Molecular mechanisms in allergy and clinical immunology
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T-cell responses to allergens

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The allergic response in human beings is engineered by $CD4^+$ T lymphocytes, which secrete T_H2 cytokines in response to activation by allergen-derived peptides. Although T_H2 cells have been well characterized, defining the properties of allergen-specific T cells has proved challenging in human beings because of their low frequency within the T-cell repertoire. However, recent studies have provided insight into the molecular signature of long-lived human memory T_H2 cells, which are allergen-specific. T-cell responses directed against allergens develop in early life and are heavily influenced by the type and dose of allergen, and possibly coexposure to microbial products. These responses are susceptible to suppression by regulatory T cells. This article highlights recent advances in the characterization of allergen-specific memory T_H2 cells and discusses the heterogeneous nature of regulatory T cells and possible mechanisms of action. The relevance of T-cell epitope mapping studies to understanding the unique nature of T-cell responses to different allergens, as well as to peptide vaccine development, is reviewed. Experimental techniques and approaches for analyzing allergen-specific T cells and identifying novel T-cell epitopes are described that may lead to new T-cell–based therapies. (J Allergy Clin Immunol 2007;119:280-94.)

Key words: *Allergens, T_H1, T_H2, effector memory T cells, central memory T cells, regulatory T cells, LPS, lipid antigens, immunostimulatory DNA sequences, T-cell epitopes, peptide vaccines*

Abbreviations used

AD:	Atopic dermatitis
APC:	Antigen-presenting cell
CLA:	Cutaneous lymphocyte-associated antigen
CRTH2:	Chemoattractant receptor-homologous molecule expressed on T_H2 cells
DC:	Dendritic cell
ISS:	Immunostimulatory DNA sequence
NKT:	Natural killer T
OVA:	Ovalbumin
T_{CM}:	Central memory T cells
T_{EM}:	Effector memory T cells
TCR:	T-cell receptor
TLR:	Toll-like receptor
Treg:	Regulatory T cell
TSLP:	Thymic stromal lymphopoietin

Although different types of T lymphocytes (eg, $CD4^+$, $CD8^+$, and natural killer T [NKT] cells) have the capacity to respond to allergens, $CD4^+$ T cells are the predominant effector population. Type 2 $CD4^+$ T lymphocytes (T_H2), which recognize peptide antigen presented by antigen-presenting cells (APCs) in the context of MHC class II molecules, are pivotal to the allergic inflammatory cascade. These cells exert their effects through production of IL-4, IL-5, and IL-13. IL-4 is not only critical to the development of T_H2 cells but also mediates antibody isotype switching to IgE synthesis, which is a major risk factor for the development of asthma.[1-3] On the other hand, IL-5 orchestrates eosinophil recruitment, which is a pathognomonic feature of this disease.[4-8] The relative roles of different T_H2 cytokines in the pathogenesis of allergic diseases such as asthma and atopic dermatitis have been widely debated.[9-13] Nevertheless, the consensus is that multiple immune pathways governed by a T_H2 cytokine network promote allergic inflammation and the manifestation of clinical symptoms. In the last decade, much has been learned about the general properties of T_H2 cells, including mechanisms of differentiation and activation, and how these relate to cytokine receptor signaling pathways and gene transcription events. However, characterizing allergen-specific T_H2 cells in human beings has proven challenging. This review highlights our current knowledge of, and recent advances in, $CD4^+$ T-cell responses to allergens in human beings.

ALLERGENS AS INITIATORS OF A T_H2 RESPONSE

Numerous murine models of asthma have shown a role for T_H2 cells in both the initiation (ie, sensitization) and

