High-Dose Allergen Exposure Leads to Tolerance

Judith A. Woodfolk

Asthma and Allergic Diseases Center, University of Virginia Health System, Charlottesville, VA;
E-mail: jaw4m@virginia.edu.

Abstract

Reports of decreased sensitization to cat allergen (Fel d 1) among individuals living with a cat or subjects exposed to high-dose cat allergen may be explained by the development of a form of high-dose tolerance resulting from natural exposure to an inhalant allergen. Although the epidemiological data regarding the relationship between exposure and sensitization to Fel d 1 are conflicting, the ability for high-dose Fel d 1 to induce a characteristic nonallergic immune response with a distinctive serum antibody profile has been established. Definition of this modified T-helper (Th)2 response to cat allergen, coupled with the renewed interest in regulatory T cells within the immunology field, has provided an avenue for exploring the mechanism by which IgE antibody-mediated responses are controlled. There is mounting evidence to suggest that the modified Th2 response is a variation of the allergic response and that the modified Th2-allergic axis is influenced by allergen dose and genetics. This article discusses putative immune mechanisms of tolerance within the context of an allergen-specific system. The relevance of high-dose allergen exposure and alternate factors such as endotoxin to the development of tolerance is considered. Fel d 1 exhibits unique molecular and immunological characteristics that may contribute to its tolerogenic properties. Major T-cell epitopes of Fel d 1 that preferentially induce regulatory factors have been defined. Furthermore, high-titer IgE antibody responses associated with atopic dermatitis are characterized by a defect in the T-cell repertoire that is specific to these epitopes. Identification of Fel d 1 epitopes that induce interleukin-10 may provide new targets for treatment.

Index Entries

Cat allergen; regulatory T cell; interleukin-10; T helper 1; T helper 2; tolerance.

Relationship Between Exposure to Cat Allergen and Sensitization

In the late 1990s, several studies reported a negative association between exposure to cats and sensitization *(1–4)*. Soon thereafter, decreased sensitization among children exposed to high-dose cat allergen (≥4.4 μg Fel d 1 per gram floor dust) was reported *(5,6)*. High-dose exposure to Fel d 1 was associated with an immune response characterized by high-titer IgG and IgG4 antibodies (Abs) to Fel d 1 without IgE and a lack of allergic symptoms. This immune response was described as a modified Th2 response based on the presence of the interleukin (IL)-4-dependent antibody IgG4. These studies have generated much debate regarding the role of cats, as well as domestic animals in general, in influencing the allergic response. Because of studies that have reported no effect of cat exposure on sensitization or an increased risk for developing IgE, the conflicting data on the exposure–sensitization relationship are difficult to reconcile *(7–16)*. This is in large part because of the different study designs used and the patient populations examined. For example, in Sweden (where cat allergen is the major source of indoor allergen), reports of decreased asthma among subjects with IgG4 Abs to cat compared to those with IgE Abs are consistent with the view that exposure to cat allergen induces clinical tolerance *(17)*. In another report from Sweden, living with a cat was inversely related both to sensitization and incidence of physician-diagnosed asthma *(18)*. It could be argued that this effect on asthma is the result of the dominant effects of cat allergen in this environment. In the United Kingdom, cat ownership has been associated with a reduced prevalence of sensitization to cat and dog, but not mite and pollen, among adults *(19)*. Adding to the complexity of this issue, in a study performed in the United States, children exposed to two or more dogs or cats in the first year of life exhibited a reduced risk of allergic sensitization to both indoor and outdoor allergens *(20)*. The implication is that exposure to cat influences the immune response to other allergens; however, the effects are not consistent. A detailed review of these studies is beyond the scope of this article; however, the variable findings raise some important issues. First, measurement of Fel d 1 in houses often is not performed, making it difficult to evaluate what actually constitutes high-dose exposure among the populations studied. Second, because the allergen environment is highly dependent on the geographic region studied, it is difficult to draw a comparison between studies performed at disparate sites. Third, any adjuvant effects resulting from co-exposure to immunomodulatory factors within the environment (e.g., chemical pollutants or endotoxin) often are not addressed.

Questions regarding the nature of the dose–response relationship to cat allergen are valid based on existing epidemiological data. Despite this, there is substantial evidence that high-dose exposure to cat allergen can induce a characteristic immune response that is not associated with allergic symptoms. For cat allergen to induce high-dose respiratory tolerance by natural exposure, both the timing and duration of exposure to allergen are likely to be critical to immune outcome. Levels of Fel d 1 in house dust may be up to 10-fold higher than the highest levels for allergens derived from dust mite *(6,21–24)*. Furthermore, cat allergen is easily rendered airborne, and, in contrast to mite allergen, it persists in the air even in undisturbed conditions because of its presence on small particles (<5 μm diameter) *(23,25,26)*. The aerodynamic properties coupled with the high allergen load in houses may facilitate efficient delivery of high-dose allergen to the respiratory tract and induction of tolerance.

What Constitutes Tolerance to an Allergen?

Classical immunological tolerance is defined as an active state of unresponsiveness by lym-

phoid cells. In this context, tolerance is an antigen-specific phenomenon that serves to prevent damage to the host (i.e., self-tolerance) while retaining immune responsiveness to foreign antigens. Three primary mechanisms of T-cell tolerance have been demonstrated, including: (a) clonal deletion with elimination of antigen-specific T cells within the thymus during development of the T-cell repertoire; (b) T-cell anergy, whereby engagement of the T-cell receptor by specific antigen results in an altered activation state that is characterized by failure to both secrete IL-2 and proliferate; and (c) induction of regulatory T (Tr) cells that act to suppress effector T-cell subsets, resulting in downregulation of the immune response. In the field of allergy, tolerance has often been used as a general term to describe a lack of allergic symptoms in subjects exposed to allergen (i.e., nonallergic subjects) or an improvement of allergic symptoms after conventional immunotherapy. The debate regarding whether a nonallergic response constitutes tolerance is ongoing. However, it seems likely that the nonallergic state represents the outcome of at least two different immune pathways. The heterogeneity of nonallergic responses observed in humans based on allergen-specific serum antibody profiles supports this statement. For example, some individuals who are not sensitized to the major cat allergen Fel d 1 exhibit no evidence of a serological response to Fel d 1 (i.e., $IgE^{neg} IgG^{neg}$), whereas in others, IgG Abs to Fel d 1 are readily detectable *(6)*.

Defining the mechanisms of allergen-specific tolerance is a considerable challenge because multiple factors may contribute to the nonallergic state. There is no doubt that during the initiation phase of the immune response to allergens, the antigen-presenting cell (APC) fulfills a central role in determining the nature of the T-cell response that is generated. In murine systems, the ability for dendritic cells (DCs) to prime distinct T-cell subsets (i.e., Th1 vs Th2) depends on the maturational stage of the DC and secretion of DC-derived factors

stimulated by adjuvants such as bacterial lipopolysaccharide (LPS; ref. *27*). Adding to the complex picture, in a murine model, co-exposure to aerosolized high-dose LPS and allergen (ovalbumin) may favor induction of Th1 cells, whereas low-dose LPS with allergen results in Th2 responses *(28)*.

Genetics is a major determinant of allergic disease, as evidenced by the linkage between asthma or elevated IgE and single nucleotide polymorphisms (SNPs) in multiple genes encoding molecules implicated in the allergic inflammatory cascade *(29)*. Furthermore, there is no doubt that numerous factors encountered within the environment (e.g., diesel particulates, bacteria, viruses) can alter immune responsiveness to allergens. Given this information, recent evidence has emerged to support the view that the ability to mount a tolerant response may be attributable to the dose of allergen received and properties of the allergen itself.

