

Selective Roles and Dysregulation of Interleukin-10 in Allergic Disease

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Current Allergy and Asthma Reports 2006, 6:40–46

Current Science Inc. ISSN 1529-7322

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There is mounting evidence to support a role for interleukin (IL)-10 in the regulation of both allergic and nonallergic immune responses. The current view is that IL-10 influences Th2-driven allergic processes by altering the interplay between Th1 and Th2 effector cells. However, lack of a clear delineation of the different types of IL-10-secreting regulatory T cells poses a major challenge in defining IL-10-mediated immune pathways, which govern the development, persistence, and modulation of allergic status. This is compounded by observations that undermine the credibility of the Th1/Th2 paradigm as a model for allergic disease. Nevertheless, enhanced IL-10 secretion by T cells during conventional immunotherapy, coupled with evidence of a link between genetics and high IL-10 production to a specific allergen, suggests that IL-10 induction is an appropriate goal of therapy. Knowledge of the targeted patient population and design of an immunogen (ie, peptide or modified allergen) within this context are likely to provide improved results over conventional immunotherapy.

Introduction

The resurgence of regulatory T cells has fostered renewed interest in the role of the inhibitory cytokine, interleukin (IL)-10, in allergic disease. Regulatory T cells generally act to suppress effector T cells that may otherwise be detrimental to the host, by maintaining tolerance to self antigens, or by limiting immune responses to foreign antigens. It has been proposed that regulatory T cells act to control the “balance” between Th1 and Th2 subsets in an IL-10-dependent manner, and thus are key determinants in the development of Th2-driven allergic processes. Although this view is likely to be overly simplistic, several studies indicate a role for IL-10 in the development of

protective immune responses directed against allergens. In this article, we review evidence supporting a role for IL-10 in the regulation of the allergic response, and discuss how failure of IL-10-mediated regulatory mechanisms may contribute to aberrant production of IgE.

Regulatory T Cells as a Source of Interleukin-10

IL-10 is a potent anti-inflammatory cytokine, as evidenced by its ability to inhibit inflammatory bowel disease and autoimmune disease, and to control infection by pathogenic agents such as the parasite, *Leishmania major* [1–6]. In addition, IL-10 has been shown to limit the in vivo expansion of naïve CD4⁺ T cells in lymphopenic mice, pointing to a central role in maintaining T-cell homeostasis [7]. Regulatory T cells (Tregs) are an important source of IL-10. Several different types of Tregs have been described in murine systems based on distinct phenotypic and functional characteristics [8,9]. Within the field of allergy, most studies have focused on defining a role for CD4⁺ regulatory T cells, which express the IL-2 receptor alpha chain, CD25. A distinction has been made between regulatory T cells that constitutively express CD25 upon egress from the thymus (“natural” Tregs) and those cells that are induced to express CD25 after encountering antigen in the periphery (“adaptive” Tregs) (Fig. 1). The forkhead/winged helix transcription factor, Foxp3, is specifically expressed in CD4⁺CD25⁺ Tregs and is critical to their development [10–12]. As such, Foxp3 has been used as a marker for these cells. However, it has recently been reported that antigen-induced IL-10-secreting regulatory T cells do not express Foxp3, despite comparable regulatory function to naturally occurring CD4⁺CD25⁺ Tregs [13]. In that study, antigen-driven (ie, “adaptive”) Tregs were generated both in vitro using anti-inflammatory drugs (1 α ,25-dihydroxyvitamin D3 and dexamethasone) and in vivo by repetitive intranasal administration of peptide antigen. The suppressive effects of these IL-10-secreting Tregs were IL-10-independent but cell contact-dependent, implying a role for cell-surface molecules. Cell-surface molecules that have been implicated in the function of regulatory T cells include glucocorticoid-induced tumor necrosis factor (TNF) receptor-related protein (GITR) and cytotoxic T-