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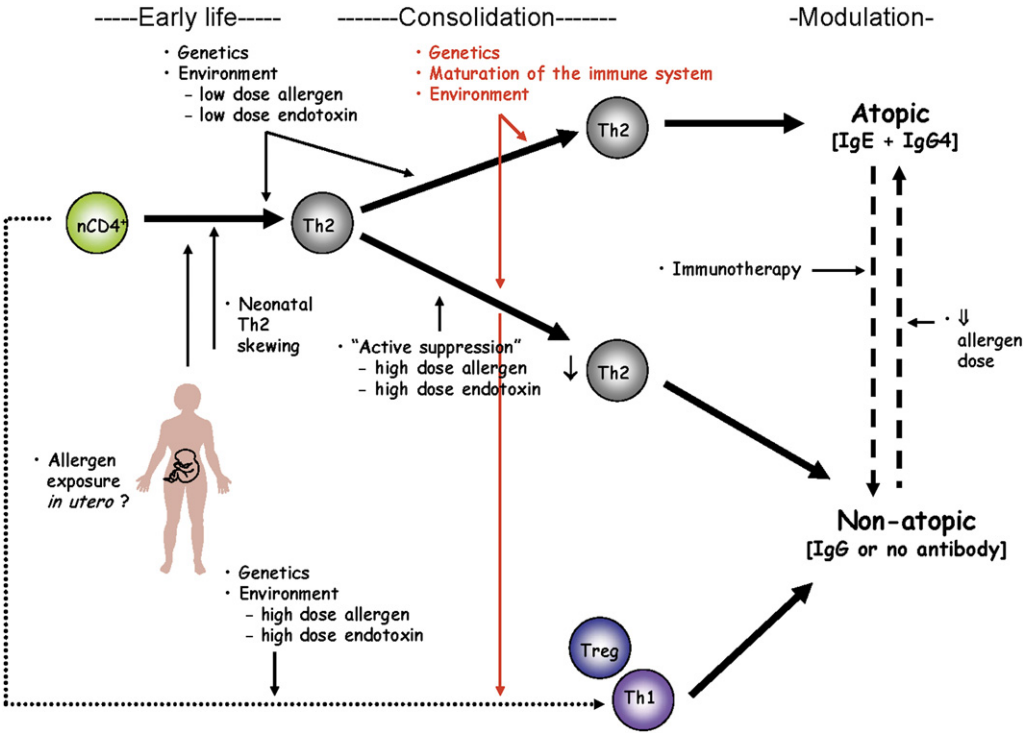

FIG 1. Evolution of T_H2 responses to allergens in human beings. Within the first months of life, multiple factors converge to influence the generation or enhancement of T_H2 responses. During the consolidation phase, T_H1 or T_H2 responses are strengthened. The atopic and nonatopic state are not fixed, but can be altered by therapeutic (immunotherapy) or environmental (change in allergen dose) intervention.

effector phases of the allergic inflammatory response. In addition to the use of mouse strains with a genetic T_H2 predisposition (BALB/c), a major criticism of murine studies has been the mode of sensitization and the nature of the antigen used. Most models incorporate an immunization regimen consisting of sensitization with ovalbumin (OVA) via the intraperitoneal route followed by respiratory challenge with OVA. Obviously, OVA is not a relevant inhalant allergen to human beings; however, regimens incorporating relevant allergens can also elicit T_H2 responses in mice. By using an intratracheal sensitization regimen, which more closely mimics the natural route in human beings, dendritic cells (DCs) have been shown to play a central role during both T-cell priming and subsequent effector T-cell responses to allergen within the lung.[14-16]

Despite considerable advances in our understanding of the nature of allergens at the molecular level, the question of why these molecules preferentially promote T_H2 responses in a subset of individuals remains enigmatic. Diverse epidemiology studies have confirmed that the allergic phenotype, as judged by the production of allergen-specific IgE antibodies, develops as early as the first 2 years of life. Whether this is promoted by allergen-driven T_H2 priming in predisposed individuals, and at what point this occurs, has been controversial. Prescott et al[17] suggested that transplacental delivery of allergen induces T_H2 priming in utero and that this phenomenon is universal (Fig 1). This was based on induction of T_H2 cytokines in cord blood mononuclear cells derived from both high-risk and low-risk infants after *in vitro* stimulation with allergen. Development of atopy was proposed to reflect failure to redirect T_H2-skewed fetal responses in early life. However, an alternative explanation for these observations is the universal propensity to T_H2 skewing in neonates, which occurs independently of allergen exposure.[18-20] This phenomenon is well recognized and reflects the unique properties of immature T cells and DCs.