In 2001, the definition of the modified Th2 response to cat allergen became the first description of tolerance induced by natural exposure to an inhalant allergen. This immune response, which is characterized by the presence of IgG and IgG4 Abs to Fel d 1 in the absence of IgE, is not associated with allergic symptoms. Because of the high titers of IgG and IgG4 Abs that develop after systemic exposure to allergen during conventional immunotherapy, the modified Th2 response may reflect a naturally occurring variant of high-dose tolerance. For the modified Th2 response to fulfill the criteria for immunological tolerance, we would expect this phenomenon to be antigen-specific and to be mediated by clonal deletion, by T-cell anergy, or through the effects of Tr cells.

Tr Cells and the Allergic Response

Suppressed T-cell reactivity to allergens or to T-cell epitopes derived from allergens has been reported after exposure to systemic or inhaled allergens *(30–33)*. It seems unlikely that this arises from clonal deletion. For example,

in in vitro culture systems, altered T-cell responsiveness associated with injection of high-dose allergen often can be restored or increased by altering the cytokine micro-environment (*34*). Suppression of T-cell reactivity to noninjected peptides derived from cat allergen (Fel d 1) after administration of a peptide vaccine also supports an alternate mechanism (*35*). Because, in this context, clonal deletion of T cells requires ligation of the T-cell receptor by a specific peptide–major histocompatibility complex (MHC) complex, this process could not occur for T cells that are specific for peptides not included in the vaccine. We recently reported the presence of a T-cell epitope-specific defect in the immune response to cat allergen in patients with high-titer IgE Abs to Fel d 1 (*36*). This defect mapped to putative regulatory epitopes of the Fel d 1 molecule—that is, epitopes that selectively induced IL-10 and interferon (IFN)-γ. One interpretation of this is that targeted deletion of T-cell clones may actually contribute to development of allergic responses, rather than being relevant to tolerant responses. However, in this scenario, altered responsiveness to the regulatory cytokine IL-10 is a more likely explanation for these observations, as discussed in the section entitled High-Titer IgE Abs and Atopic Dermatitis: Evidence of Failure to Control.

In patients who are allergic to the major bee venom allergen, phospholipase A2 (PLA$_2$), administration of bee venom as part of an immunotherapy regimen resulted in diminished T-cell proliferation and reduced production of Th1 and Th2 cytokines to whole allergen as well as to PLA$_2$-derived peptides that contained T-cell epitopes (*31*). This T-cell hyporesponsiveness, or anergy, occurred rapidly (within 28 d) and was reversed by neutralization of IL-10 in vitro. The implication was that injection of high-dose allergen induced IL-10-dependent T-cell anergy. Similar observations were made in subjects who were not allergic who had received multiple bee stings. Subsequently, a mechanism for IL-10-mediated suppression was proposed in which IL-10 binding to its receptor inhibited CD28 tyrosine phosphorylation and phosphatidylinositol 3-kinase binding (*37*). Thus IL-10 may inhibit T-cell activation through effects on the CD28 CD-stimulatory pathway.

Although there is no doubt that changes in allergen-specific T-cell responses occur with conventional immunotherapy, these changes have not been well-characterized. Diminished T-cell proliferation to allergen and/or a shift in cytokine profile have been widely reported for allergens derived from diverse sources (*30–33, 38–42*). However, the data are conflicting. For example, we and others have reported little, if any, change in T-cell proliferation to allergen or allergen-derived peptides during immunotherapy (*43–45*). Furthermore, evidence of a Th2 to Th1 "switch" is variable (*31,46,47*). In contrast, consistent with studies of patients receiving immunotherapy for bee venom allergy, recent reports have documented increases in IL-10 after injection of allergens or peptides from other sources (*44,48,49*). In one study, circulating T cells derived from allergic patients produced elevated IL-10 after 1 yr of grass pollen immunotherapy, compared to atopic controls (*44*). In an extension of this study, increased local production of IL-10 in the nasal mucosa was reported after 2 yr of immunotherapy, but this occurred only during the pollen season (*48*).

Immunotherapy is associated with marked increases in the production of allergen-specific serum IgG and IgG4 Abs, with little or no change in the production of IgE Abs (*45,50–53*). This change in serum Ab profile during immunotherapy is consistent with a shift toward a modified Th2 phenotype (i.e., IgG^{hi}/IgG4^{hi}, IgE^{neg}). In our studies, analysis of T-cell responses to Fel d 1 peptides in five patients who were sensitized to cats who started immunotherapy for cat allergens revealed enhanced production of IL-10 as well as IFN-γ production to a region of the molecule that contained T-cell epitopes implicated in development of the modified Th2

response and control of the allergic response *(43)*. These observations raise two major questions:

1. What is the source of IL-10 associated with high-dose exposure to systemic or inhaled allergen?
2. How do these cells function to modify an established allergic response and induce tolerance?

There is strong evidence to support the argument that high-dose exposure to allergen, by either the systemic or inhaled route, can lead to tolerance. The mechanism by which this arises may be more elusive. In a murine model, repeated respiratory exposure to ovalbumin induced IL-10-producing DCs within the lung and the production of CD4$^+$ T cells that secreted IL-10 *(54)*. In an adoptive transfer model, these IL-10-producing DCs or CD4$^+$ T cells engineered to express IL-10 or transforming growth factor (TGF)-β were shown to inhibit allergen-induced airway hyper-reactivity *(55,56)*. These findings implicate a specialized subset of CD4$^+$ T cells (collectively referred to as Tr cells) in the control of inflammatory responses in the lung. Tr cells were first described based on their ability to inhibit the development of autoimmune disease and transplant rejection. In recent years, a putative role for these cells in regulation of IgE Ab-mediated responses has emerged. The assumption is that these cells suppress the immune response to both self-antigens (i.e., maintenance of self-tolerance) and foreign antigens (which may include allergens) in the periphery by exerting an effect on T cells that have the potential to respond to these antigens. Such pathogenic autoreactive or allergen-specific T cells are an integral component of the T-cell repertoire, and regulation of their reactivity is pivotal to the maintenance of immune homeostasis.

Several types of CD4$^+$ Tr cells have been described based on their cytokine profile and functional properties. Th3 cells, which secrete high amounts of TGF-β, can be induced in the gut after oral administration of antigen *(57)*. These cells have been implicated in the suppression of a spectrum of autoimmune diseases *(58)*. In contrast, Tr type 1 (Tr1) cells, which secrete high levels of IL-10, were shown to suppress pathogenic autoreactive T cells in a murine model of colitis *(59)*. Subsequent studies showed that OVA-specific Tr1 cells inhibited priming of Th2 cells, suggesting that they may modulate allergic responses *(60)*.

CD4$^+$ T cells that constitutively express the IL-2 receptor α-chain (CD25) comprise another type of Tr cell. These cells are produced by the thymus as a functionally mature subpopulation, and they act in the periphery to maintain tolerance to self-antigens. Similar to the Tr1 subset, these cells exhibit a low proliferative capacity after antigenic stimulation; however, in contrast to Tr1 cells, they exert their effect in a manner that depends on both secretion of TGF-β and cell contact *(61)*. Because TGF-β is also produced by Tr1 cells, the distinction between Tr1 and CD25$^+$CD4$^+$ Tr cells was imprecise. However, recent observations suggest that CD25$^+$CD4$^+$ T cells that produce TGF-β are distinct from Tr1 cells because they do not secrete IL-10 *(62)*.

