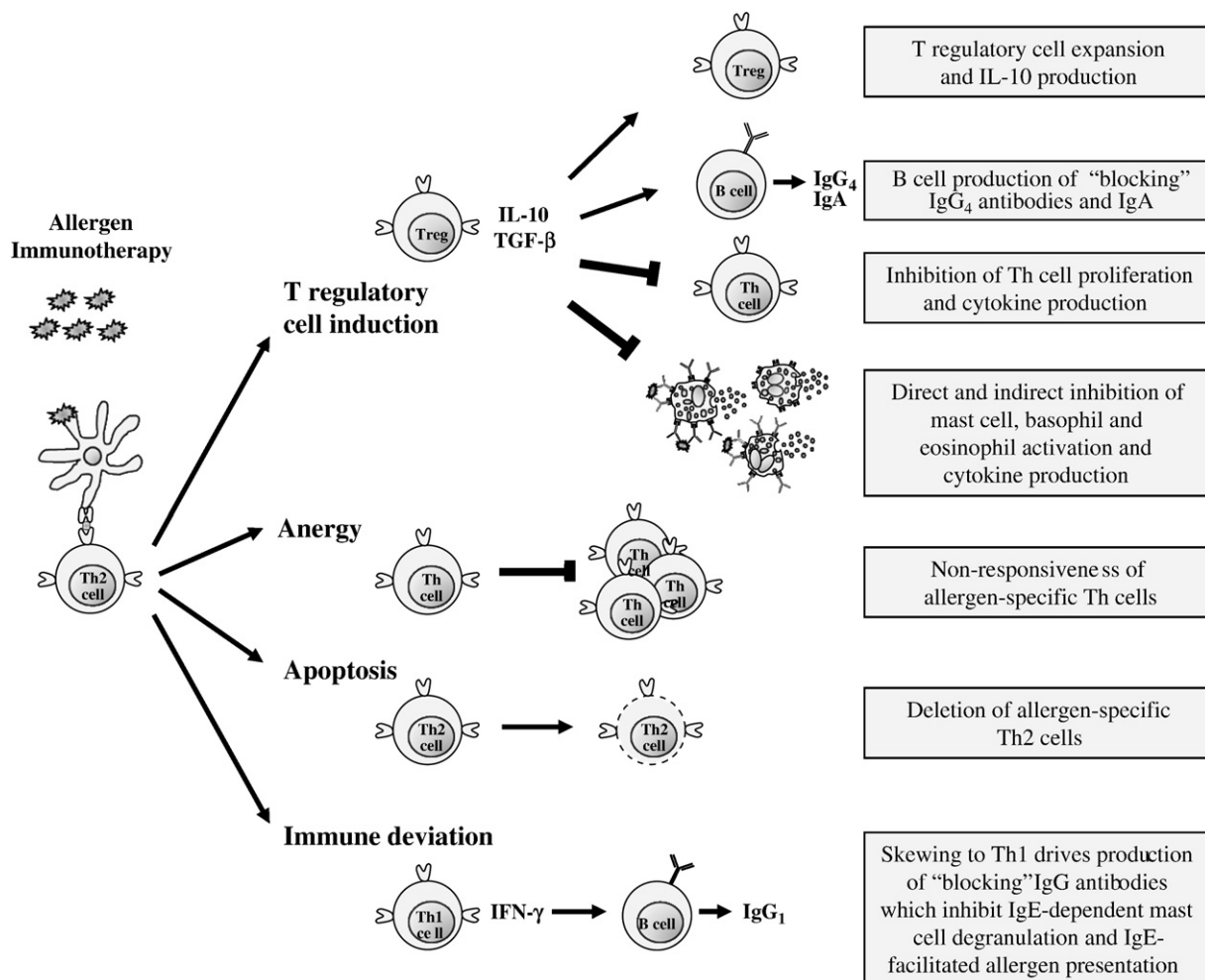


Fig. 2. Mechanisms of successful SIT. Administration of high dose allergen during immunotherapy results in an altered T cell response. SIT can induce Treg activity with production of IL-10 and/or TGF- β . IL-10 suppresses Th cell responses, induces IgG₄ production by B cells and inhibits mast cell, basophil and eosinophil activation. TGF- β induces IgA production by B cells and further supports Treg development. During SIT, allergen-specific Th cells can also become anergised or deleted by apoptosis, further contributing to decreased T cell proliferation and cytokine production to allergen. Immune deviation can also occur with a shift from Th2-type to Th1-type cytokine production post SIT. IFN- γ drives B cell class switching to IgG₁. Allergen-specific IgG₁ and IgG₄ antibodies can act as “blocking antibodies” inhibiting IgE-dependent mast cell degranulation and IgE-facilitated allergen presentation. Strategies for refining SIT will selectively target allergen-specific T cells, and in particular regulatory T cells either directly or via the antigen presenting cell.

the effect with anti-IL-10 blocking antibodies (Bellinghausen et al., 1997; Akdis et al., 1998; Bohle et al., 2007).

Another consequence of the repeated high dose antigen stimulation of T cells during SIT may be activation-induced cell death by apoptosis (Kabelitz et al., 1993). This mechanism was explored in a study of SIT for rye grass and olive tree pollen allergy (Guerra et al., 2001). After SIT, blood lymphocytes showed an increased susceptibility to undergo apoptosis when stimulated with allergen in vitro and there was preferential apoptosis of Th2 cells. Presumably due to a lack of IL-2 and other survival factors, peripheral blood T cells which had been anergized by mitogen stimulation were more susceptible to Fas-mediated apoptosis (Mollereau et al., 1997). This suggests that anergy and apoptosis may act in concert during allergen immunotherapy. In support of these mechanisms, decreased T cell proliferative response to allergen with increased apoptosis of IL-4⁺ T cells can be demonstrated in vitro by using higher dose stimulation (Gardner et al., 2004a). Thus achieving high dose allergen treatment during SIT is likely to be important for achieving efficacy, but this should not compromise safety and other factors that influence the quality of response should be taken into account as discussed later.

3.2. Immune deviation

Concomitantly with decreased proliferative responses, decreased production of Th2-type cytokines is observed in allergen-stimulated T cell cultures after SIT (see review, Rolland & O'Hehir, 1998). While all studies have not shown increased production of IFN- γ by peripheral blood T cells, overall, there is usually a decrease in the ratio of IL-4/IL-5 to IFN- γ levels to those more typical of a non-atopic subject (e.g. Jutel et al., 1995; Eusebius et al., 2002). It is likely that these changes are a result of skewing the differentiation path of a naïve allergen-specific T cell rather than altering the cytokine production of a committed Th2 cell. However, short-term changes in cytokine profile favoring IFN- γ production by both CD4⁺ and CD8⁺ T cells can be induced in polyclonal and monoclonal T cell populations when stimulated with high allergen concentrations (O'Hehir et al., 1991b; Carballido et al., 1997; Gardner et al., 2004a). Higher levels of IFN- γ will contribute to immune deviation by inhibiting Th2 IL-4 induced gene expression (Venkataraman et al., 1999). In agreement with this mechanism, increased expression of mRNA for IFN- γ and IL-12 has been reported in the nasal mucosa following grass pollen immunotherapy (Varney et al., 1993). This finding would also suggest altered trafficking of allergen-stimulated T cells during SIT as supported by in vitro studies demonstrating enhanced expression of peripheral tissue trafficking and adhesion molecules on IFN- γ ⁺ cells following stimulation with high concentrations of allergen (Gardner et al., 2004b).

Concurrent with skewing from Th2 to Th1 cytokine production is increased production of IL-10 and TGF- β following SIT (Jutel et al., 2006). This altered cytokine profile could account for the changes in specific antibody isotype from IgE to IgG₁, IgG₄ and IgA described above but importantly, increased levels of IL-10 will play a role in inducing T cell tolerance and in dampening down both Th2 and Th1 cytokine-mediated inflammation at sites of allergen exposure (Akdis & Blaser, 2001). IL-10 increases are detected as early as one week after commencing bee venom immunotherapy (Akdis et al., 1998). Of note, both T cells and DC are sources of IL-10 with important cross-regulatory potential (Mahnke et al., 2008).

3.3. Regulatory T cells

Regulatory T cells (Tregs) are considered the “master regulators” of immune homeostasis (Tang & Bluestone, 2008). They play a major role in maintaining immune self tolerance in the periphery and act as a normal counter balance to immune defence reactions, protecting against excessive activation and disease (Sakaguchi, 2000; O'Garra & Vieira, 2004; Belkaid, 2007). Evidence is accumulating for a role of

Tregs in inducing tolerance in allergen-specific T cells in healthy individuals and in allergic subjects following SIT. Tregs are defined as T cells which suppress or dampen effector T cell proliferative and cytokine responses to antigen. Treg function has been shown to be largely due to induction of cytokine deprivation-induced apoptosis of effector CD4⁺ T cells (Pandiyan et al., 2007). While anergy and apoptosis of allergen-specific effector T cells is evident after SIT as described above, these observations could also be consistent with increased Treg activity. Over the last decade there has been a wealth of information generated on the role of Tregs in several immunopathologies and it is likely that harnessing Tregs will also be a key to controlling allergic disease.

Several different subsets of Tregs are described. “Natural”, or thymic-derived, Tregs are CD4⁺ T cells constitutively expressing CD25 and surviving thymic selection by having intermediate affinity for self peptide-MHC. They are the major regulators of self-reactive T cells in the periphery acting largely by cell-contact dependent mechanisms (PD1, CTLA-4, and membrane bound TGF- β) (Sakaguchi et al., 2006). CTLA-4⁺ natural Tregs induce the expression of indoleamine 2,3-dioxygenase by APC and this enzyme degrades the essential amino acid tryptophan thus inhibiting T cell activation and promoting T cell apoptosis (Fallarino et al., 2003). Expression of the transcription factor Foxp3 is required for natural Treg function, and Treg development and expansion is dependent on TGF- β (Fontenot et al., 2003; Huber et al., 2004). Imaging studies show TGF- β -dependent impairment of the immunological synapse between helper T cells and APC by Treg (Esquerre et al., 2008). Other populations of Tregs, termed “induced Tregs”, develop in the periphery in response to repeated exogenous antigen stimulation in concert with dendritic cell and cytokine signals (Tang & Bluestone, 2006). Suppressor function of induced Tregs is mediated by secreted immunoregulatory cytokines including IL-10 (Tr1 subset) and TGF- β (Th3 subset) (O'Garra & Vieira, 2004).