Few studies have examined how the cytokine milieu in early life influences allergen-specific responses and/or the subsequent development of atopy. Analysis of PHA-activated T cells within the first 12 months of life showed no clear-cut T_H2 polarization in children with an atopic predisposition.[21] By contrast, other work implied that allergen-specific T_H2 responses are present at birth in all infants, but progressively decline during the first months of life in nonatopic children.[22] Approaching from another angle, other studies have attempted to link enhanced production of the T_H1-promoting cytokine, IL-12, to allergen-induced IFN-γ in early life.[23] However, there is little evidence to suggest that such a response in early life is protective.

On the basis of existing data, patterns of T_H2 cytokine induction in early life are inconsistent, and evidence for established allergen-specific memory T_H2 responses within the first months of life is not convincing. It seems likely that allergen-specific memory T_H2 cells develop *de novo* within the first years of life through gene/

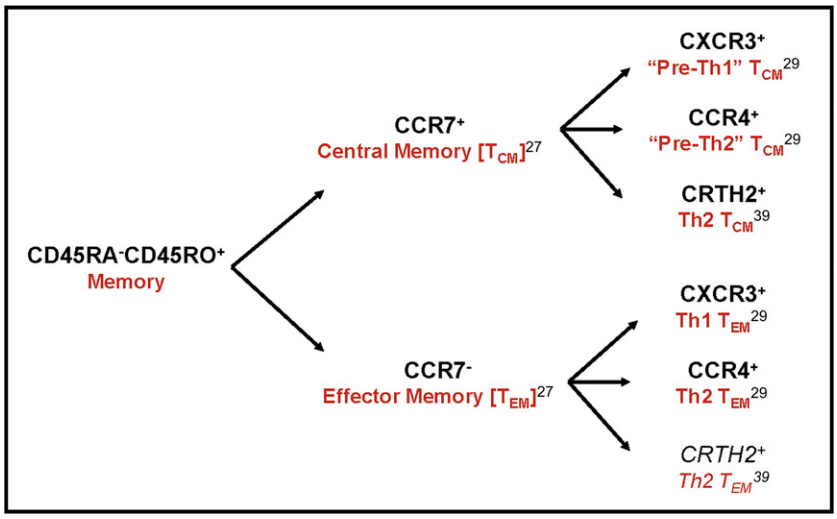

FIG 2. Phenotypic properties of human memory $CD4^+$ T-cell subsets. Numbers denote references. $CRTH2^+$ T_H2 T_{EM} cells have not been formally demonstrated; however, $CRTH2^+CD3^+$ T cells identified in skin lesions of patients with AD would be predicted to exhibit an effector memory phenotype.[39]

environment interactions (Fig 1). In the longer term, the development of sensitization and allergic disease in children across a wide age range suggests that memory T_H2 responses continue to evolve over a prolonged period.[24-26] With this in mind, monitoring memory T_H2 cells through childhood into early adulthood could yield insight into the influences of gene/environment interactions and maturation of the immune system on memory T_H2 responses.

WHAT CONSTITUTES A MEMORY T_H2 RESPONSE TO ALLERGEN IN HUMAN BEINGS?

Chemokine receptors and memory phenotype

The recurrence of allergic symptoms after a period of lack of allergen exposure (eg, in patients with seasonal allergic rhinitis) reflects the presence of allergen-specific memory T-cell responses. The nature of memory T cells has been extensively studied and is reviewed elsewhere.[27,28] Expression of the CD45RO isoform on memory T cells distinguishes them from their naive counterparts that express CD45RA. Memory $CD4^+$ T cells have been further classified into 2 distinct types on the basis of expression of the chemokine receptor, CCR7. Central memory T cells (T_{CM}) that express CCR7 preferentially home to secondary lymphoid tissues, and proliferate on antigen encounter, giving rise to a progeny of effector T cells. By contrast, effector memory T cells (T_{EM}), which lack CCR7 expression, traffic to inflamed tissues and respond rapidly on activation by secreting cytokines, thereby exerting their effector functions (Figs 2 and 3).[27] Despite what we know about memory T cells and T_H2 cells in general (eg, selective expression of the transcription factors GATA-3 and c-Maf), it has been difficult to pinpoint cell surface markers restricted to memory T_H2 cells. Distinct patterns of chemokine receptor expression distinguish pre-T_H1 and pre-T_H2 central memory cells. Specifically, T_{CM}, which express the chemokine receptor CXCR3, secrete low amounts of IFN-γ and differentiate into T_H1 effector memory cells in response to T-cell growth factors. By contrast, T_{CM} cells, which express CCR4, produce IL-4 and differentiate into T_H2 effector memory cells (Figs 2 and 3).[29] Although CCR4 has been touted as a marker of T_H2 cells, this receptor is also expressed on all skin-homing (cutaneous lymphocyte-associated antigen–positive) memory T cells and may facilitate trafficking to the skin.[30] Moreover, memory T cells with both T_H1 and T_H2 potential, as well as regulatory T cells (Tregs), have now been shown to express CCR4.[31-35] Analysis of other chemokine receptors has failed to reveal any clear-cut segregation in expression patterns among T_H1 and T_H2 cells, and expression of specific receptors is inconsistent within the T_H2 subset.[36] Thus, no reliable chemokine receptor marker has been identified for memory T_H2 cells in human beings.