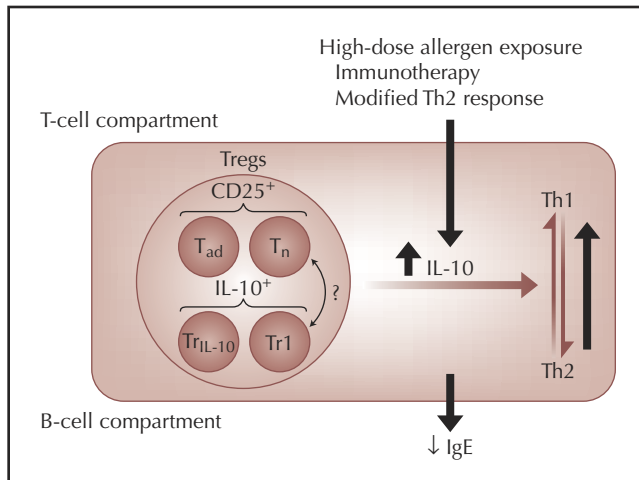


Figure 1. Influence of IL-10 on the allergic response. It has been proposed that IL-10 acts as a central regulator to influence the balance between Th1 and Th2 effector cells. Regulatory T cells comprise a heterogeneous population and are an important source of IL-10. These cells include both natural (T_n) and adaptive (T_{ad}) CD4⁺CD25⁺ T cells, as well as antigen-driven IL-10-secreting T cells (Tr_{IL-10}) and type 1 T-regulatory (Tr1) cells. Adaptive CD4⁺CD25⁺ T cells may encompass Tr_{IL-10} cells; however, the relationship between different types of regulatory T cells has not been fully defined. High-dose allergen exposure may enhance IL-10 secretion by regulatory T cells and alter the interplay between Th1 and Th2 subsets, thereby augmenting IFN- γ secretion and inhibiting Th2-driven allergic processes. The net effect of this T-cell interplay is to suppress production of IgE. *Black arrows* indicate the influence of high-dose allergen on IL-10 and consequent effects on T- and B-cell compartments. IFN—interferon; IL—interleukin.

lymphocyte antigen (CTLA-4). Triggering through GITR abrogates the suppressive effects of Tregs; however, the role of the inhibitory co-stimulation receptor, CTLA-4, in Treg suppression remains controversial [14–16]. It is not known whether GITR and CTLA-4 fulfill a functional role in IL-10-secreting CD4⁺CD25⁺ Tregs. Adding to the complex picture, it has recently been proposed that the mechanism of suppression of Tregs is dictated by the nature of the antigenic stimulus [17•]. Thus, it is possible that the same type of regulatory T cell operates preferentially through cytokines or cell surface molecules under different conditions.

In humans, the nature of regulatory T cells involved in allergic disease is ill-defined. This may be attributed to the limitations of *in vitro* systems for analyzing the properties of these cells, and confusion regarding the delineation of different types of Tregs. For example, in the murine system there appears to be considerable heterogeneity of Tregs, even among IL-10-secreting regulatory T cells (Fig. 1). This is highlighted by comparing the properties of antigen-driven IL-10-secreting CD4⁺CD25⁺ Tregs and CD4⁺ type 1 T-regulatory (Tr1) cells. Tr1 cells differentiate in the periphery, but unlike antigen-driven CD4⁺CD25⁺ Tregs, they require IL-10 for their generation and appear to exert suppressive effects through secretion

of IL-10 as opposed to through cell contact [18,19]. Moreover, these cells do not constitutively express high levels of CD25 or Foxp3 [20]. Taken together, these observations highlight the complex nature of regulatory T cells.

Allergen-specific Induction of Interleukin-10: Evidence for Protection

In humans, there is convincing evidence that allergen can induce IL-10 secretion and that, under certain conditions, this favors the development of a protective nonallergic immune response. High-dose allergen exposure, either via the systemic route, or by inhalation, may be a prerequisite for optimal IL-10 induction. Augmented IL-10 production has been reported in patients who have received immunotherapy (IT) for bee venom allergy and grass pollen allergy [21,22••,23]. In both cases, CD4⁺CD25⁺ T cells were implicated as the source of IL-10. In atopic patients receiving a rush IT regimen for bee venom allergy, increases in IL-10 in peripheral blood mononuclear cell (PBMC) cultures stimulated with the major bee venom allergen, phospholipase A2 (PLA₂), occurred within days [21]. These changes were associated with marked decreases in PLA₂-induced T-cell proliferation and production of Th1 and Th2 cytokines. Furthermore, increased staining for intracytoplasmic IL-10 was observed within CD4⁺CD25⁺ T cells in both freshly isolated samples and PLA-stimulated cultures after IT. By contrast, in patients with allergic rhinitis who received conventional IT, increased IL-10 mRNA-positive cells were detected in nasal mucosa only after 2 years, and these increases were restricted to the pollen season [22••,23]. In the same study, intracellular expression of IL-10 localized to CD4⁺CD25⁺ T cells in PBMC cultures; however, because phenotypic analyses were performed after allergen stimulation, these CD25⁺ T cells likely contained activated effector T cells and, as such, were not representative of a homogeneous regulatory T-cell population. Moreover, there was a modest increase in T-cell proliferation and marked increase in the frequency of CD4⁺CD25⁺ T cells after allergen stimulation in atopic patients receiving IT compared with patients who received no therapy. Clearly, more detailed analysis of CD4⁺CD25⁺ T cells induced after immunotherapy is warranted. Nevertheless, given the established clinical efficacy of the IT regimens described, these studies establish a link between increased production of IL-10 and modulation of allergic responses toward a protective phenotype.