Although IL-10 induction during immunotherapy can be localized to CD4$^+$ T cells, the phenotype of these cells does not fit with the distinct regulatory subsets described. For example, in patients receiving immunotherapy for bee venom allergy, IL-10-producing CD4$^+$ T cells expressed CD25 *(34)*. More recently, cells with a similar phenotype (i.e., IL-10$^+$CD25$^+$CD4$^+$) have been identified in patients receiving grass pollen immunotherapy *(44)*. In the latter study, T-cell proliferation was correlated with the percentage of CD25$^+$CD4$^+$ cells in allergic subjects but not in allergic patients undergoing immunotherapy. This observation was interpreted as indirect evidence of a poorly proliferating subset of cells, which is a characteristic of Tr cells. It has been proposed that IL-10-producing CD25$^+$CD4$^+$ T cells are distinct from naturally

occurring non-IL-10-producing CD25⁺CD4⁺ T cells and that CD25⁻CD4⁺ T cells differentiate into CD25⁺ T cells after encountering antigen in the periphery (*63*). Indeed, recent evidence suggests that CD25⁻CD4⁺ T cells can convert to TGF-β-producing CD25⁺ Tr cells in a TGF-β-dependent manner (*64,65*). These TGF-β-induced suppressor T cells were subsequently shown to prevent HDM-induced inflammatory cell infiltration in a murine asthma model (*64*). In a human system, the ability for CD25⁺CD4⁺ T cells to modulate T-cell responses to grass pollen extract has been examined (*66*). Addition of CD25⁺ T cells to the CD25⁻ subset suppressed T-cell proliferation and IL-5 production to allergen. Interestingly, the magnitude of this effect was substantially reduced in cultures from symptomatic atopic subjects compared to cultures from asymptomatic atopics as well as in cultures from asymptomatic atopics compared to cultures from nonatopics. In this system, the effects of CD25⁺ T cells were IL-10-independent. However, in contrast to other in vitro human-based allergen-specific systems, these observations implicated naturally occurring CD25⁺CD4⁺ Tr cells in downregulation of the allergic response. Moreover, this effect was evident in distinct immune responses to the same allergen, but the magnitude of this effect was the distinguishing feature between allergic and nonallergic responders. Clearly, more detailed analysis of Tr cells is warranted to further characterize the nature of these cells and the mechanism by which they function to control allergic responses.

The Significance of IgG and IgG4 Abs and the Modified Th2 Response

In grass pollen immunotherapy, inhibition of allergen–IgE complex binding to B cells by IgG Abs has been demonstrated using flow cytometry, and this activity was observed to co-elute with IgG4 (*48,67,68*). This suggests that IgG Abs disrupt the IgE network by inhibiting facilitated allergen presentation mediated through the low-affinity IgE receptor CD23, which is expressed on B cells. Furthermore, this "blocking" activity correlated with the patients' perceived improvement of allergic symptoms. The implication is that IgE Abs, but not IgG/IgG4 Abs, are pivotal to the development of allergic symptoms. This is consistent with the numerous studies showing that IgE Abs are a major risk factor for the development of allergic disease. It is not known whether allergen-specific IgG or IgG4 Abs associated with the modified Th2 response exhibit functional activity. Furthermore, although IgE Abs are not measurable in the serum of modified responders, the issue of whether IgE-switched memory B cells are present in these patients remains unanswered. In addition to observations at the T-cell level outlined below, there is extensive anecdotal evidence that the modified Th2 response is a variation of the allergic response. Indeed, it is not uncommon for patients who previously lived in a house with a cat and who experienced no allergic symptoms to report the onset of symptoms after removal of the cat from their home environment. Therefore, a change from high- to low-dose allergen exposure may result in a paradoxical worsening of symptoms. In our studies, we identified individuals who currently lived with a cat who exhibited low-titer IgE (0.35–0.7 IU/mL) in the presence of high IgG; these patients almost invariably denied allergic symptoms. We theorize that individuals with a modified Th2 response represent those with an allergic predisposition who may otherwise become allergic at low levels of exposure to cat allergen. Therefore, the aforementioned group of individuals living with a cat may represent a transitional stage between the allergic and modified Th2 phenotype. It has not been examined whether IgG Abs in these subjects exhibit inhibitory activity.

In addition to its ability to modulate T-cell responses, IL-10 has also been shown to differentially regulate the production of IgE and IgG4 Abs in both an antigen-specific and

nonantigen-specific manner *(31,69)*. In vitro studies have shown that for IL-10 to inhibit IL-4-stimulated IgE production and IgE messenger RNA ε-transcript expression in peripheral blood mononuclear cells (PBMCs), it must be present during the first 2 d of culture after addition of IL-4. This suggests that IL-10 inhibits IL-4-induced IgE switching and may explain why IgE Ab titers are refractory to the effects of IL-10 during immunotherapy (i.e., after IgE switching has occurred). Titers of serum IgG Abs to Fel d 1 are strongly correlated with Fel d 1 levels in house dust *(43)*. Therefore, there is no doubt that serum IgG Ab titers are an index of exposure. We do not know whether this IgG Ab production is driven by allergen exposure alone or by an associated increase in IL-10.

Definition of a T-Cell Regulatory Mechanism Associated With the Modified Th2 Response

Given the association between high-dose allergen exposure via the systemic or respiratory route and induction of IL-10, we hypothesized that natural exposure to high-dose Fel d 1 was associated with IL-10 production. Analysis of T-cell responses to Fel d 1 identified a novel immunodominant region mapping to the amino-terminal portion of polypeptide chain 2 that preferentially induced IL-10 and IFN-γ. Specifically, production of IL-10 and IFN-γ localized to two overlapping 17-mer peptides that were designated peptide (P)2:1 and P2:2, respectively *(43)*. Surprisingly, induction of IL-10 and IFN-γ was not restricted to T cells derived from subjects with a modified Th2 response. Indeed, cultures derived from allergic (IgE^posIgG^pos) as well as nonallergic controls (IgE^negIgG^neg) exhibited a similar pattern of cytokine responsiveness. In contrast, a second immunodominant region within chain 1 (P1:2) induced strong proliferation in PBMC from allergic and modified Th2 responders but not control subjects. Moreover, P1:2 stimulated the

highest levels of IL-5, with a trend toward increased production among allergic subjects. Thus, induction of Th2 and Th1 or regulatory cytokines localized to different regions of the Fel d 1 molecule.

These findings suggest that the T-cell repertoire for cat allergen consists of distinct Th- and Tr-cell subsets with defined T-cell receptor specificities and that expansion of chain 1 epitope-specific Th2 cells is a feature of the allergic response. Furthermore, CD4^+ T cells with potent IL-10- and IFN-γ-secreting properties are integral to the Fel d 1-specific T-cell repertoire, irrespective of allergic phenotype. Flow cytometry analysis confirmed that CD4^+ T lymphocytes stimulated with chain 2 epitopes secreted IL-10; again, this was independent of allergic status.