Chemoattractant receptor-homologous molecule expressed on T_H2 cells: A marker of allergen-specific memory T_H2 cells?

In the late 1990s, selective expression of a gene encoding a novel G protein-coupled receptor named chemoattractant receptor-homologous molecule expressed on T_H2 cells (CRTH2), was described in human T_H2 cells.[37] CRTH2 is a receptor for prostaglandin D_2, which is expressed on 0.4% to 6.5% of circulating memory ($CD45RA^-$ $CD45RO^+$) $CD4^+$ T cells. T-cell receptor (TCR) triggering was shown preferentially to induce production of IL-4, IL-5, and IL-13 by these cells.[37,38] Recent work by Yong-Jun Liu's group[39] showed that $CRTH2^+CD4^+$ cells from normal donors exhibit a central memory phenotype and respond to allergen *in vitro* (Fig 2). Interestingly, priming of DCs with the IL-7–like

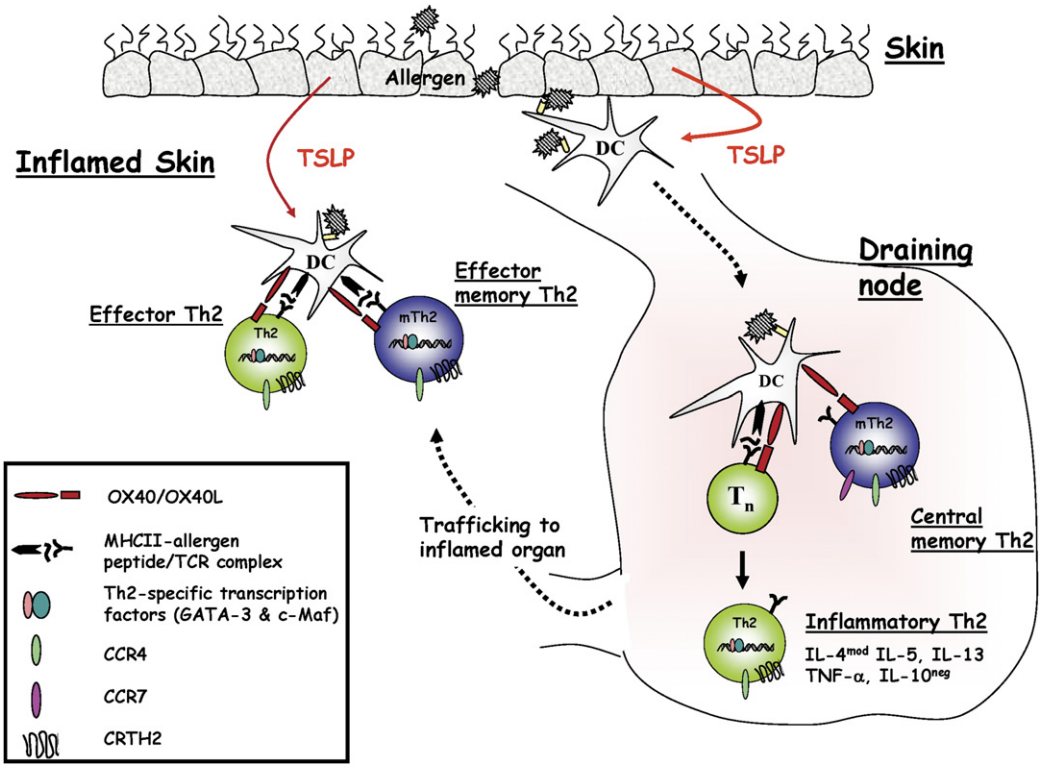

FIG 3. Immune pathways for induction and maintenance of allergen-specific T_H2 cells in human beings. Migration of TSLP-activated DCs loaded with allergen may induce inflammatory allergen-specific T_H2 cells from naive T cells. TSLP-activated DCs also maintain allergen-specific central memory T_H2 cells, possibly in the absence of allergen. Signaling through OX40/OX40 ligand is a prerequisite for both T-cell pathways. At sites of inflammation, DCs activate allergen-specific effector/memory T_H2 cells *in situ*. *mTh2*, Memory T_H2 cells; T_n, naive T cells.

cytokine, thymic stromal lymphopoietin (TSLP), was a prerequisite for generating allergen-specific recall responses in $CRTH2^+CD4^+$ T cells. These findings contradict numerous studies showing that $CD4^+$ T-cell recall responses are readily generated *in vitro* in response to allergen in the absence of exogenous TSLP. Given that $CRTH2^+CD4^+$ T cells have been identified in the lesional skin of patients with atopic dermatitis (AD), it will be important to determine whether these cells constitute an activated effector memory subset in the blood of allergic individuals.[39]

Work by Liu's group has also shown that TSLP-activated DCs can maintain $CRTH2^+CD4^+$ T cells *in vitro* without altering their central memory phenotype or T_H2 commitment.[39] This provides insight into how allergen-specific memory T_H2 cells may persist *in vivo* for many years. In the last few years, TSLP has emerged as an important mediator in allergic inflammatory processes, possibly acting as a principal architect of the T_H2 response through induction of inflammatory (TNF-α^+) T_H2 cells.