Evidence for a role for Tregs in controlling the immune response to allergens comes from studies comparing allergic and non-allergic donors. Different proportions of Th1 (IFN- γ -secreting), Th2 (IL-4-secreting) and Tr1 (IL-10-secreting) subsets were demonstrated after short-term stimulation of PBMC with allergen in vitro between healthy and allergic individuals, with Tr1 cells representing the dominant subset in the healthy subjects (Akdis et al., 2004). Interestingly, increased proportions of peripheral blood CD4⁺CD25⁺ T cells were found in children who had outgrown cow's milk allergy compared with milk allergic children (Karlsson et al., 2004) but a clear Treg phenotype of these cells was not demonstrated. Decreased allergen-specific suppressive function of CD4⁺CD25⁺ T cells has also been shown by allergic patients compared with healthy subjects. Ling et al. showed that CD4⁺CD25⁺ blood T cells from atopic subjects had reduced ability to suppress allergen-induced CD4⁺CD25[−] T cell proliferation and IL-5 production compared with atopic donors, and that for hay fever sufferers this ability was further impaired during the pollen season (Ling et al., 2004). In another study, peripheral blood CD4⁺CD25⁺ cells from birch pollen-allergic patients suppressed influenza antigen-, but not birch pollen-stimulated proliferation, while control donor CD4⁺CD25⁺ cells suppressed both responses (Thunberg et al., 2007). These results indicate that rather than having reduced total Treg numbers, allergic individuals have intrinsic defects in the ability of these cells to suppress pathogenic allergen-specific effector T cell responses.

Analysis of T cell responses during SIT suggests that Tregs play a role in down-regulating adverse Th2-polarized responses to allergen (Akdis & Akdis, 2007). Increased proportions of CD4⁺IL-10⁺ cells are observed in allergen-stimulated peripheral blood T cell cultures after HDM and grass pollen SIT compared to before treatment, with the IL-10⁺ cells co-expressing CD4 and CD25 (Francis et al., 2003; Gardner et al., 2004c). The IL-10⁺ cells also express peripheral tissue trafficking surface markers (Gardner et al., 2004c), consistent with the observed increase in IL-10⁺ cells in skin biopsies of allergen-induced cutaneous

late phase responses following wasp venom SIT (Nasser et al., 2001). Increased Treg numbers following venom immunotherapy correlate with a shift in the ratio of venom-specific IgG₄/IgE (Pereira-Santos et al., 2008).

Thus it is likely that as for self antigen, Foxp3⁺CD25⁺CD4⁺ Tregs are an essential component of the normal immune system for maintaining immune homeostasis to exogenous antigens. Insight into the mechanisms by which Tregs are induced and expanded in response to antigen stimulation points to strategies for improving efficacy of SIT and, in particular, for designing the most appropriate allergen preparations. Factors associated with the local environment are pivotal for the immune system's discrimination between innocuous and pathogenic situations. These include the local cytokine milieu, co-stimulatory molecules and danger signals mediated by Toll-like receptors (TLR) and other pattern recognition receptors (PRR) (Kapsenberg, 2003). Treg homeostasis is regulated by IL-2 (Scheffold et al., 2005) and IL-1, IL-6 and IL-12 produced by mature APC can potentiate Treg responsiveness to IL-2, increasing Treg proliferation (Kubo et al., 2004). Treg development, expansion and suppressive function are modulated by certain co-stimulatory molecules in the direct interaction between APC and Treg (Tang & Bluestone, 2006). Murine studies show that engagement of CD40 and CD80/CD86 molecules induces Treg expansion while glucocorticoid-induced TNFR family related gene (GITR) and Receptor activator of nuclear factor- κ B (RANK) interaction prevents Treg-mediated suppression (Green et al., 2002; McHugh et al., 2002; Yamazaki et al., 2003; Fehervari & Sakaguchi, 2004). Human Tregs can be induced by costimulation with Campath-1H, a humanized anti-CD52 monoclonal antibody (Watanabe et al., 2006). Treg activity can also be influenced by direct interaction with microbes and viruses via TLR. Tregs express several TLR as do effector T cells, but TLR4, 5, 7 and 8 are expressed at much higher levels on Treg than on effector T cells (Caramalho et al., 2003). Triggering of Tregs via TLR induces increased expression of Foxp3 and in particular, TLR4 and TLR5 ligand interaction enhances suppressor function (Caramalho et al., 2003; Crellin et al., 2005). In this way, APC activating signals such as from allergen cofactors and adjuvants which control expression of co-stimulatory molecules in turn influence Treg activity and determine whether the immune response is dampened or promoted.

4. New approaches to allergen immunotherapy

Allergic diseases are the only group of immune disorders for which antigen-specific immunomodulation is a current clinically successful and routine treatment. Nevertheless, as discussed above, SIT is associated with side effects which, for some allergens and for severe asthmatics, prohibits routine use. Advances in understanding of the factors which influence the type and magnitude of an immune response to allergen stimulation point to new directions for improving efficacy and safety of SIT. In particular, the form and concentration of allergen are important as well as concurrent administration of adjuvant molecules and adjunct therapies. APC subsets differ with respect to PRR and may therefore be targeted selectively by use of particular allergen preparations and adjuvants. The heterogeneity of APC subsets and function at different sites of the body will also play a role in determining the most effective route for allergen administration. Thus different compartments of the immune system can be targeted in new SIT regimens.

4.1. Allergen structure and function

The major allergens of most clinically important allergen sources have been cloned and sequenced providing an important foundation on which to consider structural modifications for optimal immunomodulation in clinical practice. The Allergen Nomenclature Subcommittee of the World Health Organization and International Union

of Immunological Societies maintains a Protein Database which provides a register of validated information (<http://www.allergen.org/Allergen.aspx>). Allergens are organised into families with related structure and function, but allergen variations or isoforms and cross-reactivity between allergenic components from related species must be taken into account when designing allergen “vaccines”. More recently, the importance of structural biology and function of allergen molecules is recognized in determining the response to allergen encounter. Crystal structures have been obtained for many well characterised allergen molecules revealing sites of IgE epitopes on exposed outer surfaces of tertiary and quaternary structure (eg Verdino et al., 2008). Higher order structures may also influence allergen processing by APC and subsequent presentation of peptides for T cell recognition.

An emerging field is the characterization of allergen sources with respect to molecular components which bind to different PRR of APC and even Tregs. Unpurified allergen sources contain a complex mixture of chemical components including lipids, lipid-derived plant hormones and polysaccharides as well as proteins and glycoproteins (Haavik et al., 1985; Spiewak et al., 1996; Traidl-Hoffmann et al., 2005). Analysis of these components and their influence on the immune outcome will determine appropriate procedures in preparing purified allergen extracts for SIT. For example a recent study showed that the phospholipids phosphatidylcholine and phosphatidylethanolamine from cypress pollen could be presented by CD1, stimulating the proliferation and production of IL-4, IFN- γ , IL-10 and TGF- β by T cells and to a lesser extent NKT cells (Agea et al., 2005). Whether or not these endogenous adjuvant-type components should be retained in allergen preparations for effective immunotherapy requires further research.

In many cases the biological function, such as enzyme activity, of an allergen can influence immune reactivity (Stewart & Robinson, 2003; Chapman et al., 2007). For example, the protease activity of the major HDM allergen, Der p 1, results in cleavage of surface CD23 and CD25 receptors on B and T cells, thereby promoting Th2-type responses (Shakib et al., 1998; Furmonaviciene et al., 2007). Enzymic activity of Der p 1 was shown to be crucial for immunogenicity of the allergen in murine studies (Gough et al., 2003). NAD(P)H oxidase of pollen allergens also has Th2 adjuvant activity (Dharajiyi et al., 2008). Thus, retention of enzymic activity of allergens is not advantageous for immunotherapy due to Th2-inducing properties. Moreover, addition of allergens with protease activity to other allergens in mixes for immunotherapy is not recommended due to resultant allergen degradation (Nelson, 2007).

4.2. Hypoallergenic preparations for SIT

It is generally accepted that successful SIT is associated with altered T cell response to allergen and that side effects from SIT are largely IgE mediated. As discussed previously, high allergen concentrations can promote allergen-specific T cell anergy, deletion and immune deviation from Th2-type to Th1-type. Thus the development of hypoallergenic preparations which retain T cell reactivity and can be used at high dose will permit safer but more effective application of SIT. Approaches include chemical modification of allergen molecules, preparation of recombinant allergens with altered critical residues for IgE binding and synthetic peptides based on dominant T cell epitopes.

4.2.1. Chemical modification of allergens

An early approach to decreasing anaphylactic reactions in SIT was the production of “allergoids” by chemical modification of allergens. Clinical trials of formaldehyde- and glutaraldehyde-treated allergens of grass pollen and ragweed showed better tolerance of the allergoids with retained clinical and immunological efficacy (Marsh et al., 1981; Bousquet et al., 1988; Negro et al., 1999). An alternative chemical treatment for decreasing IgE reactivity of allergens is potassium

cyanate treatment at neutral pH which substitutes ϵ -amino groups of lysine residues producing a carbamylated allergoid (Mistrello et al., 1996; Ariano et al., 2005). Such monomeric dust mite and tree pollen allergoids were trialled for SLIT with improved symptom and medication scores (Passalacqua et al., 1998; Ariano et al., 2005; Cosmi et al., 2006; La Grutta et al., 2007). Decreased allergen-specific IgE and increased IFN- γ and IL-10 production followed SLIT with dust mite and grass pollen carbamylated allergoids (Cosmi et al., 2006; Burastero et al., 2008). Treatment with hypoallergenic allergoids can provide clinical benefit and immunomodulation safely but the methods for preparation of these extracts are poorly standardized and characterization of allergoids is difficult.

4.2.2. Recombinant allergens

More precise alteration of IgE epitopes of allergens is possible using recombinant technologies. DNA sequences for most major allergens are known (<http://www.allergen.org/Allergen.aspx>) and there are a range of approaches for generating hypoallergenic recombinant molecules depending on the particular features of the allergen molecule. For allergens with predominant IgE reactivity associated with carbohydrate moieties (eg the latex allergen Hev b 2, Yagami et al., 2002), bacterial expression of non-glycosylated allergens provides a first line approach, but for most allergens, site-directed mutagenesis of critical residues for conformational IgE epitopes is the rational strategy. For example, disruption of intramolecular disulphide bonds by substitution of cysteine residues reduces or ablates IgE reactivity of allergens of dust mite (Der p 2, Der f 2, Lep d 2), cow dander (Bos d 2), Parietaria pollen (Par j 1) and natural rubber latex (Hev b 6.01) (Olsson et al., 1998; Smith et al., 1998; Kauppinen et al., 1999; Korematsu et al., 2000; Bonura et al., 2001; Drew et al., 2004; Rolland & O'Hehir, 2008). For the allergens Bet v 1, Lol p 5, and Ara h 1, 2 and 3, residues pivotal for IgE binding were identified either by alanine screening, prediction analyses or from crystal structures and targeted successfully by site-directed mutagenesis (Ferreira et al., 1998; Singh et al., 1999; Rabjohn et al., 2002; Swoboda et al., 2002; Spangfort, 2003; Holm et al., 2004). Additional approaches include recombinant generation of deletion mutants, oligomers, fragments and allergen chimeras based on knowledge of structural properties of particular allergens (Zeiler et al., 1997; Okada et al., 1998; Schramm et al., 1999; Vrtala et al., 1999; Vrtala et al., 2000). Full immunological characterization of recombinant hypoallergenic molecules is not complete in all cases and importantly retained T cell reactivity will be essential for clinical efficacy in SIT. Careful purification of recombinant allergens and elimination of bacterial contaminants especially endotoxin is essential for clinical use. An advantage of the recombinant hypoallergen approach is that the molecule will retain multiple T cell epitopes and thus target the polyclonal allergen-specific T cell population in patients. Several clinical trials of recombinant birch and grass pollen allergen-based vaccines have been undertaken with promising results (see review, Valenta & Niederberger, 2007).