Induction of different cytokines by epitopes mapping to separate polypeptide chains of Fel d 1 suggests that activity of distinct T-cell subsets is compartmentalized within the microenvironment in vivo. It is possible that IFN-γ- and IL-10-inducing epitopes from the same region of Fel d 1 are generated within the same endosomal compartment in the antigen processing pathway. This may facilitate interactions between Th1 and Tr cells at the surface of the APC. There is evidence to suggest that Fel d 1-induced IL-10 acts preferentially on Th1 responses. For example, in the presence of anti-IL-10 monoclonal Ab, production of IFN-γ, but not IL-5, was enhanced in cultures stimulated with Fel d 1 or pooled chain 2 peptides. In fact, chain 2 peptides stimulated unusually high levels of IFN-γ secretion (up to 10 ng/mL) when IL-10 was blocked; these levels were up to eightfold higher than those induced by whole allergen *(43)*.

Selective activation of chain 2 epitope-specific T cells after injection of cat extract in allergic subjects implicated these cells in tolerance induction. This observation provided evidence that chain 2 epitopes were generated naturally from whole allergen by antigen processing pathways in vivo. Furthermore, the

ability for chain 2 epitope-specific cells to respond rapidly after systemic administration of allergen (i.e., within 1–2 mo of initiation of immunotherapy) suggested that they are not quiescent during an established allergic response.

It is likely that irrespective of allergic phenotype, IL-10 fulfills a key regulatory function during an established immune response. Although regulation of the allergic state by T cells may occur in early life, the function of these cells may be altered in later life by a change in environment—that is, allergen dose. For example, there are numerous anecdotal reports of individuals who have grown up with a cat in the home who, after living in a cat-free environment, experience the onset of allergic symptoms in the presence of a cat. These observations, coupled with altered T-cell reactivity in patients receiving immunotherapy, suggest that both the modified Th2 response and the allergic response are plastic and that a bidirectional immune pathway links each state.

Numerous studies have demonstrated that genetic factors are a major determinant of allergic disease. A striking observation in our study was the high prevalence of expression of the human leukocyte antigen (HLA)-DR allele *0701 among subjects with a modified Th2 response (50%) compared to allergic subjects (7%). Interestingly, the IL-10-inducing peptide P2:1 and the IFN-γ-inducing peptide P2:2 were identified as putative promiscuous HLA-DR ligands capable of binding to multiple HLA-DR molecules. The ability of these peptides to induce strong T-cell proliferation in cultures from patients with diverse HLA types, regardless of allergic status, was consistent with this finding. Indeed, of the four DR7 peptide-binding motifs identified within Fel d 1, two localized to P2:1 and P2:2, whereas the remaining two mapped to weakly stimulatory peptides, suggesting that these were subdominant or not relevant in vivo. Production of IL-10 was enhanced in PBMCs from DR7-positive modified responders compared to their DR7-negative

counterparts for cultures stimulated with P2:1 and other peptides within chain 1 and chain 2 of the molecule. Furthermore, mean levels of peptide-induced IFN-γ and the ratio of peptide-induced IL-10 to IFN-γ were enhanced in DR7-positive modified responders compared to the allergic group *(43)*. One interpretation of this is that development of the modified Th2 response in DR7-positive individuals represents optimal regulation mediated through chain 2 epitopes.

Based on the premise that the modified Th2 response reflects a variation of the allergic response, we speculate that exposure to high-dose cat allergen among DR7-positive subjects with an allergic predisposition favors IL-10 induction within the respiratory tract beyond a critical threshold, resulting in tolerance. The finding that increased production of IL-10 was observed for all the Fel d 1-derived peptides in cultures of T cells from DR7-positive modified responders may have resulted from intramolecular epitope spreading. Indeed, such a mechanism has recently been implicated in the induction of an immune response to epitopes of Fel d 1 not included in a Fel d 1 peptide vaccine *(35)*.

The issue of why HLA-DR7 expression in modified Th2 responders should favor IL-10 production is complex. Increased affinity and/or stability of chain 2 peptide binding to DR7, coupled with increased density of DR7–peptide complexes at the APC surface, could alter T-cell signaling events to favor production or activation of Tr cells. Studies using altered peptide ligands have shown that the affinity of the peptide–MHC interaction with the T-cell receptor (TCR) can influence whether a CD4 T cell produces Th1 or Th2 cytokines *(70–72)*. Furthermore, it has been proposed that TCR structure is influenced by the cytokine milieu and this may alter TCR triggering and subsequent development of different T-cell subsets *(70)*. It is tempting to speculate that DR7-chain 2 peptide complexes favor induction of Th1 or Tr cells through similar mechanisms.

Modified Th2 or Modified Th1?

Our findings point to a central role for IL-10 in regulation of the immune response to cat allergen. Because IL-10 acts preferentially on Th1 (IFN-γ) cytokines, rather than Th2 (IL-5 and IL-13) cytokines, it could be argued that tolerance to cat allergen represents a modified Th1 response. Thus, an indirect effect of IL-10 on Th2 cells via its effects on Th1 cells cannot be excluded. There is little evidence to suggest that the nonallergic response represents a Th1 response. For example, common inhalant allergens do not induce delayed-type hypersensitivity (DTH) skin tests, which is the hallmark of a classical Th1 response. Furthermore, where DTH responses to allergens have been demonstrated (i.e., fungal allergens), the titer of IgG Abs is markedly lower in subjects with DTH compared to those with immediate hypersensitivity *(73)*. This observation suggests that the presence of high-titer allergen-specific IgG Abs is more consistent with an allergic response. As an adjunct to this, production of IFN-γ is a frequent observation in allergen-stimulated lymphocyte cultures derived from patients with allergy. Thus, it could be argued that IFN-γ is not a Th1 marker *per se* but, under certain conditions, is a feature of Th2 responses. Indeed, IFN-γ has recently been reported to enhance Th2 priming in vivo *(74)*. Features of the modified Th2 response that suggest it is a variation of the allergic response (as opposed to a Th1-mediated nonallergic response) include: (a) presence of the IL-4-dependent IgG4 Ab at high titers; (b) elevated IL-5 levels in peptide-stimulated cultures from modified Th2 subjects, compared to nonallergic controls; (c) enhancement of IL-10 and IFN-γ production to chain 2 epitopes, which are targeted during immunotherapy in allergic patients.

The fact that the regulatory effects of IL-10 do not appear to be confined to nonallergic responses is not surprising because of the complex homeostatic mechanisms that operate to regulate immune responses to other antigens.

Expansion and contraction of effector T cells specific for pathogen-derived antigens within the T-cell compartment is tightly regulated during acute episodes of infection. Because exposure to cat allergen is chronic and airborne levels are high relative to other allergens, control of T-cell activation within the Fel d 1-specific T-cell repertoire may be important for preventing deleterious allergic inflammatory responses. In contrast to reports of T-cell hyporesponsiveness in patients receiving immunotherapy, we found no evidence for diminished T-cell activation associated with increased allergen exposure via the respiratory route (i.e., a cat in the house) *(43)*. On the contrary, our results suggest that activation of T cells targeting a defined region of the Fel d 1 molecule is pivotal to control of distinct allergen-specific responses.