[40-46] TSLP is expressed at high levels in keratinocytes of skin lesions in patients with AD. The presence of TSLP at the environmental interface raises the important question of how allergen contributes to its induction and subsequent differentiation of allergen-specific effector/memory T_H2 cells (Fig 3).

Evidence for long-lived allergen-specific memory T cells

Whether memory cells arise from effector cells or from naive T-cell precursors has been widely debated.[28,47] In human beings, the kinetics for generation of allergen-specific memory T_H2 cells are not known. Moreover, it is difficult to assess whether the memory T-cell repertoire directed against allergens is shaped by chronic allergen exposure. It is clear that allergen-specific memory T cells are long-lived on the basis of the persistence of allergen-specific T-cell clones for many years in the blood of allergic subjects.[48] One school of thought is that *in vivo* persistence of memory T cells is antigen-independent. As an example, asthma can be induced in mice as long as 400 days after a sensitization and challenge regimen in which antigen depots are avoided by administering allergen in the absence of adjuvants.[49] In considering the molecular pathways involved, interactions between the costimulatory molecule OX40 expressed on memory $CD4^+$ T cells, and OX40 ligand expressed on DCs, have been implicated in the persistence of allergen-specific memory T cells, as well as in recall responses to allergen in both human beings and mice (Fig 3).[39,46,50] Although persistence of memory T_H2 cells may be antigen-independent, it is possible that these cells are maintained by allergen-induced factors (eg, TSLP) that exert their effect in the absence

of allergen. Regardless, the long-lived nature of allergen-specific memory T_H2 cells highlights the importance of designing therapeutic interventions that target these cells.

PLASTICITY OF THE T-CELL RESPONSE TO ALLERGENS

It seems logical that allergen-specific T-cell responses that develop in early life in parallel with maturation of the immune system would be more susceptible to change than established T-cell responses in adulthood. However, T-cell responses to allergen can be influenced at all stages of life through changes in natural exposure to environmental antigens and therapeutic intervention.