Recently the targeted delivery of allergen to the MHC class II presentation pathway in APC using recombinant technology has been explored as a strategy for increasing the level of allergen presentation to T cells. This could potentially alter T cell responses more efficiently without administering increased doses of allergen to the patient. Fusion proteins were created consisting of a [HIS]₆-tag for protein purification, a TAT (transactivator of transcription) peptide for converting extracellular to cytoplasmic protein, a truncated invariant chain (Ii) peptide for protein targeting to endosomal/lysosomal compartments and an allergen (Bet v 1, PLA₂, Der p 1 or Fel d 1) (Cramer et al., 2007). Stimulation of allergic donor PBMC with these fusion proteins induced T cell proliferation at 10–100 fold lower dose than seen for the control proteins not containing the TAT or Ii peptides. At this concentration range, a shift in cytokine profile from Th2- to Th1-type with concurrent IL-10 production was also observed. Thus this recombinant approach may be ideal for the design of safe and

effective allergy vaccines and further research is warranted to validate the effectiveness of these preparations in vivo.

4.2.3. T cell epitope based peptides

The use of short, linear peptides corresponding to T cell epitopes of allergens is an attractive approach for targeting allergen-specific T cells without adverse IgE mediated effects. Peptides of about 12–15 amino acids are too short to cross-link inflammatory cell-bound IgE. Conventional approaches to mapping T cell epitopes are labour intensive and the culturing of T cell lines and clones is technically demanding (O'Hehir et al., 1993a). A more specific approach to increase cloning efficiency, is to enrich for allergen-reactive T cells by in vitro allergen stimulation of CFSE-labelled PBMC and flow cytometric sorting of divided, CFSE^{lo}, cells (Mannering et al., 2003). Allergen-enriched T cell populations can then be mapped for peptide specificity to determine predominant epitopes. Computer-based programs such as ProPred (<http://bic.uams.edu/mirror/propred/index.html>) have been designed to predict MHC class II binding regions in proteins (Singh & Raghava, 2001). While such algorithms are useful for predicting allergen T cell epitopes, in our experience complete agreement with data generated by in vitro methods is not observed. Another approach, yet untested for identification of allergen T cell epitopes, is to screen T cells with random peptide libraries (Gundlach et al., 1996; Sparbier & Walden, 1999). T cell epitopes discovered using this method are not necessarily natural epitopes, but are mimics or structural equivalents, and thus are termed mimotopes. Both agonistic and antagonistic mimotopes can be selected depending on the type of immune response required.

Although T cell epitope mapping generally reveals multiple T cell reactive sites throughout allergen molecules (O'Hehir et al., 1993b; Gardner et al., 2003; Larche, 2007; O'Hehir et al., 2007), sites of dominance are apparent. Importantly, the high degree of promiscuity observed in presentation of T cell reactive peptides in population studies obviates the need to individually tailor peptides for particular MHC class II haplotypes. In one novel allergen vaccine design, covalently-linked T cell epitope peptides were incorporated to encompass multiple T cell-reactive sites, but it is not clear whether multiple allergen-specific T cell populations are best targeted in vivo by such peptide constructs or by cocktails of individual peptides. Importantly candidate peptide vaccines must be tested for lack of IgE reactivity and biological toxicity before clinical trial. Lack of IgE binding can be suggested by ELISA but the more biologically relevant basophil activation assay is recommended before testing in patients (Drew et al., 2004; Ebo et al., 2008). For several allergens, preclinical testing has included evaluation in murine models for down-regulation of Th2 responses. Candidate T cell peptide vaccines to treat allergy to cat dander, ragweed and bee venom are at various stages of clinical trial with promising results. The early finding in cat peptide immunotherapy studies that some patients experienced shortness of breath within hours of peptide treatment was attributed to cytokines released from peptide-stimulated T cells (Haselden et al., 1999). These reactions were subsequently managed by careful up dosing (Oldfield et al., 2002). In addition to the safety benefit of peptide immunotherapy, this approach has the important advantages of ease of manufacture and standardization.

4.3. Adjuvants and adjunct therapies

Despite well advanced strategies for selective targeting of allergen-specific T cells with hypoallergenic preparations as described above, the best delivery regimens are still under evaluation and in particular the need to target accessory cells especially APC to achieve optimal suppression of adverse immune response is an area of active ongoing research. Whether efficacy of SIT can be improved by co-administration of adjuvants either endogenous (as discussed above) or exogenous, and whether safety can be improved by adjunct anti-inflammatory therapies are areas of active research.

4.3.1. Adjuvants

Conventional SIT uses either alum-precipitated or aqueous solutions of allergen for subcutaneous delivery. SLIT preparations are generally aqueous but tablet formulations are receiving interest for increased safety and more reliable dosing (O'Hehir et al., 2007; Dahl et al., 2008; Frew, 2008). Insight into the molecular mechanisms for antigen and adjuvant recognition by APC and subsequent intracellular signalling points to a new generation of allergen preparations for SIT. With any antigen and adjuvant molecule, the particular PRR used for uptake can influence the antigen-stimulated T cell cytokine profile. An intriguing question is whether those PRR/TLR expressed by Tregs as described previously can be targeted with SIT allergen formulations. Early studies on suitable SIT adjuvants focused on strategies to preferentially drive Th1 responses for down-regulating adverse Th2 responses. An inverse correlation observed between Th1-type hypersensitivity to *Mycobacterium tuberculosis* and atopy in Japanese schoolchildren (Shirakawa et al., 1997) led investigators to explore the use of heat-killed or live *Mycobacteria* to down-regulate Th2 responses to allergens. Despite promising results for prophylactic treatment of allergic inflammation and asthma in murine models using preparations of non-pathogenic *Mycobacterium vaccae* (Wang & Rook, 1998; Yazi et al., 2008), clinical trials for treatment of allergic asthma have been disappointing (Renz, 2004). Interestingly murine studies showed that the beneficial effects of *Mycobacterial* treatment were more related to induction of Treg responses rather than enhanced Th1 responses (Zuany-Amorim et al., 2002). These studies indicate that more research is required to elucidate the precise mechanisms for driving particular types of T cell response to microorganisms and how these may be exploited by using relevant components as adjuvant preparations for allergy vaccines.

Bacterial cell wall components are being investigated as adjuvants for SIT due to their immunomodulatory properties. Of particular promise is monophosphoryl lipid A, a non-toxic lipopolysaccharide derived from *Salmonella minnesota*. Monophosphoryl lipid A can directly bind TLR4, and has been shown to activate human DC, and induce production of IFN- γ by PBMC and IL-10 and IL-12 by monocytes (Ismaili et al., 2002; Puggioni et al., 2005). This adjuvant is already in clinical use in the Pollinex® Quattro vaccine for pollen-induced seasonal allergic rhinitis (Patel & Salapatek, 2006). The vaccine is clinically well tolerated and four pre-seasonal injections were shown to reduce rhinitis symptoms and medication use, and blunt the elevation of allergen-specific IgE during the pollen season. Another approach has been to create a fusion protein consisting of the bacterial surface protein (S-layer) from *Geobacillus stearothermophilus* ATCC 12980 and the birch pollen allergen, Bet v 1 (Gerstmayr et al., 2007). This adjuvant induced IL-10 and IL-12 production by DC from allergic individuals in vitro possibly by engagement of TLR2. The synthetic lipopeptide Pam3CSK4, another TLR2 ligand, effectively induced Th1-type and Treg responses in vitro, and sublingual administration together with allergen reversed airway hyperresponsiveness in OVA-sensitized BALB/c mice (Lombardi et al., in press).

Microbial preparations to be avoided when designing vaccines for allergic disease are those termed "superallergens" for their prominent induction of Th2-type responses, for example protein A and L from *Staphylococcus aureus* and *Peptostreptococcus magnus*, respectively (Marone et al., 2007). Alternatively, other 'regulatory-type PAMPs' on bacterial, fungi, parasites and viruses induce IL-10 or TGF- β production by DC and thus promote Treg development (reviewed by Mills, 2004). Such PAMPs provide a tantalizing new adjuvant approach for SIT development.

Considerable attention has been given to the use of bacterial immunostimulatory DNA sequences (ISS) for immunomodulation. CpG-rich bacterial DNA binds TLR-9 with strong Th1 adjuvant properties, promoting IFN- γ production by natural killer cells and induction of type I interferons and IL-12 by plasmacytoid DCs (see review, Kline, 2007). There is evidence that CpG DNA modulates

allergic responses by regulating the activity of indoleamine 2,3-dioxygenase (Kline, 2007) and therefore generation of kynurenines, potent immunomodulatory molecules that regulate T cell function. Synthetic CpG-rich oligodeoxynucleotide analogues (ODN) demonstrated impressive adjuvant effects in the treatment of anaphylaxis (Horner et al., 2000), asthma (Broide et al., 1998) and allergic conjunctivitis (Magone et al., 2000) in murine models. Different types of CpG-ODN are described, differing in backbone structure, stability and immune reactivities (Klinman, 2004). CpG-ODN could be used in treatments for allergy either alone or in combination with allergen as a genetic construct or co-administered. In a pilot study of six weekly injections of a vaccine consisting of the ragweed-pollen Amb a 1 allergen conjugated to ISS-DNA in 25 ragweed-allergic adults, symptom scores were significantly improved compared with placebo-treated patients in the subsequent two pollen seasons and there was no pattern of vaccine-associated systemic reactions (Creticos et al., 2006). Commercial preparations are under clinical evaluation for treatment of allergic disease either alone or with SIT (Aventis: AVE7279 and AVE0675 and Dynavax: 1018 ISS). Although it might be expected that Th1 pathologies such as autoimmune reactions may result from repeated clinical use of CpG DNA, such reactions have not been observed in patients treated with ISS-ODN. Of interest is the report that CpG-ODN packaging into virus-like-particles protects the ODN from DNase I digestion and delivers it directly to TLR-9 in the endolysosome without systemic side-effects (Storni et al., 2004).

4.3.2. Corticosteroid, anti-IgE and other drug co-administration

Mainstay pharmacotherapy for allergic diseases includes a panel of anti-inflammatory and immunosuppressive agents (Barnes, 2006). Detailed analysis of the effects of these agents on key regulatory points of the immune response to allergen suggests the potential for enhanced efficacy of allergen-specific treatments by using appropriate adjunct therapies. It is generally accepted that immature DC promote Treg expansion and function (Lutz & Schuler, 2002), and therefore T cells can be modulated indirectly by targeting DC. By inhibiting DC maturation through inhibition of NF- κ B activation, rosiglitazone, a selective peroxisome proliferator-activated receptor-gamma (PPAR- γ) agonist, was shown to induce Tr1 cells and to suppress allergic inflammation in a murine model of asthma (Hammad et al., 2004).