We propose a model in which the dose of allergen combined with the appropriate HLA-DR type dictates the efficiency of activation of chain 2 epitope-specific T cells. Expression of HLA-DR7, coupled with high-dose exposure to cat allergen, could favor optimal induction of Tr cells in parallel with Th1 cells that are specific for chain 2 epitopes. When this occurs during the initiation phase of the immune response, it may result in "imprinting" of the epitope-specific regulatory mechanisms necessary to prevent expansion of Th2 cells. These early immune events could be sufficient not only to induce a modified Th2 response but also to maintain it later in life under the appropriate environmental conditions (i.e., persistent high-dose allergen; *see* Fig. 1). In contrast, low-dose allergen exposure in the absence of HLA-DR7 could result in preferential stimulation of Th2 cells specific for chain 1 epitopes (i.e., P1:2). In this scenario, diminished activation of Th1 and Tr cells could lead to expansion of the Th2 subset and establishment of the allergic phenotype (Fig. 1). The view that a small Th2 compartment within the Fel d 1-specific T-cell repertoire is sufficient to establish the allergic response is inherent in this model.
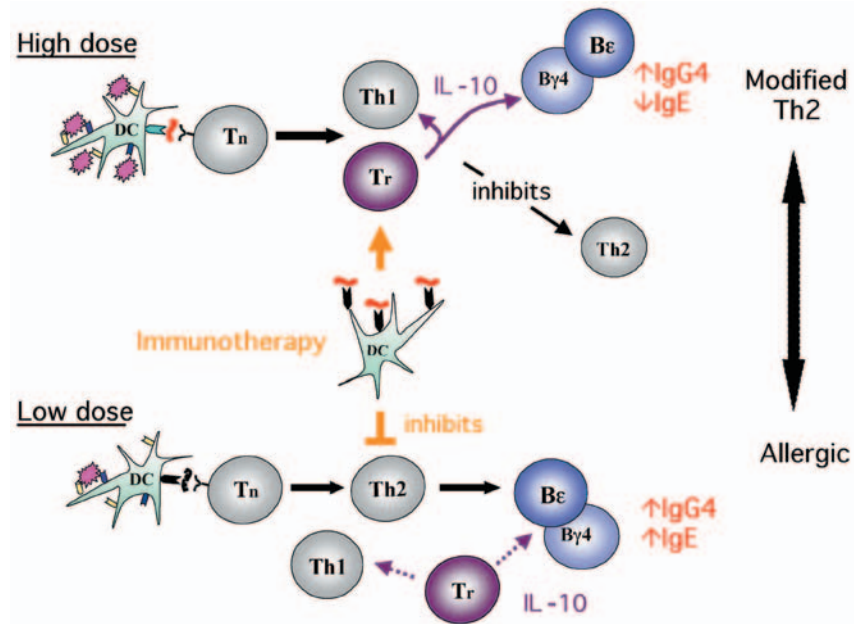
Fig. 1. Tr cells and Th1 cells coordinately regulate the development and modulation of distinct immune responses to cat allergen. Exposure to high-dose allergen (magenta) coupled with expression of HLA-DR7 (turquoise) favors presentation of chain 2 epitopes (red) at the APC surface. This results in preferential induction of Tr cells in parallel with Th1 cells. The subsequent interplay between Tr cells and Th1 cells inhibits expansion of Th2 cells within the T-cell repertoire. The differential effects of IL-10 on IgE and IgG4 manifest as the modified Th2 response. Low-dose exposure in conjunction with expression of non-HLA-DR7 (black) favors recognition of chain 1 epitopes (black) and preferential induction of Th2 cells. The associated diminished activation of Th1 and Tr cells results in development of the allergic phenotype. The immune pathway between the modified Th2 and allergic state is bidirectional. Presentation of chain 2 peptides at high dose in the context of non-HLA-DR7 molecules could favor induction of chain 2 epitope-specific T cells and development of tolerance in allergic subjects (immunotherapy). DC, dendritic cell; Tn, naïve T cell; Tr, regulatory T cell.

Therefore, optimal activation of chain 2 epitope-specific T cells during early responses could be a critical determinant of sensitization status to cat allergen.

In cultures of lymphocytes from allergic subjects who were DR7-negative, IFN-γ production to chain 2 epitopes was enhanced in the presence of anti-IL-10 monoclonal Ab *(43)*. This observation, which points to a role for IL-10 in the control of Th1 responses during an established allergic response, suggests that treat-ment strategies that enhance the interactions between Tr cells and Th1 cells may be efficacious. Administration of high-dose chain 2 peptides in DR7-negative allergic subjects could selectively target Fel d 1-specific Tr cells and Th1 subsets, thereby inhibiting expansion of the Th2 subset. Therefore, coordinated regulation by Th1 and Tr cells may not only be pivotal to the development of distinct immune responses but may also act to limit the magnitude or severity of an established allergic response.

High-Titer IgE Abs and Atopic Dermatitis: Evidence of Failure to Control

If Tr cells control the allergic response, then an aberration of Tr-cell development or function may lead to allergic symptoms. This could occur through decreased IL-10 or increased Th2 cytokine production. Our findings (reviewed earlier) point to induction of Tr cells by respiratory exposure to allergen; however, studies of patients with atopic dermatitis (AD) suggest that altered responsiveness to IL-10 (rather than diminished IL-10 production) could also be relevant to the development of allergic responses.

In population-based studies, titers of IgE Abs to cat allergen are significantly lower than for mite allergen among school-age children exposed to high levels of both mite and cat allergen *(75)*. Therefore, control of the allergic response to cat is evident at the B-cell and the T-cell level among sensitized subjects. Such downregulation of IgE Abs was shown to be deficient in cat-sensitized patients with AD *(36)*. Among these individuals, mean titers of Fel d 1-specific IgE Abs were significantly elevated compared to titers of cat-sensitized patients without AD (11.2 vs 2.3 IU/mL; $p = 0.001$). This observation, coupled with the very high titers of total IgE in many of these patients, suggested that dysregulation of the allergic response is a feature of this disease. Consistent with this finding, development of the modified Th2 response occurred infrequently in patients with AD compared with a random population of subjects without AD (4 of 55 vs. 14 of 57; $p = 0.013$). Based on the ability for IL-10 to suppress IgE Abs, high-titer IgE Abs to Fel d 1 in patients with AD could reflect a deficiency in chain 2 epitope-specific T-cell activation. Consistent with this, PBMCs from sensitized patients with AD showed markedly reduced T-cell proliferation and IFN-γ production to chain 2 peptides as well as an altered pattern of IL-10 secretion *(36)*. Furthermore, blocking IL-10 using Abs against IL-10 or against the IL-10 receptor (unpublished observations) failed to enhance IFN-γ production to chain 2 epitopes. Moreover, decreased production of epitope-specific IL-10 was accompanied by a paradoxical decrease in peptide-induced IFN-γ in cultures from cat-sensitized patients with AD who had undergone an allergen avoidance regimen *(36)*. These findings suggest that decreased IFN-γ production in cultures from patients with AD was not explained by the effects of IL-10. Thus, a deficiency in regulatory mechanisms governed by chain 2 epitopes could contribute to the development of high-titer IgE Abs associated with AD.

A recent study examining CD25+CD4+ T cells derived from patients with AD reported an increased frequency of these cells, compared with asthmatic subjects and normal controls *(76)*. These cells exhibited properties consistent with Tr cells (i.e., anergy to anti-CD3 stimulation and suppression of proliferation of CD25– CD4+ T cells) after stimulation with anti-CD3. In contrast, culture in the presence of Staphylococcal enterotoxin B abrogated these effects. The implication is that chronic exposure to superantigens produced by skin-colonizing bacteria subverts the activity of Tr cells. Because the majority of patients with AD do not live with cats, these observations raise the intriguing issue of whether chronic low-dose exposure to inhaled allergen exerts a similar effect on Tr cells specific for Fel d 1.