Innate signals mold the T-cell response to allergens in early life

Beginning in early childhood, the environment is a major determinant of atopic status, as evidenced by the decreased prevalence of atopy and asthma among children living on farms who are exposed to high levels of endotoxin.[51,52] A similar effect has been reported for children living with multiple pets, which could also reflect endotoxin exposure.[53] In keeping with this, increased domestic endotoxin exposure in early life has been linked to increased circulating $CD4^+$ T cells that express IFN-γ.[54] The idea that enhanced T_H1 immunity arising from increased exposure to microbial products inhibits the subsequent development of T_H2 responses to allergens is a basic tenet of the hygiene hypothesis. Accordingly, murine models of asthma have shown that intranasal coexposure to high-dose LPS favors the induction of a T_H1 response to allergen, whereas low-dose LPS induces a T_H2 response (Fig 1).[55] Although these observations appear to provide a simple explanation for the protective effects of bacterial exposure, the situation is likely far more complex. Key issues that remain to be addressed include the following: (1) What constitutes a protective dose of endotoxin/LPS, if any, in the indoor environment, given that domestic levels are likely much lower than those found on farms? (2) Is coexposure to endotoxin and allergen a prerequisite for protection from allergy? (3) What mechanisms are involved at the T-cell level, and is the timing of exposure relevant?

Considering these points, intranasal exposure to LPS within the first week of life inhibits subsequent OVA-induced asthma in mice through induction of putative regulatory T cells ($CD25^+IL\text{-}10^+$).[56] Surprisingly, asthma is also inhibited after exposure to respiratory allergen during the neonatal period, through induction of a T_H1 response. These findings suggest that protective responses within the respiratory tract in very early life do not require innate signals, but can be induced by allergen alone. In addition, murine studies have demonstrated an influence of the sequence of exposures to bacterial products and allergen on the type of allergen-specific T-cell responses generated.[57,58] Few studies have addressed these issues in human beings. However, there is evidence of increased responsiveness to LPS in nasal mucosa derived from children compared with adults as judged by increased expression of IL-10.[59] This highlights how LPS exposure in early life may influence allergen-specific T-cell responses.

Influence of high-dose allergen exposure on T-cell responses in early life

As mentioned, T-cell exposure to allergen in early life may elicit a protective response under certain conditions. A decreased prevalence of cat allergy has been reported among children living in a house with a cat.[60] Whether this effect arises from increased endotoxin levels or high-dose allergen exposure remains controversial. However, there is increasing evidence to support the latter theory. This includes the following: (1) low levels of endotoxin in houses with cats, which are comparable to houses without animals; (2) decreased rates of sensitization to cat among children exposed to high-dose allergen (>20 μg Fel d 1 per gram dust); (3) the presence of a unique allergen-specific serum antibody profile (IgG^{high} IgE^{neg}) in the absence of allergic symptoms in these children, termed a "modified T_H2" response; and (4) enhanced production of IL-10 induced by defined regions of the Fel d 1 molecule in PBMC cultures from adults with a modified T_H2 (or tolerant) response.[25,61-63] Collectively, these data suggest that the effects of high-dose exposure to Fel d 1 are allergen-specific and are mediated at the T-cell level. Interestingly, among college students in Central Virginia, atopy is uncommon among subjects with a modified T_H2 response to cat allergen (Woodfolk and Platts-Mills, personal observations, April 2005). This could be explained by decreased atopic predisposition at the genetic level or an influence of Fel d 1 exposure on the immune response to other allergens. It is tempting to speculate that high-dose exposure to cat allergen enhances IL-10 production, possibly through induction of regulatory T cells in early life, thereby suppressing T_H2 responses to other allergens.

Role of regulatory T cells

A patient history describing allergic symptoms to cats on returning home after 3 months in college is familiar to many physicians. Given the role for regulatory T cells (Tregs) in controlling the allergic response, reduced activity of these cells could explain this phenomenon. The implication is that the modified T_H2 response induced by high-dose exposure to cat allergen is susceptible to change and requires high-dose exposure for persistence. The prevailing T-cell paradigm in relation to allergic disease dictates that Tregs influence the balance of allergen-specific T_H1 and T_H2 cells (Fig 4). As such, the activity of Tregs may be critical in the immune outcome to allergen and hence the development of allergic versus nonallergic responses. Some investigators would argue that enhanced Treg activity favors T_H1 skewing and the development of nonallergic responses. This could occur by increasing the frequency of T_H1 cells within the allergen-specific T-cell repertoire, or by diminishing the frequency of T_H2 cells. Working within this framework, the plasticity of the