Other drug therapies may promote suppression of adverse T cell responses to allergen by targeting T cell receptor/CD3 signal transduction pathways. For example, the widely used immunosuppressive drug cyclosporin A blocks activation of NF-AT by inhibiting its dephosphorylation, thereby decreasing transcription of several cytokine genes. In asthma patients, the anti-inflammatory effects of cyclosporine A include inhibition of IL-5 production (Mori et al., 1995). Glucocorticoids also target cytokine genes including IL-5 by a similar mechanism including others (Barnes, 2006). Although cyclosporine A side effects currently limit the use of this drug to short-term therapy for allergic disease, glucocorticoids are mainstay chronic asthma therapy. In addition to targeting Th2 cell effector molecules, in vitro and in vivo studies suggest that glucocorticoid treatment may have added benefit for asthma treatment by potentiating the immunosuppressive effects of Tregs through increased IL-10 production and/or FOXP3 expression (Dao Nguyen & Robinson, 2004; Karagiannidis et al., 2004). 1,25(OH) $_2$ vitamin D3 was shown to enhance the glucocorticoid effects on Tregs in vitro (Barrat et al., 2002).

Anti-IgE therapy is being explored as a strategy for minimising IgE-mediated side effects of SIT especially during induction. The humanized anti-IgE monoclonal antibody omalizumab decreases free serum IgE as well as levels of surface Fc ϵ R1 and bound IgE on inflammatory cells (Beck et al., 2004; Lin et al., 2004). Nine weeks pretreatment with omalizumab led to a 5-fold reduction in frequency of acute reactions after rush SIT for patients receiving ragweed for allergic rhinitis (Casale et al., 2006). Similarly, high dose omalizumab effectively controlled recurrent reactions during bee venom

immunotherapy (Schulze et al., 2007; Kontou-Fili, 2008), but was ineffective in controlling systemic reactions in another bee venom allergic subject (Soriano Gomis et al., 2008). Further clinical studies are ongoing to fully evaluate the role of this adjunct therapy in SIT.

Finally, drugs which disrupt T cell co-stimulation and trafficking are potential adjunct therapies for allergy treatment. The T cell co-stimulatory molecule CTLA-4 (CD152) is expressed late in activation and competes with CD28 for engagement with CD80 and CD86, delivering inhibitory signals rather than activation as for CD28 co-stimulation (Walunas et al., 1996). Taking advantage of this immunosuppressive function of CTLA-4, a chimeric fusion protein comprising the extracellular domain of CTLA-4 and the heavy-chain constant region of human IgG₁, CTLA-4-Ig, was constructed (Abatacept, Ornetecia®) and is under clinical trial for treatment of autoimmune disorders (Kremer et al., 2005). In vitro and murine experimental asthma models show that CTLA-4-Ig inhibits allergen-induced CD4⁺ T cell proliferation and IL-5 production and has therapeutic potential for mild asthma (Tsuzyki et al., 1997; Keane-Myers et al., 1998; Jaffar et al., 1999; Deurloo et al., 2001). Drugs interfering with chemokines and chemokine receptors to halt trafficking of effector T cells to sites of allergic inflammation show promise in animal models of asthma and clinical studies are eagerly awaited (Caramori et al., 2004; Garcia et al., 2005).

5. Conclusions

Allergic diseases are a major health concern worldwide with an allergy “epidemic” particularly affecting Western countries and the likelihood of a secondary epidemic wave in newly developed countries with increasing globalization. To date, allergen SIT is the only treatment able to modify the natural history of allergic diseases. Although clinically effective, current SIT is limited to certain allergens and selected patients due to risks of adverse reactions. Conventional SCIT is the most established form of SIT but increasingly SLIT is gaining popularity due to increased safety and ease of administration and proven efficacy. Understanding allergen structure and function and the mechanisms of effective SIT informs the development of new treatment strategies. Burgeoning research activity focuses on T cell epitope mapping, Treg induction, allergen modifications, adjuvants and adjunct therapies and hypoallergenic approaches to more potent allergens. Together these approaches offer exciting opportunities to further refine allergen-related approaches to immunotherapy with the goal of preventing life threatening allergic reactions or at least substantially raising the threshold for the induction of severe allergic reactions.

Acknowledgments

The work of the authors is supported by the Cooperative Research Centre for Asthma and Airways, Australia, the National Health & Medical Research Council of Australia, and the Alfred Hospital, Melbourne.

References

- Abramson, M. J., Puy, R. M., & Weiner, J. M. (2003). Allergen immunotherapy for asthma. *Cochrane Database Syst Rev*(4) (CD001186).
- Agea, E., Russano, A., Bistoni, O., Mannucci, R., Nicoletti, I., Corazzi, L., et al. (2005). Human CD1-restricted T cell recognition of lipids from pollens. *J Exp Med* 202(2), 295–308.
- Akdis, M., & Akdis, C. A. (2007). Mechanisms of allergen-specific immunotherapy. *J Allergy Clin Immunol* 119(4), 780–791.
- Akdis, C. A., & Blaser, K. (2001). Mechanisms of interleukin-10-mediated immune suppression. *Immunology* 103(2), 131–136.
- Akdis, C. A., Akdis, M., Blesken, T., Wymann, D., Alkan, S. S., Muller, U., et al. (1996). Epitope-specific T cell tolerance to phospholipase A2 in bee venom immunotherapy and recovery by IL-2 and IL-15 in vitro. *J Clin Invest* 98(7), 1676–1683.
- Akdis, C. A., Blesken, T., Akdis, M., Wuthrich, B., & Blaser, K. (1998). Role of interleukin 10 in specific immunotherapy. *J Clin Invest* 102(1), 98–106.
- Akdis, M., Verhagen, J., Taylor, A., Karamloo, F., Karagiannidis, C., Cramer, R., et al. (2004). Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. *J Exp Med* 199 (11), 1567–1575.
- Antico, A., Pagani, M., & Crema, A. (2006). Anaphylaxis by latex sublingual immunotherapy. *Allergy* 61(10), 1236–1237.
- Ariano, R., Panzani, R. C., & Mistrello, G. (2005). Efficacy of sublingual coseasonal immunotherapy with a monomeric allergoid in *Cupressaceae* pollen allergy—preliminary data. *Eur Ann Allergy Clin Immunol* 37(3), 103–108.
- Asher, M. I., Montefort, S., Bjorksten, B., Lai, C. K., Strachan, D. P., Weiland, S. K., et al. (2006). Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC phases one and three repeat multicountry cross-sectional surveys. *Lancet* 368(9537), 733–743.
- Bagnasco, M., Mariani, G., Passalacqua, G., Motta, C., Bartolomei, M., Falagiani, P., et al. (1997). Absorption and distribution kinetics of the major *Parietaria judaica* allergen (Par j 1) administered by noninjectable routes in healthy human beings. *J Allergy Clin Immunol* 100(1), 122–129.
- Barnes, P. J. (2006). Drugs for asthma. *Br J Pharmacol* 147(Suppl 1), S297–S303.
- Barrat, F. J., Cua, D. J., Boonstra, A., Richards, D. F., Crain, C., Savelkoul, H. F., et al. (2002). In vitro generation of interleukin 10-producing regulatory CD4(+) T cells is induced by immunosuppressive drugs and inhibited by T helper type 1 (Th1)- and Th2-inducing cytokines. *J Exp Med* 195(5), 603–616.
- Beck, L. A., Marcotte, G. V., MacGlashan, G., Togias, A., & Saini, S. (2004). Omalizumab-induced reductions in mast cell Fcε receptor 1 expression and function. *J Allergy Clin Immunol* 114(3), 527–530.
- Belkaid, Y. (2007). Regulatory T cells and infection: a dangerous necessity. *Nat Rev Immunol* 7(11), 875–888.
- Bellinghausen, I., Metz, G., Enk, A. H., Christmann, S., Knop, J., & Saloga, J. (1997). Insect venom immunotherapy induces interleukin-10 production and a Th2-to-Th1 shift, and changes surface marker expression in venom-allergic subjects. *Eur J Immunol* 27(5), 1131–1139.
- Bohle, B., Kinaciyan, T., Gerstmayr, M., Radakovic, 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(3), 707–713.
- Bonura, A., Amoroso, S., Locorotondo, G., Di Felice, G., Tinghino, R., Geraci, D., et al. (2001). Hypoallergenic variants of the *Parietaria judaica* major allergen Par j 1: a member of the non-specific lipid transfer protein plant family. *Int Arch Allergy Immunol* 126(1), 32–40.
- Botcher, M. F., Haggstrom, P., Bjorksten, B., & Jemmal, M. C. (2002). Total and allergen-specific immunoglobulin A levels in saliva in relation to the development of allergy in infants up to 2 years of age. *Clin Exp Allergy* 32(9), 1293–1298.
- Bousquet, J., Lockey, R., & Malling, H. J. (1998). Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. *J Allergy Clin Immunol* 102(4 Pt 1), 558–562.
- Bousquet, J., Maasch, H., Martinot, B., Hejjajoui, A., Wahl, R., & Michel, F. B. (1988). Double-blind, placebo-controlled immunotherapy with mixed grass-pollen allergoids. II. Comparison between parameters assessing the efficacy of immunotherapy. *J Allergy Clin Immunol* 82(3 Pt 1), 439–446.
- Broide, D., Schwarze, J., Tighe, H., Gifford, T., Nguyen, M. D., Malek, S., et al. (1998). Immunostimulatory DNA sequences inhibit IL-5, eosinophilic inflammation, and airway hyperresponsiveness in mice. *J Immunol* 161(12), 7054–7062.
- Burastero, S. E., Mistrello, G., Falagiani, P., Paolucci, C., Breda, D., Roncarolo, D., et al. (2008). Effect of sublingual immunotherapy with grass monomeric allergoid on allergen-specific T-cell proliferation and interleukin 10 production. *Ann Allergy Asthma Immunol* 100(4), 343–350.
- Calamita, Z., Sacconato, H., Pela, A. B., & Atallah, A. N. (2006). Efficacy of sublingual immunotherapy in asthma: systematic review of randomized-clinical trials using the Cochrane collaboration method. *Allergy* 61(10), 1162–1172.
- Calderon, M. A., Alves, B., Jacobson, M., Hurwitz, B., Sheikh, A., & Durham, S. (2007). Allergen injection immunotherapy for seasonal allergic rhinitis. *Cochrane Database Syst Rev*(1) (CD001936).
- Canonica, G. W., & Passalacqua, G. (2003). Noninjection routes for immunotherapy. *J Allergy Clin Immunol* 111(3), 437–448 (quiz 449).
- Caramalho, I., Lopes-Carvalho, T., Ostler, D., Zelenay, S., Haury, M., & Demengeot, J. (2003). Regulatory T cells selectively express toll-like receptors and are activated by lipopolysaccharide. *J Exp Med* 197(4), 403–411.
- Caramori, G., Ito, K., & Adcock, I. M. (2004). Targeting Th2 cells in asthmatic airways. *Curr Drug Targets Inflamm Allergy* 3(3), 243–255.
- Carballido, J. M., Faith, A., Carballido-Perrig, N., & Blaser, K. (1997). The intensity of T cell receptor engagement determines the cytokine pattern of human allergen-specific T helper cells. *Eur J Immunol* 27(2), 515–521.
- Casale, T. B., Busse, W. W., Kline, J. N., Ballas, Z. K., Moss, M. H., Townley, R. G., et al. (2006). Omalizumab pretreatment decreases acute reactions after rush immunotherapy for ragweed-induced seasonal allergic rhinitis. *J Allergy Clin Immunol* 117(1), 134–140.
- Chapman, M. D., Wunschmann, S., & Pomes, A. (2007). Proteases as Th2 adjuvants. *Curr Allergy Asthma Rep* 7(5), 363–367.
- Cosmi, L., Santarlasci, V., Angeli, R., Liotta, F., Maggi, L., Frosali, F., et al. (2006). Sublingual immunotherapy with *Dermatophagoides* monomeric allergoid down-regulates allergen-specific immunoglobulin E and increases both interferon-gamma- and interleukin-10-production. *Clin Exp Allergy* 36(3), 261–272.
- Cox, L. (2008). Advantages and disadvantages of accelerated immunotherapy schedules. *J Allergy Clin Immunol* 122(2), 432–434.
- Cox, L. S., Linnemann, D. L., Nolte, H., Weldon, D., Finegold, I., & Nelson, H. S. (2006). Sublingual immunotherapy: a comprehensive review. *J Allergy Clin Immunol* 117(5), 1021–1035.
- Cramer, R., Fluckiger, S., Daigle, I., Kundig, T., & Rhyner, C. (2007). Design, engineering and in vitro evaluation of MHC class-II targeting allergy vaccines. *Allergy* 62(2), 197–206.
- Crellin, N. K., Garcia, R. V., Hadisfar, O., Allan, S. E., Steiner, T. S., & Levings, M. K. (2005). Human CD4⁺ T cells express TLR5 and its ligand flagellin enhances the suppressive capacity and expression of FOXP3 in CD4⁺CD25⁺ T regulatory cells. *J Immunol* 175(12), 8051–8059.