Relevance of the Hygiene Hypothesis to Allergen-Specific Tolerance

It has been argued that the modified Th2 response to cat allergen arises not by virtue of high-dose exposure to Fel d 1 *per se*, but from increased exposure to endotoxin resulting from the presence of animals in the home. This is based on the premise that endotoxin exposure favors induction of a Th1 response resulting in inhibition of the allergic response. At the cellular level, there is no doubt that endotoxin can

modulate the immune response through binding to toll-like receptors (TLRs) present on various cell types. However, only a single study has examined the relationship between environmental endotoxin and T-cell responses in humans. In that study, endotoxin levels in house dust were correlated with IFN-γ production by CD4⁺ T cells, and this was proposed to protect against allergen sensitization in infants *(77)*. In a murine model of asthma, low-level inhaled endotoxin was shown to be necessary to induce Th2 responses to allergen, whereas high levels of endotoxin favored a Th1 response *(28)*. This would provide a mechanism to explain the epidemiological data showing decreased sensitization associated with high endotoxin exposure *(78)*. In contrast, in a different model in which mice were sensitized to Fel d 1 via the subcutaneous route, simultaneous injection of LPS was required to generate a strong specific Ab response for both IgE and IgG1 (Th2 profile) as well as for IgG2a (Th1 profile) *(79)*. Therefore, in that system, the adjuvant effects of LPS did not appear to polarize the immune response in either direction.

The decreased prevalence of atopy among children raised on farms in Europe led to the theory that exposure to high levels of endotoxin fulfilled a protective role against the development of allergic disease *(78)*. However, more recent data from a birth cohort study in Germany have refuted this assertion *(80)*. Given the current state of methodology for measuring endotoxin exposure, it is difficult to evaluate what constitutes low vs high endotoxin levels. However, because endotoxin levels in houses with and without cats in the United States are comparable *(81)*, it seems unlikely that downregulation of the allergic response in tolerant subjects living with cats is explained by endotoxin exposure. Although we cannot exclude an adjuvant effect of endotoxin on Fel d 1-stimulated IL-10 resulting from natural exposure, there is convincing evidence that IL-10 induction in vitro arises from intrinsic properties of the molecule and that this gives

rise to Tr cells. These data include: (a) localization of IL-10 induction to chain 2 epitopes containing promiscuous HLA class II ligands, which stimulate strong T-cell proliferation; (b) identification of IL-10⁺CD4⁺ T cells in cultures stimulated with chain 2 peptides; and (c) absence of endotoxin in Fel d 1 peptide preparations. It seems likely that both the dose and route of allergen exposure, coupled with intrinsic properties of the allergen, contribute to IL-10 secretion.

We have established that Fel d 1 induces a modified Th2 response independent of sensitization to other allergens—even among subjects who express HLA-DR7 (unpublished observations, 2004). The implication is that the protective effect of high-dose exposure to cat allergen does not influence the immune response to other allergens—that is, tolerance to cat allergen is an antigen-specific phenomenon. Consistent with this finding, in studies carried out in children living in New Zealand (where exposure to both cat and mite allergens is high), neither the prevalence nor the mean titer of IgE Abs to dust mite was influenced by exposure to cat. In contrast, the prevalence of sensitization to cat was significantly diminished in houses with a cat *(75)*. The observation that cat-specific IgE Ab is suppressed relative to mite supports the view that Fel d 1 has unique immunological properties. The reduced prevalence of sensitization to cat compared with mite (approx 10 vs approx 30%) in Australia and New Zealand is consistent with this statement *(4)*. The issue of whether the effects of Fel d 1 on the immune response can be explained solely by the epitope-specific regulatory mechanism described earlier is open to debate.

The Fel d 1 molecule is a member of the secretoglobin family of proteins, and it has limited amino acid sequence identity (approx 20%) with Clara cell secretory protein (CCSP; also termed uteroglobin). However, the crystal structure of Fel d 1 exhibits striking similarity to the three-dimensional structure of utero-

globin *(82)*. CCSP is secreted by Clara cells in the epithelium of the lung, and it has been reported to have anti-inflammatory and immunomodulatory properties *(83–85)*. If Fel d 1 exerted a similar biological role, then it would be difficult to explain how the same molecule could induce a proinflammatory allergic response and an anti-inflammatory tolerant response at different doses. From the immunological perspective, the structural similarity between Fel d 1 and CCSP suggests that these proteins are crossreactive. Thus, one would predict that elevated IgG Abs associated with the modified Th2 response could block the biological effects of CCSP, resulting in increased inflammatory responses in the lung. Clearly, this presents a paradox based on the fact that the modified Th2 response appears to be associated with decreased symptoms in the respiratory tract. In summary, intrinsic properties of Fel d 1 may contribute to the development of the tolerant response. However, the possibility that other environmental factors, such as endotoxin, potentiate the effects of high-dose airborne allergen cannot be excluded.

Implications for the Allergic Patient

Allergen avoidance is the primary treatment for patients who are sensitized to indoor allergens. However, the "cat paradox," in which increased exposure leads to decreased prevalence of sensitization, presents a dilemma for the clinician. There is no doubt that an allergic patient living in a house with a cat will benefit from removal of the pet. This statement is supported by numerous clinical observations. Once an allergic response is established, persistent allergen exposure only serves to perpetuate inflammatory responses within the respiratory tract. However, in terms of primary prevention, it is not clear at this point whether children from families with an allergic history would benefit from a cat-free environment from birth. Epidemiological data support the view that children born into a household with

animals are at decreased risk for sensitization *(20,86,87)*. It seems likely that to induce a modified Th2 response, high-dose allergen exposure from early life is a prerequisite. Indeed, the majority of subjects who have a modified Th2 response report the presence of a cat in the house from early childhood.

So, what should we recommend for an atopic individual who is determined to obtain a cat? Development of the modified Th2 response can occur in the presence of sensitization to other allergens. Once an individual has become sensitized to multiple allergens, it is not known whether exposure to high-dose cat allergen later in life could induce a modified Th2 response. Indeed, among subjects older than age 23 yr, it has been shown that sensitization to other allergens is a risk factor for the development of IgE to cat *(8)*. In an atopic individual, the prediction is that exposure to high-dose cat allergen favors a modified Th2 response in the context of DR7, whereas lack of expression of DR7 presents an increased risk for sensitization. If the dose of allergen received during maturation of the immune system is a critical determinant of the immune pathway, then high-dose respiratory exposure to cat allergen in later life may not be sufficient to override an established allergic response. Consistent with this finding, exposure to cat allergen in early childhood, but not in adulthood, was related to a decreased prevalence of skin prick test positivity to cat, and this was restricted to children exposed before age 2 yr *(88)*. As mentioned previously, there is substantial anecdotal evidence that a modified Th2 response can transition to an allergic response after a change from high- to low-dose allergen exposure. However, the converse (i.e., development of the modified Th2 response after change in environment from low- to high-dose allergen) cannot be said. Therefore, the allergic response may represent a "default pathway" that can be subverted by high-dose, persistent exposure to cat allergen from early life.

Conventional immunotherapy with cat extract induces an immune response with many

features that are characteristic of the modified Th2 response. The duration of such treatment is a major drawback for many patients; however, few advances in vaccine development for cat allergen are on the horizon. In the mid-1990s, epitope-mapping studies identified immunodominant epitopes of Fel d 1 chain 1 of the molecule *(89,90)*. This led to development of a vaccine that incorporated chain 1 peptides (ALLERVAX CAT); however, therapy was associated with adverse reactions *(91)*. In subsequent studies, shorter peptides were used that spanned chain 1 or both chains of the Fel d 1 molecule *(33,35,49)*. In those studies, intradermal injection of peptides was associated with late asthmatic reactions in a subset of patients with cat allergies. However, these reactions were diminished after a second injection, and peptide-specific T-cell hyporesponsiveness persisted for several months. It is notable that peptides spanning the amino-terminus of Fel d 1 chain 2 were not administered as part of the peptide preparations. Identification of tolerogenic epitopes of Fel d 1 chain 2 may provide an avenue for the design of a more tailored peptide-based vaccine that preferentially induces T-cell responses targeting a defined region of the allergen molecule.