In studies by our group, enhanced IL-10 production was a feature of PBMCs from nonallergic individuals exposed to high-dose cat allergen (Fel d 1) that was present in the environment [24••,25]. These subjects had a serum antibody profile consistent with a modified Th2 response, which is characterized by the presence of high-titer IgG ab to Fel d 1 in the absence of IgE. Because the

Table 1. Characteristics of distinct immune responses to the major cat allergen, Fel d 1

Classification	Anti-Fel d 1 ab		T-cell reactivity to P2:1*		HLA-DR7+†
	IgE	IgG	Proliferation	IL-10	
Allergic‡	++	+/-	++	+	~10%
Post-immunotherapy	+	++	++	+++	
Modified Th2	-	++	++	+++	50%
Control	-	-	++	+	~35%
Atopic eczema	+++	++	-	-/+	~10%

*An immunodominant epitope of Fel d 1 that is located within the amino-terminal region of polypeptide chain 2 of the molecule.

†Prevalence of subjects who express the major HLA-DRB1 allele, *701.

‡Allergic (atopic) individuals were defined on the basis of positive skin prick test to cat extract and the presence of serum IgE ab to Fel d 1.

modified Th2 response is not associated with IgE production, nor with allergic symptoms, we have proposed that it represents a form of high-dose respiratory tolerance. Thus, in this scenario, increased IL-10 secretion may confer protection. Although PBMC from both allergic (IgE^{pos}) and nonallergic (modified Th2) individuals secreted high levels of IL-10 in vitro in the presence of Fel d 1, enhanced secretion of IL-10 in cultures from modified Th2 subjects was only revealed after stimulation with a specific Fel d 1 peptide. This peptide (designated Peptide (P) 2:1) localized to the amino-terminal region of polypeptide chain 2 of the molecule (Table 1). It is noteworthy that increased IL-10 secretion to P2:1 was restricted to those modified responders who expressed the major HLA-DRB1 allele, *0701. We proposed that expression of DR7 may favor enhanced recognition of this IL-10-inducing CD4⁺ T-cell epitope of Fel d 1, because this peptide contains a putative DR7-binding core epitope [24••]. Although the cell source of IL-10 was not identified in our studies, blocking of IL-10 using a monoclonal antibody directed against the IL-10 receptor (IL-10R) enhanced allergen-induced secretion of the Th1 cytokine, interferon (IFN)- γ , but had no effect on the Th2 cytokines, IL-5 and IL-13. This suggested a preferential effect of allergen-induced IL-10 on IFN- γ . Approximately 50% of modified responders did not express DR7 and exhibited low levels of Fel d 1-induced IL-10 secretion. These observations underscore the influence of genetics in regulation of the allergic response and suggest that alternative factors to IL-10 are involved in the development and persistence of protective nonallergic responses. In addition, and perhaps more importantly, our findings support the view that IL-10 is a feature of both allergic and nonallergic responses, and indicate that the magnitude of the IL-10 response to allergen is relevant to immune outcome.