- Creticos, P. S., Schroeder, J. T., Hamilton, R. G., Balcer-Whaley, S. L., Khattignavong, A. P., Lindblad, R., et al. (2006). Immunotherapy with a ragweed-toll-like receptor 9 agonist vaccine for allergic rhinitis. *N Engl J Med* 355(14), 1445–1455.
- Creticos, P. S., Van Metre, T. E., Mardiny, M. R., Rosenberg, G. L., Norman, P. S., & Adkinson, N. F., Jr. (1984). Dose response of IgE and IgG antibodies during ragweed immunotherapy. *J Allergy Clin Immunol* 73(1 Pt 1), 94–104.
- D'Amato, G., Liccardi, G., Russo, M., Saggese, M., & D'Amato, M. (1996). Measurement of serum levels of eosinophil cationic protein to monitor patients with seasonal respiratory allergy induced by *Parietaria* pollen (treated and untreated with specific immunotherapy). *Allergy* 51(4), 245–250.
- Dahl, R., Kapp, A., Colombo, G., de Monchy, J. G., Rak, S., Emminger, W., et al. (2008). Sublingual grass allergen tablet immunotherapy provides sustained clinical benefit with progressive immunologic changes over 2 years. *J Allergy Clin Immunol* 121(2), 512–518 (e512).
- Dao Nguyen, X., & Robinson, D. S. (2004). Fluticasone propionate increases CD4CD25 T regulatory cell suppression of allergen-stimulated CD4CD25 T cells by an IL-10-dependent mechanism. *J Allergy Clin Immunol* 114(2), 296–301.
- Des Roches, A., Paradis, L., Menardo, J. L., Bouges, S., Daures, J. P., & 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(4), 450–453.
- Deurloo, D. T., van Esch, B. C., Hofstra, C. L., Nijkamp, F. P., & van Oosterhout, A. J. (2001). CTLA4-IgG reverses asthma manifestations in a mild but not in a more "severe" ongoing murine model. *Am J Respir Cell Mol Biol* 25(6), 751–760.
- Dharajiya, N., Boldogh, I., Cardenas, V., & Sur, S. (2008). Role of pollen NAD(P)H oxidase in allergic inflammation. *Curr Opin Allergy Clin Immunol* 8(1), 57–62.
- Drew, A. C., Eusebius, N. P., Kenins, L., de Silva, H. D., Suphioglu, C., Rolland, J. M., et al. (2004). Hypoallergenic variants of the major latex allergen Hev b 6.01 retaining human T lymphocyte reactivity. *J Immunol* 173(9), 5872–5879.
- Durham, S. R., Walker, S. M., Varga, E. M., Jacobson, M. R., O'Brien, F., Noble, W., et al. (1999). Long-term clinical efficacy of grass-pollen immunotherapy. *N Engl J Med* 341(7), 468–475.
- Durham, S. R., Ying, S., Varney, V. A., Jacobson, M. R., Sudderick, R. M., Mackay, I. S., et al. (1996). Grass pollen immunotherapy inhibits allergen-induced infiltration of CD4+ T lymphocytes and eosinophils in the nasal mucosa and increases the number of cells expressing messenger RNA for interferon-gamma. *J Allergy Clin Immunol* 97(6), 1356–1365.
- Ebo, D. G., Britts, D. H., Hagendorens, M. M., Aerts, N. E., De Clerck, L. S., & Stevens, W. J. (2008). Basophil activation test by flow cytometry: present and future applications in allergology. *Cytometry B Clin Cytom* 74(4), 201–210.
- Esquerre, M., Tauzin, B., Guiraud, M., Muller, S., Saoudi, A., & Valitutti, S. (2008). Human regulatory T cells inhibit polarization of T helper cells toward antigen-presenting cells via a TGF-beta-dependent mechanism. *Proc Natl Acad Sci U S A* 105(7), 2550–2555.
- Eusebius, N. P., Papalia, L., Suphioglu, C., McLellan, S. C., Varney, M., Rolland, J. M., et al. (2002). Oligoclonal analysis of the atopic T cell response to the group 1 allergen of *Cynodon dactylon* (bermuda grass) pollen: pre- and post-allergen-specific immunotherapy. *Int Arch Allergy Immunol* 127(3), 234–244.
- Fallarino, F., Grohmann, U., Hwang, K. W., Orabona, C., Vacca, C., Bianchi, R., et al. (2003). Modulation of tryptophan catabolism by regulatory T cells. *Nat Immunol* 4(12), 1206–1212.
- Fehervari, Z., & Sakaguchi, S. (2004). Control of Foxp3+ CD25+CD4+ regulatory cell activation and function by dendritic cells. *Int Immunol* 16(12), 1769–1780.
- Ferreira, F., Ebner, C., Kramer, B., Casari, G., Briza, P., Kungl, A. J., et al. (1998). Modulation of IgE reactivity of allergens by site-directed mutagenesis: potential use of hypoallergenic variants for immunotherapy. *FASEB J* 12(2), 231–242.
- Floistrup, H., Swartz, J., Bergstrom, A., Alm, J. S., Scheynius, A., van Hage, M., et al. (2006). Allergic disease and sensitization in Steiner school children. *J Allergy Clin Immunol* 117(1), 59–66.
- Fontenot, J. D., Gavin, M. A., & Rudensky, A. Y. (2003). Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat Immunol* 4(4), 330–336.
- Francis, J. N., Till, S. J., & Durham, S. R. (2003). Induction of IL-10+CD4+CD25+ T cells by grass pollen immunotherapy. *J Allergy Clin Immunol* 111(6), 1255–1261.
- Frew, A. J. (1993). Injection immunotherapy. British Society for Allergy and Clinical Immunology Working Party. *BMJ* 307(6909), 919–923.
- Frew, A. J. (2008). Sublingual immunotherapy. *N Engl J Med* 358(21), 2259–2264.
- Furmanovicene, R., Ghaemmaghami, A. M., Boyd, S. E., Jones, N. S., Bailey, K., Willis, A. C., et al. (2007). The protease allergen Der p 1 cleaves cell surface DC-SIGN and DC-SIGNR: experimental analysis of in silico substrate identification and implications in allergic responses. *Clin Exp Allergy* 37(2), 231–242.
- Garcia, G., Godot, V., & Humbert, M. (2005). New chemokine targets for asthma therapy. *Curr Allergy Asthma Rep* 5(2), 155–160.
- Gardner, L. M., O'Hehir, R. E., & Rolland, J. M. (2003). T cell targeted allergen derivatives for improved efficacy and safety of specific immunotherapy for allergic disease. *Curr Med Chem – Anti-inflammatory and Anti-Allergy Agents* 2, 351–365.
- Gardner, L. M., O'Hehir, R. E., & Rolland, J. M. (2004a). High dose allergen stimulation of T cells from house dust mite-allergic subjects induces expansion of IFN-gamma+ T cells, apoptosis of CD4+IL-4+ T cells and T cell anergy. *Int Arch Allergy Immunol* 133(1), 1–13.
- Gardner, L. M., Spyroglou, L., O'Hehir, R. E., & Rolland, J. M. (2004b). Increased allergen concentration enhances IFN-gamma production by allergic donor T cells expressing a peripheral tissue trafficking phenotype. *Allergy* 59(12), 1308–1317.
- Gardner, L. M., Thien, F. C., Douglass, J. A., Rolland, J. M., & O'Hehir, R. E. (2004c). Induction of T 'regulatory' cells by standardized house dust mite immunotherapy: an increase in CD4+ CD25+ interleukin-10+ T cells expressing peripheral tissue trafficking markers. *Clin Exp Allergy* 34(8), 1209–1219.
- Gerstmayr, M., Ilk, N., Schabussova, I., Jahn-Schmid, B., Egelseer, E. M., Sleytr, U. B., et al. (2007). A novel approach to specific allergy treatment: the recombinant allergen-S-layer fusion protein rSbsC-Bet v 1 matures dendritic cells that prime Th0/Th1 and IL-10-producing regulatory T cells. *J Immunol* 179(11), 7270–7275.