Summary

Definition of the modified Th2 response has provided a framework for examining how IgE Ab-mediated responses are controlled. IL-10 is pivotal to regulation of the allergic response; however, there is likely to be considerable overlap in the role of different Tr cells in modulation of the allergic response. Exposure to high-dose allergen either by inhalation or by injection appears to enhance control mechanisms. Analysis of T-cell responses to Fel d 1 suggests that coordinated regulation mediated by IL-10- and IFN-γ-producing CD4+ T cells is relevant to tolerance induction. Properties of the allergen molecule, coupled with genotype, are pivotal to this regulatory process. Dissection of the immune pathways involved in down-regulation of the allergic response associated with exposure to high-dose allergen could provide new directions for the treatment of allergic disease.

References

1. Hesselmar, B., Aberg, N., Aberg, B., Eriksson, B., and Bjorksten, B. (1999), *Clin Exp Allergy* **29,** 611–617.
2. Braback, L., Kjellman, N.I., Sandin, A., and Bjorksten, B. (2001), *Pediatr Allergy Immunol* **12,** 4–10.
3. Svanes, C., Jarvis, D., Chinn, S., and Burney, P. (1999), *J Allergy Clin Immunol* **103,** 415–420.
4. Roost, H.P., Kunzli, N., Schindler, C., et al. (1999), *J Allergy Clin, Immunol* **104,** 941–947.
5. Sporik, R., Squillace, S.P., Ingram, J.M., Rakes, G., Honsinger, R.W., and Platts-Mills, T.A. (1999), *Thorax* **54,** 675–680.
6. Platts-Mills, T., Vaughan, J., Squillace, S., Woodfolk, J., and Sporik, R. (2001), *Lancet* **357,** 752–756.
7. Holscher, B., Frye, C., Wichmann, H.E., and Heinrich, J. (2002), *Pediatr Allergy Immunol* **13,** 334–341.
8. Linneberg, A., Nielsen, N.H., Madsen, F., Frolund, L., Dirksen, A., and Jorgensen, T. (2003), *Allergy* **58,** 21–26.
9. Kuehr, J., Frischer, T., Karmaus, W., et al. (1992), *J Allergy Clin Immunol* **90,** 358–363.
10. Wahn, U., Lau, S., Bergmann, R., et al. (1997), *J Allergy Clin Immunol* **99,** 763–769.
11. Suoniemi, I., Bjorksten, F., Haahtela, T. (1981), *Allergy* **36,** 263–268.
12. Arshad, S.H. (1991), *Br J Clin Pract* **45,** 88, 89.
13. Custovic, A., Simpson, B.M., Simpson, A., Kissen, P., and Woodcock, A. (2001), *Lancet* **358,** 188–193.
14. Lindfors, A., Wickman, M., Hedlin, G., Pershagen, G., Rietz, H., and Nordvall, S.L. (1995), *Arch Dis Child* **73,** 408–412.
15. Melen, E., Wickman, M., Nordvall, S.L., van Hage-Hamsten, M., and Lindfors, A. (2001), *Allergy* **56,** 646–652.
16. Almqvist, C., Egmar, A.C., Hedlin, G., et al. (2003), *Clin Exp Allergy* **33,** 1190–1197.
17. Hesselmar, B., Aberg, B., Eriksson, B., Bjorksten, B., and Aberg, N. (2003), *Clin Exp Allergy* **33,** 1681–1685.
18. Perzanowski, M., Ronmark, E., Platts-Mills, T.A., and Lundback, B. (2002), *Am J Respir Crit Care Med* **166,** 696–702.
19. Custovic, A., Wimpson, B.M., Simpson, A., et al. (2003), *J Allergy Clin Immunol* **111,** 402–407.
20. Ownby, D.R., Johnson, C., and Peterson, E.L. (2002) *JAMA* **288,** 963–972.
21. Ingram, J.M., Sporik, R., Rose, G., Honsinger, R., Chapman, M.D., and Platts-Mills T.A.E. (1995), *J Allergy Clin Immunol* **96,** 449–456.

22. Custovic, A., Simpson, B., Simpson, A., Hallam, C., Craven, M., and Woodcock, A. (1999), *Allergy* **54,** 612–616.

23. Custovic, A., Simpson, A., Padhi, H., Green, R.M., Chapman, M.D., and Woodcock, A. (1998), *Thorax* **53,** 33–38.

24. DeLucca, S.D., O'Meara, T.J., and Tovey, E.R. (2000), *J Allergy Clin Immunol* **106,** 874–879.

25. Luczynska, C.M., Li, Y., Chapman, M.D., and Platts-Mills T.A.E. (1990), *Am Rev Respir Dis* **141,** 361–367.

26. DeBlay, F., Heymann, P.W., Chapman, M.D., and Platts-Mills, T.A.E. (1991), *J Allergy Clin Immunol* **88,** 919–926.

27. Lambrecht, B.N. and Hammad, H. (2003), *Nat Rev Immunol* **3,** 994–1003.

28. Eisenbarth, S.C., Piggott, D.A., Huleatt, J.W., Visintin, I., Herrick, C.A., and Bottomly, K. (2002), *J Exp Med* **196,** 1645–1651.

29. Halapi, E. and Hakonarson, H. (2004), *Curr Opin Pulm Med* **10,** 22–30.

30. Ebner, C., Siemann, U., Bohle, B., et al. (1997), *Clin Exp Allergy* **27,** 1007–1015.

31. Akdis, C.A., Blesken, T., Akdis, M., Wuthrich, B., and Blaser, K. (1998), *J Clin Invest* **102,** 98–106.

32. Bellinghausen, I., Metz, G., Enk, A.H., Christmann, S., Knop, J., and Saloga, J. (1997) *Eur J Immunol* **27,** 1131–1139.

33. Oldfield, W.L., Kay, A.B., and Larche, M. (2001), *J Immunol* **167,** 1734–1739.

34. Akdis, C.A. and Blaser, K. (1999), *FASEB* **13,** 603–609.

35. Oldfield, W.L.G., Stillion, R., Kay, A.B., and Larche, M. (2002), *J Allergy Clin Immunol* **109,** S261. (Abstr.).

36. Carneiro, R., Reefer, A., Wilson, B., et al. (2004), *J Invest Dermatol* **122,** 927–936.

37. Akdis, C.A., Joss, A., Akdis, M., Faith, A., and Blaser, K. (2000), *FASEB* **14,** 1666–1668

38. Jutel, M., Pichler, W.J., Skrvic, D., Urwyler, A., Dahinden, C., and Muller, U.R. (1995), *J Immunol* **154,** 4187–4194.

39. Meissner, N., Kochs, S., Coutelle, J., et al. (1999), *Clin Exp Allergy* **29,** 618–625.

40. Oda, N., Yamashita, N., Minoguchi, K., Rakeno, M., Kaneko, S., and Adachi, M. (1998), *Cell Immunol* **190,** 43–50.

41. Secrist, H., Chelen, C.J., Wen, Y., Marshall, J.D., and Umetsu, D.T. (1993), *J Exp Med* **178,** 2123–2130.

42. Hamid, Q.A., Schotman, E., Jacobson, M.R., Walker, S.M., and Durham, S.R. (1997) *J Allergy Clin Immunol* **99,** 254–260.