Several animal studies support observations in human-based systems that implicate IL-10 in the development of protective responses to allergens. Akbari et al. [26••] demonstrated the induction of IL-10-secreting CD4⁺ T cells in the lung after high-dose respiratory exposure to antigen. Development of these "Tr1-like" cells was stimulated by mature (B7^{hi}) IL-10-secreting dendritic cells (DCs). Moreover,

adoptive transfer of pulmonary DCs from IL-10^{+/+} but not IL-10-knockout mice that were exposed to respiratory antigen was shown to induce antigen-specific unresponsiveness (ie, tolerance) in recipient mice. Similarly, adoptive transfer of CD4⁺ T helper cells that were genetically engineered to express IL-10 suppressed airway hyperresponsiveness and lung inflammation [27]. These findings point to modulation of the allergic response by IL-10 during the initiation phase of allergen-specific responses. However, there is also evidence of a role for IL-10 during an established allergic response (ie, the effector phase) based on reversal of the beneficial effects of IT in mice after neutralization of IL-10 [28].

Factors that Influence Allergen-specific Induction of Interleukin-10

Generation of the allergic response in humans is a complex process dictated by a variety of host and environmental factors. Such factors are relevant to induction of IL-10 by allergen and the influence of this cytokine on immune outcome. These include but are not limited to: 1) the timing of IL-10 production (ie, during the initiation phase versus the effector phase of the allergic response); 2) the dose and nature of the allergen; 3) co-exposure to other allergens and environmental adjuvants; and 4) genetics of the host. In relation to the allergen molecule, there is evidence that some allergens are better than others at inducing tolerance. Epidemiologic studies suggest that development of the modified Th2 response to cat allergen is an allergen-specific phenomenon [29,30]. This raises the important question of whether some antigens such as those derived from dust mite are more potent allergens than others. Cat allergen (Fel d 1) induces very low levels of Th2 cytokines in human T cells, irrespective of the allergic status of the donor, consistent with the view that this molecule is a "suboptimal" allergen [24••]. Furthermore, Fel d 1 induces secretion of IL-10 in PBMC cultures from both allergic and nonallergic subjects [24••]. This may be related to an intrinsic capacity to induce IL-10 as evidenced by identification of an immunodominant IL-10-inducing T-cell epitope. Alternatively, this molecule may exhibit anti-inflammatory or immunomodulatory properties based

on its highly conserved three-dimensional structure, which mirrors that of Clara cell secretory protein [31]. Clara cell secretory protein (also known as CC16, CC10, or uteroglobin) is secreted by nonciliated bronchiolar Clara cells and has been shown to modulate cytokine expression by Th1 and Th2 cells [32]. In addition to these factors, an important consideration is that levels of exposure to other allergens may never approach the levels of cat allergen in the environment. Whether development of tolerance to cat allergen is strictly a dose-dependent phenomenon, or also an effect of the properties of the allergen molecule itself, remains open to debate.

As already mentioned, IL-10 is secreted by antigen-presenting cells, and it is possible that the principal effects of IL-10 induction by allergen operate through these cells. The bacterial wall components of gram-negative bacteria (lipopolysaccharide [LPS]) are ubiquitous in the environment and, through binding to toll-like receptors, are potent inducers of IL-10 in a variety of cell types, including dendritic cells and macrophages. An extensive review of the effects of LPS on allergen-specific immune responses is beyond the scope of this article. However, it should be stressed that exposure to allergens does not occur in isolation, but is inevitably associated with co-exposure to bacterial products (and other environmental factors), which may exert an adjuvant effect. Many allergen preparations used in *in vivo* and *in vitro* systems contain LPS, making it difficult to ascertain whether LPS influences the quality of the allergen-specific immune response observed in these experimental systems. However, it was recently shown that high- versus low-dose co-exposure to LPS with inhaled antigen *in vivo* predisposes to a Th1 versus Th2 response in mice, and this effect is dependent upon activation of dendritic cells [33]. This observation was significant in that it provided a potential explanation for the protective effects of increased endotoxin exposure on the development of atopy, otherwise known as the "hygiene hypothesis." However, a definitive link between IL-10 and endotoxin as an adjuvant has not been established in allergen-specific responses.

Interleukin-10 as a Central Regulator of the Allergic Response

The lack of understanding of the properties of IL-10-secreting regulatory T cells and production of IL-10 by different cell types poses a problem when interpreting observations in allergen-specific systems and defining mechanisms of action of IL-10. It is clear that immune cells from both atopic and nonatopic individuals have the capacity to secrete IL-10 in response to allergen. Several studies support the view that the development of distinct allergic phenotypes is dependent upon the degree of control exerted by regulatory T cells. The current view is that IL-10 acts as a key regulator whereby decreased suppression promotes atopy while increased suppression favors a nonatopic response.