- Gidaro, G. B., Marcucci, F., Sensi, L., Incorvaia, C., Frati, F., & Ciprandi, G. (2005). The safety of sublingual-swallow immunotherapy: an analysis of published studies. *Clin Exp Allergy* 35(5), 565–571.
- Gleich, G. J., Zimmermann, E. M., Henderson, L. L., & Yunginger, J. W. (1982). Effect of immunotherapy on immunoglobulin E and immunoglobulin G antibodies to ragweed antigens: a six-year prospective study. *J Allergy Clin Immunol* 70(4), 261–271.
- Gough, L., Campbell, E., Bayley, D., Van Heeke, G., & Shakib, F. (2003). Proteolytic activity of the house dust mite allergen Der p 1 enhances allergenicity in a mouse inhalation model. *Clin Exp Allergy* 33(8), 1159–1163.
- Green, E. A., Choi, Y., & Flavell, R. A. (2002). Pancreatic lymph node-derived CD4(+)/CD25(+) Treg cells: highly potent regulators of diabetes that require TRANCE-RANK signals. *Immunity* 16(2), 183–191.
- Guerra, F., Carracedo, J., Solana-Lara, R., Sanchez-Guijo, P., & Ramirez, R. (2001). TH2 lymphocytes from atopic patients treated with immunotherapy undergo rapid apoptosis after culture with specific allergens. *J Allergy Clin Immunol* 107(4), 647–653.
- Gundlach, B. R., Wiesmuller, K. H., Junt, T., Kienle, S., Jung, G., & Walden, P. (1996). Determination of T cell epitopes with random peptide libraries. *J Immunol Methods* 192(1–2), 149–155.
- Haavik, S., Smestad Paulsen, B., & Wold, J. K. (1985). Glycoprotein allergens in pollen of timothy. I. Investigation of carbohydrates extracted from pollen of timothy (*Phleum pratense*) and purification of a carbohydrate-containing allergen by affinity chromatography on concanavalin A-sepharose. *Int Arch Allergy Appl Immunol* 78(2), 197–205.
- Hammad, H., de Heer, H. J., Soullie, T., Angeli, V., Trottein, F., Hoogsteden, H. C., et al. (2004). Activation of peroxisome proliferator-activated receptor-gamma in dendritic cells inhibits the development of eosinophilic airway inflammation in a mouse model of asthma. *Am J Pathol* 164(1), 263–271.
- Haselden, B. M., Kay, A. B., & Larche, M. (1999). Immunoglobulin E-independent major histocompatibility complex-restricted T cell peptide epitope-induced late asthmatic reactions. *J Exp Med* 189(12), 1885–1894.
- Hejjajoui, A., Dhiwert, H., Michel, F. B., & Bousquet, J. (1990). Immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract. IV. Systemic reactions according to the immunotherapy schedule. *J Allergy Clin Immunol* 85(2), 473–479.
- Higgins, J. A., Lamb, J. R., Marsh, S. G., Tonks, S., Hayball, J. D., Rosen-Bronson, S., et al. (1992). Peptide-induced nonresponsiveness of HLA-DP restricted human T cells reactive with *Dermatophagoides* spp. (house dust mite). *J Allergy Clin Immunol* 90(5), 749–756.
- Holm, J., Gajhede, M., Ferreras, M., Henriksen, A., Ipsen, H., Larsen, J. N., et al. (2004). Allergy vaccine engineering: epitope modulation of recombinant Bet v 1 reduces IgE binding but retains protein folding pattern for induction of protective blocking-antibody responses. *J Immunol* 173(8), 5258–5267.
- Horne, A. A., Nguyen, M. D., Ronaghy, A., Cinman, N., Verbeek, S., & Raz, E. (2000). DNA-based vaccination reduces the risk of lethal anaphylactic hypersensitivity in mice. *J Allergy Clin Immunol* 106(2), 349–356.
- Hsieh, K. H., & Ng, C. K. (1993). Increased plasma platelet-activating factor in children with acute asthmatic attacks and decreased in vivo and in vitro production of platelet-activating factor after immunotherapy. *J Allergy Clin Immunol* 91(2), 650–657.
- Huber, S., Schramm, C., Lehr, H. A., Mann, A., Schmitt, S., Becker, C., et al. (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(11), 6526–6531.
- Ismaili, J., Rennesson, J., Aksoy, E., Vekemans, J., Vincart, B., Amraoui, Z., et al. (2002). Monophosphoryl lipid A activates both human dendritic cells and T cells. *J Immunol* 168(2), 926–932.
- Jacobsen, L., Niggemann, B., Dreborg, S., Ferdousi, H. A., Halken, S., Host, A., et al. (2007). Specific immunotherapy has long-term preventive effect of seasonal and perennial asthma: 10-year follow-up on the PAT study. *Allergy* 62(8), 943–948.
- Jaffar, Z. H., Stanciu, L., Pandit, A., Lordan, J., Holgate, S. T., & Roberts, K. (1999). Essential role for both CD80 and CD86 costimulation, but not CD40 interactions, in allergen-induced Th2 cytokine production from asthmatic bronchial tissue: role for alphabeta, but not gammadelta, T cells. *J Immunol* 163(11), 6283–6291.
- James, L. K., & Durham, S. R. (2008). Update on mechanisms of allergen injection immunotherapy. *Clin Exp Allergy* 38(7), 1074–1088.
- Joint Task Force on Practice Parameters. (2007). Allergen immunotherapy: a practice parameter second update. *J Allergy Clin Immunol* 120, S25–S85.
- Jutel, M., Akdis, M., Blaser, K., & Akdis, C. A. (2006). Mechanisms of allergen specific immunotherapy—T-cell tolerance and more. *Allergy* 61(7), 796–807.
- Jutel, M., Pichler, W. J., Skrbic, D., Urwyler, A., Dahinden, C., & Muller, U. R. (1995). Bee venom immunotherapy results in decrease of IL-4 and IL-5 and increase of IFN-gamma secretion in specific allergen-stimulated T cell cultures. *J Immunol* 154(8), 4187–4194.
- Kabelitz, D., Pohl, T., & Pechhold, K. (1993). Activation-induced cell death (apoptosis) of mature peripheral T lymphocytes. *Immunol Today* 14(7), 338–339.
- Kapsenberg, M. L. (2003). Dendritic-cell control of pathogen-driven T-cell polarization. *Nat Rev Immunol* 3(12), 984–993.
- Kapsenberg, M. L., Jansen, H. M., Bos, J. D., & Wierenga, E. A. (1992). Role of type 1 and type 2 T helper cells in allergic diseases. *Curr Opin Immunol* 4(6), 788–793.
- Karagiannidis, C., Akdis, M., Holopainen, P., Woolley, N. J., Hense, G., Ruckert, B., et al. (2004). Glucocorticoids upregulate FOXP3 expression and regulatory T cells in asthma. *J Allergy Clin Immunol* 114(6), 1425–1433.
- Karlsson, M. R., Rugtveit, J., & Brandtzaeg, P. (2004). Allergen-responsive CD4+CD25+ regulatory T cells in children who have outgrown cow's milk allergy. *J Exp Med* 199(12), 1679–1688.
- Kauppinen, J., Zeiler, T., Rautiainen, J., Rytönen-Nissinen, M., Taivainen, A., Mantylarvi, R., et al. (1999). Mutant derivatives of the main respiratory allergen of cow are less allergenic than the intact molecule. *Clin Exp Allergy* 29(7), 989–996.
- Kay, A. B. (2001). Allergy and allergic diseases. First of two parts. *N Engl J Med* 344(1), 30–37.