43. Reefer, A.J., Carneiro, R.M., Custis, N.J., et al. (2004), *J Immunol* **172,** 2763–2772.

44. Francis, J.N., Till, S.J., and Durham, S.R. (2003), *J Allergy Clin Immunol* **111,** 1255–1261.

45. Ewbank, P.A., Murray, J., Sanders, K., Curran-Everett, D., Dreskin, S., and Nelson, H.S. (2003), *J Allergy Clin Immunol* **111,** 155–161.

46. O'Brien, R.M., Byron, K.A., Varigos, G.A., and Thomas, W.R. (1997), *Clin Exp Allergy* **27,** 46–51.

47. Moverare, R., Elfman, L., Bjornsson, E., and Stalenhiem, G. (2000), *Immunol Lett* **3,** 51–56.

48. Nouri-Aria, K.T., Wacholz, P.A., Francis, J.N., et al. (2004), *J Immunol* **172,** 3252–3259.

49. Oldfield, W.L., Larche, M., and Kay, A.B. (2002), *Lancet* **360,** 47–53.

50. Ohman, J.L., Findlay, S.R., and Leitermann, K.M. (1984), *J Allergy Clin Immunol* **74,** 230–239.

51. Aalberse, R.C., van der Gaag, R., and van Leuwen, J. (1983), *J Immunol* **130,** 722–726.

52. Djurup, R. and Osterballe, O. (1984), *Allergy* **39,** 433–441.

53. Durham, S.R., Kay, A.B., and Hamid, Q. (1995), *Int Arch Allergy Immunol* **107,** 282–284.

54. Akbari, O., DeKruyff, R.H., and Umetsu, D.T. (2001), *Nat Immunol* **2,** 725–731.

55. Hansen, G., McIntire, J.J., Yeung, V.P., et al. (2000), *J Clin Invest* **105,** 61–70.

56. Oh, J.W., Seroogy, C.M., Meyer, E.H., et al. (2002), *J Allergy Clin Immunol* **110,** 460–468.

57. Chen, Y., Kuchroo, V.K., Inobe, J., Hafler, D.A., and Weiner, H.L. (1994), *Science* **265,** 1237–1240.

58. Prud'homme, G.J. and Piccirillo, C.A. (2000), *J Autoimmun* **14,** 23–42.

59. Groux, H., O'Garra, A., Bigler, M., et al. (1997), *Nature* **389,** 737–742.

60. Cottrez, F., Hurst, S.D., Coffman, R.L., and Groux, H. (2000), *J Immunol* **165,** 4848–4853.

61. Powrie, F., Read, S., Mottet, C., Uhlig, H., and Maloy, K. (2003), *Novartis Found Symp* **252,** 92–98.

62. Levings, M.K., Sangregorio, R., Sartirana, C., et al. (2002), *J Exp Med* **196,** 1335–1346.

63. Levings, M.K., Bacchetta, R., Schulz, U., and Roncarolo, M.G. (2002), *Int Arch Allergy Immunol* **129,** 263–276.

64. Chen, W., Jin, W., Hardegen, N., et al. (2003), *J Exp Med* **198,** 1875–1886.

65. Fantini, M.C., Becker, C., Monteleone, G., Pallone, F., Galle, P.R., and Neurath, M.F. (2004), *J Immunol* **172,** 5149–5153.

66. Ling, E.M., Smith, T., Nguyen, X.D., et al. (2004), *Lancet* **363,** 608–615.

67. Wacholz, P.A., Soni, N.K., Till, S.J. (2003), *J Allergy Clin Immunol* **112,** 915–922.

68. van Neerven, R.J.J., Wikborg, T., Lund, G., et al. (1999) *J Immunol* **163,** 2944–2952.

69. Jeannin, P., Lecoanet, S., Delneste, Y., Gauchat, J-F., and Bonnefoy, J-Y. (1998) *J Immunol* **160,** 3555–3561.

70. Boyton, R.J., Zaccai, N., Jones, E.Y., and Altmann, D.M. (2002), *J Immunol* **168,** 1018–1027.

71. Pfeiffer, C., Stein, J., Southwood, S., Ketelaar, H., Sette, A., and Bottomly, K. (1995), *J Exp Med* **181,** 1569–1574.

72. Nicholson, L.B., Greer, J.M., Sobel, R.A., Lees, M.B., and Kuchroo, V.K. (1995), *Immunity* **3,** 397–405.

73. Woodfolk, J.A., Sung, S.S., Benjamin, D.C., Lee, J.K., and Platts-Mills, T.A. (2000), *J Immunol* **165,** 4379–4387.

74. Bocek, P., Foucras, G.L., and Paul, W.E. (2004), *J Exp Med* **199,** 1619–1630.

75. Erwin, E.A., Wickens, K., Custis, N.J., et al. (2005), *J Allergy Clin Immunol* **115,** 74–79.

76. Ou, L-S., Golevan, E., Hall, C., and Leung, D.Y.M. (2004), *J Allergy Clin Immunol* **113,** 756–763.

77. Gereda, J.E., Leung, D.Y., Thatayatikom, A., et al. (2000), *Lancet*, **13,** 1680–1683.

78. von Mutius, E., Braun-Fahrlander, C., Schierl, R., et al. (2000), *Clin Exp Allergy* **30,** 1230–1234.

79. Ormstad, H., Groeng, E.C., Duffort, O., and Lovik, M. (2003), *Toxicology* **188,** 309–318.

80. Bolte, G., Bischof, W., Borte, M., Lehmann, I., Wichmann, H.E., Heinrich, J., LISA Study Group. (2003), *Clin Exp Allergy* **33,** 770–776.

81. Custis, N.J., Woodfolk, J., and Platts-Mills, T. (2004), *J Allergy Clin Immunol* **113,** S259 (Abstr.).

82. Kaiser, L., Gronlund, H., Sandalova, T., et al. (2003), *J Biol Chem* **278,** 37,730–37,735.

83. Wang, S.Z., Rosenberger, C.L., Bao Y.X., Stark, J.M., and Harrod, K.S. (2003), *J Immunol* **171,** 1051–1060.

84. Dierynck, I., Bernard, A., Roels, H., and De Ley, M. (1996), *Mult Scler* **1,** 385–387.

85. Singh, G. and Katyal, S.L. (1997), *Am J Respir Cell Mol Biol* **17,** 141–143.

86. Celedon, J.C., Litonjua, A.A., Ryan, L., Platts-Mills, T., Weiss, S.T., and Gold, D.R. (2002), *Lancet* **360,** 781,782.

87. Nafstad, P., Magnus, P., Gaarder, P.I., and Jaakkola, J.J. (2001), *Allergy* **56,** 307–312.

88. Oryszczyn, M.P., Annesi-Maesano, I., Charpin, D., and Kauffman, F. (2003), *Allergy* **58,** 1136–1143.

89. Counsell, C.M., Bond, J.F. Ohman, J.L., Greenstein, J.L., and Garman, R.D. (1996), *J Allergy Clin Immunol* **98,** 884–894

90. Mark, P.G., Segal, D.B. Dallaire, M.L., and Garman, R.D. (1996), *Clin Exp Allergy* **26,** 1316–1328

91. Maguire, P., Nicodemus, C., Robinson, D., Aaronson, D., and Umetsu, D.T. (1999), *Clin Immunol* **93,** 222–231.