However, two major issues remain to be addressed: 1) What is the nature of the regulatory T cell involved? and 2) What is the mechanism of suppression? A decreased suppressive effect of CD4⁺CD25⁺ T cells on allergen-stimulated proliferation and Th2 cytokine responses has been reported for grass pollen-allergic subjects compared with nonatopic individuals [34••]. Similar differences in suppression have been described in birch-allergic and nonallergic subjects, but only during birch season [35]. This latter observation was attributed to a selective failure to suppress Th2 cytokines during pollen season in birch-allergic subjects. Interestingly, evidence of suppression of both Th1 and Th2 cytokines was present in both birch-allergic and nonallergic subjects outside of the pollen season. However, a mechanism for "reduced suppression" in allergic versus nonallergic subjects was not addressed in either of these studies. A defect in regulatory T-cell function or failure of effector T cells to respond to regulatory T cells are two possible explanations. In both the studies described, the effects of CD4⁺CD25⁺ T cells on CD4⁺CD25⁻ responder cells were examined using co-cultures of CD25⁺ and CD25⁻ cells. This system assumes that CD4⁺CD25⁻ T cells represent an unactivated effector T cell population. By contrast, the CD4⁺CD25⁺ subset likely encompasses both natural and adaptive regulatory T cells, as well as any activated effector T cells that may be present in the circulation. We and others have shown that CD4⁺CD25⁺ T cells are expanded in the peripheral blood of patients with atopic eczema who have markedly elevated levels of IgE [36]. These cells appear to represent an atypical subset with both regulatory and effector T-cell properties (Woodfolk et al., Unpublished data). This raises the question of whether, under conditions of chronic allergen exposure, CD4⁺CD25⁺ T cells acquire an effector phenotype. If this is the case, then observations made during allergy season in atopic individuals using CD4⁺CD25⁺/CD4⁺CD25⁻ co-cultures may simply reflect enhanced activation of effector T cells within the CD4⁺CD25⁺ pool rather than diminished suppression *per se*. In support of this, increased proliferation and cytokine secretion has been observed in purified CD4⁺CD25⁺ T cells from atopic donors after activation with allergen [37]. Interestingly, although such CD25⁺ T cells produced increased IL-10 compared with their CD25⁻ negative counterparts, blocking IL-10 had no effect on proliferation or cytokine responses in CD25⁺/CD25⁻ co-cultures containing these cells.

If, indeed, IL-10 acts as a central regulator to influence the "balance" between Th1 and Th2 responses, it would seem logical that its protective effects reflect preferential inhibition of allergy-promoting Th2 responses (Fig. 1). However, thinking beyond the constraints of the Th1/Th2 paradigm, it is becoming increasingly apparent that other T helper cell subsets, including Th1 cells, may actually augment allergic inflammatory processes, particularly in the chronic phase [38]. An effect of allergen-induced IL-10 on both Th1 and Th2 cytokines has been reported [21]. By con-

trast, our studies of cat allergen implied a preferential effect of IL-10 on the Th1 cytokine, IFN- γ [24••]. Despite this, levels of IFN- γ were elevated in concert with IL-10 in allergen-stimulated cultures from DR7⁺ modified responders. Similarly, both IL-10 and IFN- γ secretion were increased in PBMC cultures stimulated with chain 2 peptides of Fel d 1 shortly after initiating conventional IT in cat-allergic patients. These changes were associated with a shift in serum antibody profile analogous to that associated with a modified Th2 response (Table 1). Taken together, these observations point to a coordinated interplay between IL-10-secreting regulatory T cells and Th1 cells, which may inhibit Th2 responses. Akdis et al. [39••] recently proposed that the balance between Tr1 and Th2 cells is a key determinant in the development of allergy. This was based on differences in the frequencies of Th2 and "Tr1-like" T cells between allergic and nonallergic subjects. Specifically, allergic subjects showed an increased frequency of circulating IL-4-secreting allergen-specific T cells whereas nonallergic individuals showed increases in IL-10-secreting allergen-specific T cells. These IL-10-secreting "Tr1-like" cells were reported to inhibit allergen-stimulated proliferation in PBMC cultures from nonallergic donors through multiple suppressor factors. This was based on the observation that neutralization of IL-10, transforming growth factor (TGF)- β , and CTLA-4 significantly enhanced proliferation in allergen-stimulated PBMC cultures reconstituted with "Tr1-like" cells. However, the possibility that other regulatory T-cell types within the PBMC pool may be operating in this system was not excluded.