- Keane-Myers, A. M., Gause, W. C., Finkelman, F. D., Xhou, X. D., & Wills-Karp, M. (1998). Development of murine allergic asthma is dependent upon B7-2 costimulation. *J Immunol* 160(2), 1036–1043.
- Kleine-Tebbe, J., Ribel, M., & Herold, D. A. (2006). Safety of a SQ-standardised grass allergen tablet for sublingual immunotherapy: a randomized, placebo-controlled trial. *Allergy* 61(2), 181–184.
- Kline, J. N. (2007). Eat dirt: CpG DNA and immunomodulation of asthma. *Proc Am Thorac Soc* 4(3), 283–288.
- Klinman, D. M. (2004). Immunotherapeutic uses of CpG oligodeoxynucleotides. *Nat Rev Immunol* 4(4), 249–258.
- Kontou-Fili, K. (2008). High omalizumab dose controls recurrent reactions to venom immunotherapy in indolent systemic mastocytosis. *Allergy* 63(3), 376–378.
- Korematsu, S., Tanaka, Y., Hosoi, S., Koyanagi, S., Yokota, T., Mikami, B., et al. (2000). C8/119S mutation of major mite allergen Derf-2 leads to degenerate secondary structure and molecular polymerization and induces potent and exclusive Th1 cell differentiation. *J Immunol* 165(5), 2895–2902.
- Kremer, J. M., Dougados, M., Emery, P., Durez, P., Sibilia, J., Shergy, W., et al. (2005). Treatment of rheumatoid arthritis with the selective costimulation modulator abatacept: twelve-month results of a phase iib, double-blind, randomized, placebo-controlled trial. *Arthritis Rheum* 52(8), 2263–2271.
- Kubo, T., Hatton, R. D., Oliver, J., Liu, X., Elson, C. O., & Weaver, C. T. (2004). Regulatory T cell suppression and anergy are differentially regulated by proinflammatory cytokines produced by TLR-activated dendritic cells. *J Immunol* 173(12), 7249–7258.
- La Grutta, S., Arena, A., D'Anneo, W. R., Gammeri, E., Leonardi, S., Trimarchi, A., et al. (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(2), 40–44.
- Lamb, J. R., Skidmore, B. J., Green, N., Chiller, J. M., & Feldmann, M. (1983). Induction of tolerance in influenza virus-immune T lymphocyte clones with synthetic peptides of influenza hemagglutinin. *J Exp Med* 157(5), 1434–1447.
- Larche, M. (2007). Peptide immunotherapy for allergic diseases. *Allergy* 62(3), 325–331.
- Larche, M., Akdis, C. A., & Valenta, R. (2006). Immunological mechanisms of allergen-specific immunotherapy. *Nat Rev Immunol* 6(10), 761–771.
- Lerch, E., & Muller, U. R. (1998). Long-term protection after stopping venom immunotherapy: results of re-stings in 200 patients. *J Allergy Clin Immunol* 101(5), 606–612.
- Lin, H., Boesel, K. M., Griffith, D. T., Prussin, C., Foster, B., Romero, F. A., et al. (2004). Omalizumab rapidly decreases nasal allergic response and FcεpsilonRI on basophils. *J Allergy Clin Immunol* 113(2), 297–302.
- Lim, E. M., Smith, T., Nguyen, X. D., Pridgeon, C., Dallman, M., Arbery, J., et al. (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(9409), 608–615.
- Lombardi, V., Van Overtvelt, L., Horiot, S., Moussu, H., Chabre, H., Louise, A., et al. (in press). Toll-like receptor 2 agonist Pam3CSK4 enhances the induction of antigen-specific Th17 tolerance via the sublingual route. *Clin Exp Allergy*. [Jul 17, Electronic publication for ahead of print].
- Lutz, M. B., & Schuler, G. (2002). Immature, semi-mature and fully mature dendritic cells: which signals induce tolerance or immunity? *Trends Immunol* 23(9), 445–449.
- Magone, M. T., Chan, C. C., Beck, L., Whitcup, S. M., & Raz, E. (2000). Systemic or mucosal administration of immunostimulatory DNA inhibits early and late phases of murine allergic conjunctivitis. *Eur J Immunol* 30(7), 1841–1850.
- Mahnke, K., Bedke, T., & Enk, A. H. (2008). Regulatory conversation between antigen presenting cells and regulatory T cells enhance immune suppression. *Cell Immunol*.
- Malling, H. J., Abreu-Nogueira, J., Alvarez-Cuesta, E., Bjorksten, B., Bousquet, J., Caillot, D., et al. (1998). Local immunotherapy. *Allergy* 53(10), 933–944.
- Malling, H. J., Lund, L., Ipsen, H., & Poulsen, L. (2006). Safety and immunological changes during sublingual immunotherapy with standardized quality grass allergen tablets. *J Investig Allergol Clin Immunol* 16(3), 162–168.
- Mannering, S. I., Morris, J. S., Jensen, K. P., Purcell, A. W., Honeyman, M. C., van Endert, P. M., et al. (2003). A sensitive method for detecting proliferation of rare autoantigen-specific human T cells. *J Immunol Methods* 283(1–2), 173–183.
- Marone, G., Rossi, F. W., Detoraki, A., Granata, F., Genovese, A., & Spadaro, G. (2007). Role of superallergens in allergic disorders. *Chem Immunol Allergy* 93, 195–213.
- Marsh, D. G., Norman, P. S., Roebber, M., & Lichtenstein, L. M. (1981). Studies on allergoids from naturally occurring allergens. III. Preparation of ragweed pollen allergoids by aldehyde modification in two steps. *J Allergy Clin Immunol* 68(6), 449–459.
- Masoli, M., Fabian, D., Holt, S., & Beasley, R. (2004). The global burden of asthma: executive summary of the GINA Dissemination Committee report. *Allergy* 59(5), 469–478.
- McHugh, R. S., Whitters, R. J., Piccirillo, C. A., Young, D. A., Shevach, E. M., Collins, M., et al. (2002). CD4+CD25(+) immunoregulatory T cells: gene expression analysis reveals a functional role for the glucocorticoid-induced TNF receptor. *Immunity* 16(2), 311–323.
- Mills, K. H. (2004). Regulatory T cells: friend or foe in immunity to infection? *Nat Rev Immunol* 4(11), 841–855.
- Mistrello, G., Brenna, O., Roncarolo, D., Zanoni, D., Gentili, M., & Falagiani, P. (1996). Monomeric chemically modified allergens: immunologic and physicochemical characterization. *Allergy* 51(1), 8–15.
- Moingeon, P., Batard, T., Fadel, R., Frati, F., Sieber, J., & Van Overtvelt, L. (2006). Immune mechanisms of allergen-specific sublingual immunotherapy. *Allergy* 61(2), 151–165.
- Moller, C., Dreborg, S., Ferdousi, H. A., Halken, S., Host, A., Jacobsen, L., et al. (2002). Pollen immunotherapy reduces the development of asthma in children with seasonal rhinoconjunctivitis (the PAT-study). *J Allergy Clin Immunol* 109(2), 251–256.
- Mollereau, B., Blanchard, D., Deas, O., Dumont, C., Metivier, D., Bernard, A., et al. (1997). Relationship between proliferation and susceptibility to CD95- and CD2-mediated apoptosis in stimulated primary T lymphocytes: T cells manifesting proliferative unresponsiveness are preferentially susceptible to CD95-mediated apoptosis. *J Immunol* 159(6), 2668–2677.
- Mori, A., Suko, M., Nishizaki, Y., Kaminuma, O., Kobayashi, S., Matsuzaki, G., et al. (1995). IL-5 production by CD4+ T cells of asthmatic patients is suppressed by glucocorticoids and the immunosuppressants FK506 and cyclosporin A. *Int Immunol* 7(3), 449–457.
- Mueller, D. L., Jenkins, M. K., & Schwartz, R. H. (1989). An accessory cell-derived costimulatory signal acts independently of protein kinase C activation to allow T cell proliferation and prevent the induction of unresponsiveness. *J Immunol* 142(8), 2617–2628.
- Muller, U., Helbling, A., & Bischof, M. (1989). Predictive value of venom-specific IgE, IgG and IgG subclass antibodies in patients on immunotherapy with honey bee venom. *Allergy* 44(6), 412–418.
- Murphy, K. M., & Reiner, S. L. (2002). The lineage decisions of helper T cells. *Nat Rev Immunol* 2(12), 933–944.
- Nasser, S. M., Ying, S., Meng, Q., Kay, A. B., & Ewan, P. W. (2001). Interleukin-10 levels increase in cutaneous biopsies of patients undergoing wasp venom immunotherapy. *Eur J Immunol* 31(12), 3704–3713.
- Negro, J. M., Wheeler, A. W., Hernandez, J., Youten, L. J., Pascual, A., Garcia-Selles, F. J., et al. (1999). Comparison of the efficacy and safety of two preseasonal regimens of glutaraldehyde modified, tyrosine-adsorbed *parietaria* pollen extract over a period of three years in monosensitive patients. *Allergol Immunopathol (Madr)* 27(3), 153–164.
- Nelson, H. S. (2007). Allergen immunotherapy: where is it now? *J Allergy Clin Immunol* 119(4), 769–779.
- Noon, L. (1911). Prophylactic inoculation against hayfever. *Lancet* 1, 1572–1573.
- O'Garra, A., & Vieira, P. (2004). Regulatory T cells and mechanisms of immune system control. *Nat Med* 10(8), 801–805.
- O'Hehir, R. E., Garman, R. D., Greenstein, J. L., & Lamb, J. R. (1991a). The specificity and regulation of T-cell responsiveness to allergens. *Annu Rev Immunol* 9, 67–95.
- O'Hehir, R. E., Yssel, H., Verma, S., de Vries, J. E., Spits, H., & Lamb, J. R. (1991b). Clonal analysis of differential lymphokine production in peptide and superantigen induced T cell anergy. *Int Immunol* 3(8), 819–826.
- O'Hehir, R. E., Askonas, B. A., & Lamb, J. R. (1993a). *Cell culture: lymphocyte clones*, Vol. 3. Heidelberg: Springer-Verlag.
- O'Hehir, R. E., Hoyne, G. F., Thomas, W. R., & Lamb, J. R. (1993b). House dust mite allergy: from T-cell epitopes to immunotherapy. *Eur J Clin Invest* 23(12), 763–772.
- O'Hehir, R. E., Sandrini, A., Anderson, G. P., & Rolland, J. M. (2007). Sublingual allergen immunotherapy: immunological mechanisms and prospects for refined vaccine preparation. *Curr Med Chem* 14(21), 2235–2244.
- Okada, T., Swoboda, I., Bhalla, P. L., Toriyama, K., & Singh, M. B. (1998). Engineering of hypoallergenic mutants of the *Brassica* pollen allergen, Bra 1, for immunotherapy. *FEBS Lett* 434(3), 255–260.
- Oldfield, W. L., Larche, M., & Kay, A. B. (2002). Effect of T-cell peptides derived from Fel d 1 on allergic reactions and cytokine production in patients sensitive to cats: a randomised controlled trial. *Lancet* 360(9326), 47–53.
- Olsson, S., van Hage-Hamsten, M., & Whitley, P. (1998). Contribution of disulphide bonds to antigenicity of Lep d 2, the major allergen of the dust mite *Lepidoglyphus destructor*. *Mol Immunol* 35(16), 1017–1023.
- Pandiyani, P., Zheng, L., Ishihara, S., Reed, J., & Lenardo, M. J. (2007). CD4+CD25+Foxp3+ regulatory T cells induce cytokine deprivation-mediated apoptosis of effector CD4+ T cells. *Nat Immunol* 8(12), 1353–1362.
- Passalacqua, G., Albano, M., Fregonese, L., Riccio, A., Pronzato, C., Mela, G. S., et al. (1998). Randomised controlled trial of local allergoid immunotherapy on allergic inflammation in mite-induced rhinoconjunctivitis. *Lancet* 351(9103), 629–632.
- Passalacqua, G., Guerra, L., Compalati, E., & Canonica, G. W. (2007). The safety of allergen specific sublingual immunotherapy. *Curr Drug Saf* 2(2), 117–123.
- Patel, P., & Salapatek, A. M. (2006). Pollinex Quattro: a novel and well-tolerated, ultra short-course allergy vaccine. *Expert Rev Vaccines* 5(5), 617–629.
- Pereira-Santos, M. C., Baptista, A. P., Melo, A., Alves, R. R., Soares, R. S., Pedro, E., et al. (2008). Expansion of circulating Foxp3+CD25bright CD4+ T cells during specific venom immunotherapy. *Clin Exp Allergy* 38(2), 291–297.
- Pilette, C., Nouri-Aria, K. T., Jacobson, M. R., Wilcock, L. K., Detry, B., Walker, S. M., et al. (2007). Grass pollen immunotherapy induces an allergen-specific IgA2 antibody response associated with mucosal TGF-β expression. *J Immunol* 178(7), 4658–4666.
- Puggioni, F., Durham, S. R., & Francis, J. N. (2005). Monophosphoryl lipid A (MPL) promotes allergen-induced immune deviation in favour of Th1 responses. *Allergy* 60(5), 678–684.
- Purello-D'Ambrosio, F., Gangemi, S., Merendino, R. A., Isola, S., Puccinelli, P., Parmiani, S., et al. (2001). Prevention of new sensitizations in monosensitized subjects submitted to specific immunotherapy or not. A retrospective study. *Clin Exp Allergy* 31(8), 1295–1302.
- Rabjohn, P., West, C. M., Connaughton, C., Sampson, H. A., Helm, R. M., Burks, A. W., et al. (2002). Modification of peanut allergen Ara h 3: effects on IgE binding and T cell stimulation. *Int Arch Allergy Immunol* 128(1), 15–23.
- Renz, H. (2004). Usefulness of mycobacteria in redirecting the immune response in atopic disease. *Clin Exp Allergy* 34(2), 167–169.
- Rienzo, V. D., Minelli, M., Musarra, A., Sambugaro, R., Pecora, S., Canonica, W. G., et al. (2005). Post-marketing survey on the safety of sublingual immunotherapy in children below the age of 5 years. *Clin Exp Allergy* 35(5), 560–564.
- Rolland, J., & O'Hehir, R. (1998). Immunotherapy of allergy: anergy, deletion, and immune deviation. *Curr Opin Immunol* 10(6), 640–645.
- Rolland, J. M., & O'Hehir, R. E. (2008). Latex allergy: a model for therapy. *Clin Exp Allergy* 38(6), 898–912.
- Rolland, J. M., Douglass, J., & O'Hehir, R. E. (2000). Allergen immunotherapy: current and new therapeutic strategies. *Expert Opin Investig Drugs* 9(3), 515–527.
- Romagnani, S. (1994). Lymphokine production by human T cells in disease states. *Annu Rev Immunol* 12, 227–257.
- Sakaguchi, S. (2000). Regulatory T cells: key controllers of immunologic self-tolerance. *Cell* 101(5), 455–458.

- Sakaguchi, S., Ono, M., Setoguchi, R., Yagi, H., Hori, S., Fehervari, Z., et al. (2006). Foxp3+ CD25+ CD4+ natural regulatory T cells in dominant self-tolerance and autoimmune disease. *Immunol Rev* 212, 8–27.
- Scheffold, A., Huhn, J., & Hofer, T. (2005). Regulation of CD4+CD25+ regulatory T cell activity: it takes (IL-)two to tango. *Eur J Immunol* 35(5), 1336–1341.
- Schramm, G., Kahlert, H., Suck, R., Weber, B., Stuwe, H. T., Muller, W. D., et al. (1999). "Allergen engineering": variants of the timothy grass pollen allergen Phl p 5b with reduced IgE-binding capacity but conserved T cell reactivity. *J Immunol* 162(4), 2406–2414.
- Schulze, J., Rose, M., & Zielen, S. (2007). Beekeepers anaphylaxis: successful immunotherapy covered by omalizumab. *Allergy* 62(8), 963–964.
- Shakib, F., Schulz, O., & Sewell, H. (1998). A mite subversive: cleavage of CD23 and CD25 by Der p 1 enhances allergenicity. *Immunol Today* 19(7), 313–316.
- Shirakawa, T., Enomoto, T., Shimazu, S., & Hopkin, J. M. (1997). The inverse association between tuberculin responses and atopic disorder. *Science* 275(5296), 77–79.
- Singh, H., & Raghava, G. P. (2001). ProPred: prediction of HLA-DR binding sites. *Bioinformatics* 17(12), 1236–1237.
- Singh, M. B., de Weerd, N., & Bhalla, P. L. (1999). Genetically engineered plant allergens with reduced anaphylactic activity. *Int Arch Allergy Immunol* 119(2), 75–85.
- Smith, A. M., Chapman, M. D., Taketomi, E. A., Platts-Mills, T. A., & Sung, S. S. (1998). Recombinant allergens for immunotherapy: a Der p 2 variant with reduced IgE reactivity retains T-cell epitopes. *J Allergy Clin Immunol* 101(3), 423–425.
- Soriano Gomis, V., Gonzalez Delgado, P., & Niveiro Hernandez, E. (2008). Failure of omalizumab treatment after recurrent systemic reactions to bee-venom immunotherapy. *J Invest Allergol Clin Immunol* 18(3), 225–226.
- Spangfort, M. D. (2003). Engineering and validation of recombinant proteins for allergen vaccines. *Arb Paul Ehrlich Inst Bundesamt Sera Impfstoffe Frankfurt A M* 94, 151–154.
- Sparbier, K., & Walden, P. (1999). T cell receptor specificity and mimotopes. *Curr Opin Immunol* 11(2), 214–218.
- Spiewak, R., Kryszyska-Traczyk, E., Sitkowska, J., & Dutkiewicz, J. (1996). Microflora of allergenic pollens – a preliminary study. *Ann Agric Environ Med* 3(2), 127–130.
- Stewart, G. A., & Robinson, C. (2003). The immunobiology of allergenic peptidases. *Clin Exp Allergy* 33(1), 3–6.
- Storni, T., Ruedl, C., Schwarz, K., Schwendener, R. A., Renner, W. A., & Bachmann, M. F. (2004). Nonmethylated CG motifs packaged into virus-like particles induce protective cytotoxic T cell responses in the absence of systemic side effects. *J Immunol* 172(3), 1777–1785.
- Swoboda, I., De Weerd, N., Bhalla, P. L., Niederberger, V., Sperr, W. R., Valent, P., et al. (2002). Mutants of the major ryegrass pollen allergen, Lol p 5, with reduced IgE-binding capacity: candidates for grass pollen-specific immunotherapy. *Eur J Immunol* 32(1), 270–280.
- Tang, Q., & Bluestone, J. A. (2006). Plasmacytoid DCs and T(reg) cells: casual acquaintance or monogamous relationship? *Nat Immunol* 7(6), 551–553.
- Tang, Q., & Bluestone, J. A. (2008). The Foxp3+ regulatory T cell: a jack of all trades, master of regulation. *Nat Immunol* 9(3), 239–244.
- Thunberg, S., Akdis, M., Akdis, C. A., Gronneberg, R., Malmstrom, V., Trollmo, C., et al. (2007). Immune regulation by CD4+CD25+ T cells and interleukin-10 in birch pollen-allergic patients and non-allergic controls. *Clin Exp Allergy* 37(8), 1127–1136.
- Traidl-Hoffmann, C., Mariani, V., Hochrein, H., Karg, K., Wagner, H., Ring, J., et al. (2005). Pollen-associated phytoprostanes inhibit dendritic cell interleukin-12 production and augment T helper type 2 cell polarization. *J Exp Med* 201(4), 627–636.
- Tsuyuki, S., Tsuyuki, J., Einsle, K., Kopf, M., & Coyle, A. J. (1997). Costimulation through B7-2 (CD86) is required for the induction of a lung mucosal T helper cell 2 (TH2) immune response and altered airway responsiveness. *J Exp Med* 185(9), 1671–1679.
- Turcanu, V., Maleki, S. J., & Lack, G. (2003). Characterization of lymphocyte responses to peanuts in normal children, peanut-allergic children, and allergic children who acquired tolerance to peanuts. *J Clin Invest* 111(7), 1065–1072.
- Valenta, R., & Niederberger, V. (2007). Recombinant allergens for immunotherapy. *J Allergy Clin Immunol* 119(4), 826–830.
- van der Heijden, F. L., Joost van Neerven, R. J., van Katwijk, M., Bos, J. D., & Kapsenberg, M. L. (1993). Serum-IgE-facilitated allergen presentation in atopic disease. *J Immunol* 150(8 Pt 1), 3643–3650.
- van Neerven, R. J., Wikborg, T., Lund, G., Jacobsen, B., Brinch-Nielsen, A., Arned, J., et al. (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(5), 2944–2952.
- Varney, V. A., Gaga, M., Frew, A. J., Aber, V. R., Kay, A. B., & Durham, S. R. (1991). Usefulness of immunotherapy in patients with severe summer hay fever uncontrolled by antiallergic drugs. *BMJ* 302(6771), 265–269.
- Varney, V. A., Hamid, Q. A., Gaga, M., Ying, S., Jacobson, M., Frew, A. J., et al. (1993). Influence of grass pollen immunotherapy on cellular infiltration and cytokine mRNA expression during allergen-induced late-phase cutaneous responses. *J Clin Invest* 92(2), 644–651.
- Venkataraman, C., Leung, S., Salvekar, A., Mano, H., & Schindler, U. (1999). Repression of IL-4-induced gene expression by IFN-gamma requires Stat1 activation. *J Immunol* 162(7), 4053–4061.
- Verdino, P., Barderas, R., Villalba, M., Westritschnig, K., Valenta, R., Rodriguez, R., et al. (2008). Three-dimensional structure of the cross-reactive pollen allergen Che a 3: visualizing cross-reactivity on the molecular surfaces of weed, grass, and tree pollen allergens. *J Immunol* 180(4), 2313–2321.
- Vervloet, D., Birnbaum, J., Laurent, P., Hugues, B., Fardeau, M. F., Massabie-Bouchat, Y. P., et al. (2007). Safety and efficacy of *Juniperus ashei* sublingual-swallow ultra-rush pollen immunotherapy in cypress rhinoconjunctivitis. A double-blind, placebo-controlled study. *Int Arch Allergy Immunol* 142(3), 239–246.
- Vrtala, S., Akdis, C. A., Budak, F., Akdis, M., Blaser, K., Kraft, D., et al. (2000). T cell epitope-containing hypoallergenic recombinant fragments of the major birch pollen allergen, Bet v 1, induce blocking antibodies. *J Immunol* 165(11), 6653–6659.
- Vrtala, S., Hirtenlehner, K., Susani, M., Hufnagl, P., Binder, B. R., Vangelista, L., et al. (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(2–4), 218–219.
- Wachholz, P. A., & Durham, S. R. (2003). Induction of 'blocking' IgG antibodies during immunotherapy. *Clin Exp Allergy* 33(9), 1171–1174.
- Walker, S. M., Pajno, G. B., Lima, M. T., Wilson, D. R., & Durham, S. R. (2001). Grass pollen immunotherapy for seasonal rhinitis and asthma: a randomized, controlled trial. *J Allergy Clin Immunol* 107(1), 87–93.
- Walunas, T. L., Bakker, C. Y., & Bluestone, J. A. (1996). CTLA-4 ligation blocks CD28-dependent T cell activation. *J Exp Med* 183(6), 2541–2550.
- Wang, C. C., & Rook, G. A. (1998). Inhibition of an established allergic response to ovalbumin in BALB/c mice by killed *Mycobacterium vaccae*. *Immunology* 93(3), 307–313.
- Warner, J. O., Price, J. F., Soothill, J. F., & Hey, E. N. (1978). Controlled trial of hyposensitisation to *Dermatophagoides pteronyssinus* in children with asthma. *Lancet* 2(8096), 912–915.
- Watanabe, T., Masuyama, J., Sohma, Y., Inazawa, H., Horie, K., Kojima, K., et al. (2006). CD52 is a novel costimulatory molecule for induction of CD4+ regulatory T cells. *Clin Immunol* 120(3), 247–259.
- Wilson, D. R., Lima, M. T., & Durham, S. R. (2005). Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. *Allergy* 60(1), 4–12.
- Yagami, T., Osuna, H., Kouno, M., Haishima, Y., Nakamura, A., & Ikezawa, Z. (2002). Significance of carbohydrate epitopes in a latex allergen with beta-1,3-glucanase activity. *Int Arch Allergy Immunol* 129(1), 27–37.
- Yamazaki, S., Iyoda, T., Tarbell, K., Olson, K., Velinzon, K., Inaba, K., et al. (2003). Direct expansion of functional CD25+ CD4+ regulatory T cells by antigen-processing dendritic cells. *J Exp Med* 198(2), 235–247.
- Yazi, D., Akkoc, T., Yesil, O., Ozdemir, C., Aydogan, M., Koksalan, K., et al. (2008). Treatment with *Mycobacterium vaccae* ameliorates airway histopathology in a murine model of asthma. *Allergy Asthma Proc* 29(1), 67–73.
- Zeiler, T., Taivainen, A., Rytönen, M., Rautiainen, J., Karjalainen, H., Mantylä, R., et al. (1997). Recombinant allergen fragments as candidate preparations for allergen immunotherapy. *J Allergy Clin Immunol* 100(6 Pt 1), 721–727.
- Zuany-Amorim, C., Sawicka, E., Manlius, C., Le Moine, A., Brunet, L. R., Kemeny, D. M., et al. (2002). Suppression of airway eosinophilia by killed *Mycobacterium vaccae*-induced allergen-specific regulatory T-cells. *Nat Med* 8(6), 625–629.