Interleukin-10 Dysregulation in Allergic Disease

Although this article is focused primarily on the effects of IL-10 at the T-cell level, it should be stressed that this cytokine also acts on B cells to selectively suppress production of IgE while enhancing IgG4 ab responses [40,41]. This has been demonstrated in both allergen-specific and non-antigen-specific systems. Production of IgG4 ab is a prominent feature of serologic responses associated with high-dose allergen exposure, and there is evidence that these antibodies may block allergen uptake by antigen-presenting cells and, thus, T-cell activation [22••]. This strengthens the link between IL-10 and the development of protective immune responses. Based on the premise that high-titer IgE ab may reflect failure of IL-10-mediated control, our group investigated whether there was evidence of IL-10 dysregulation at the T-cell level in individuals with markedly elevated IgE [42••]. Patients selected for study were those with atopic eczema who were sensitized to cat allergen. Titers of cat-specific IgE in these patients were significantly elevated compared with those observed in cat-sensitized subjects who did not have atopic eczema. Interestingly, PBMC from atopic eczema patients failed to proliferate to IL-10- and IFN- γ -inducing T-cell epitopes within poly-

peptide chain 2 of the Fel d 1 molecule. Moreover, there was an altered pattern of IL-10 secretion and lack of IFN- γ production in cultures stimulated with peptides mapping to this putative regulatory region of chain 2 (Table 1). When IL-10 was neutralized, neither T-cell proliferation nor IFN- γ secretion to chain 2 epitopes was restored. This was in contrast with cultures from cat-sensitized subjects without eczema and modified Th2 responders in whom blocking IL-10 enhanced T-cell reactivity to chain 2 epitopes. Interestingly, allergen avoidance was associated with a paradoxical decrease in both IL-10 and IFN- γ production by peptide-stimulated PBMC from eczema patients. We concluded that control of IFN- γ production to chain 2 epitopes by IL-10 may be relevant to allergic status and that this mechanism fails to operate in patients with atopic eczema. Because PBMCs from these patients retain the capacity to secrete IL-10 in response to allergen, a key issue that remains unresolved is whether our findings reflect changes in IL-10-secreting regulatory T cells or changes in responsiveness of effector T cells to IL-10. Either of these scenarios may influence, directly or indirectly, the production of IgE through effects on the B-cell compartment. As already mentioned, we have identified an atypical population of skin-homing CD4⁺CD25⁺ T cells that is expanded in the circulation of AD patients (Woodfolk et al., Unpublished data). These cells express the chemokine receptor CCR4, and high levels of Foxp3. Analysis of the properties of these cells points to altered regulatory T-cell function, and we are currently investigating whether secretion of IL-10 is a feature of this cell population.

Conclusions

It is increasingly apparent that IL-10 is relevant to regulation of the allergic response. However, when examining the role of IL-10 in allergic disease, we have only begun to scratch the surface. Detailed phenotypic and functional studies of human IL-10-secreting T lymphocytes are warranted to define the relationship between these cells and other types of regulatory T cells, and to advance our knowledge of how these cells influence allergic disease. Based on a substantial body of evidence, it is apparent that enhancing induction of IL-10 is an appropriate goal of therapy. A key issue that remains is how this should be achieved to optimize protection. For example, individuals who have a genetic predisposition to secrete low levels of IL-10 may pose a challenge. As already mentioned, the nature of the immunogen is a critical component of immune outcome. Although IL-10-inducing peptide-based vaccines may be useful for treatment of cat allergy, further work is required to determine whether such a strategy would be feasible for other allergens such as those from dust mite. Finally, regardless of the type of allergen, perhaps the greatest challenge is to develop a treatment that consistently achieves suppression of effector T cells that drive allergic inflammatory processes.

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This article identifies T-cell epitope-specific defects in the T-cell repertoire directed against cat allergen in atopic eczema patients with high-titer IgE ab to cat allergen. These changes were associated with altered responsiveness to IL-10.