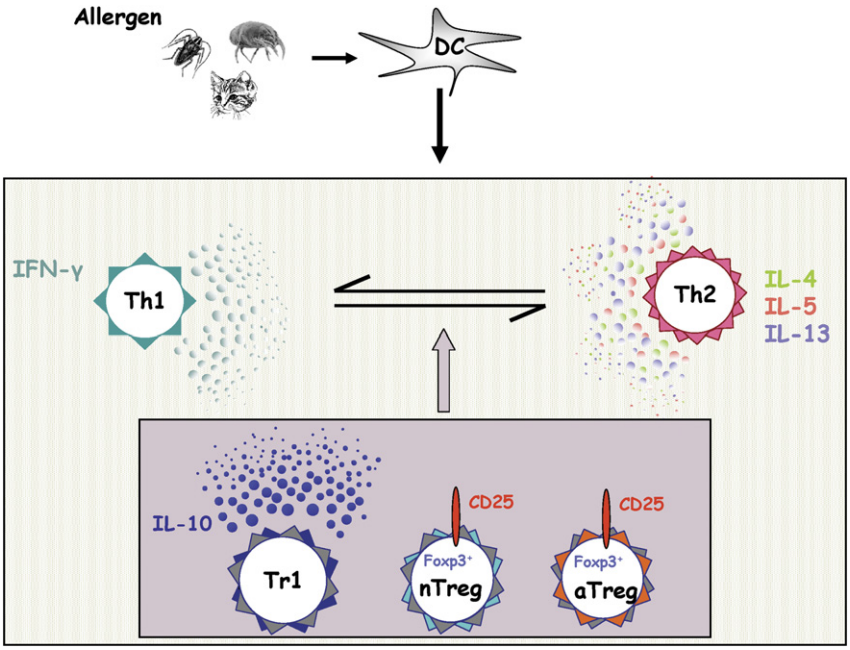

FIG 4. Regulatory T cells act to control the T_H1/T_H2 balance. Enhanced control by Tregs induces T_H1 skewing, whereas decreased regulation favors T_H2 skewing. The Treg compartment is composed of a heterogenous population of cells including IL-10–secreting T regulatory 1 cells *(Tr1)*, as well as natural *(nTreg)* and adaptive *(aTreg)* Tregs.

modified T_H2 response associated with changes in exposure to cat allergen suggests that the proportion of allergen-specific T_H2 cells versus regulatory T cells is allergen-dependent. Specifically, increased exposure to cat allergen may increase the suppressive effect of Tregs, resulting in tolerance, whereas decreased exposure reduces this effect, resulting in allergic symptoms. Thus, Tregs may act to control both allergic and nonallergic responses, with the *degree* of control determining immune outcome.

The mechanisms whereby allergen induces and maintains Tregs *in vivo* are unknown. Studies that have examined IL-10 as a surrogate marker of Tregs in early life have found no evidence of enhanced levels induced by allergen or LPS in T cells from children or infants living in an endotoxin-high environment.[51,64] Most studies of Tregs in allergic disease have focused on atopic and nonatopic adults with established immune responses. Akdis et al[65] reported an increased frequency of allergen-specific IL-10–secreting Tregs in nonatopic donors. Induction of putative Tregs ($CD4^+CD25^+IL\text{-}10^+$) has also been described in the development of protective responses resulting from conventional immunotherapy and in tolerance to bee venom in subjects who have received repeated bee stings.[66,67] A role for $CD4^+CD25^+$ T cells in suppressing T-cell responses to grass pollen allergen has also been described; however, that study did not examine IL-10.[68] Conversely, several studies have implied that the suppressive function of Tregs is compromised in a variety of atopic diseases in a manner dependent on natural exposure to allergen or products produced by skin-colonizing bacteria.[68,69]

There are conflicting data on the nature of Tregs involved in suppression of the allergic response (Fig 4). Specifically, the properties of the cells are not consistent with specific types of Tregs described in the immunology literature. Both natural and adaptive Tregs express the IL-2 receptor α-chain, CD25, and the transcription factor Foxp3. Natural Tregs are produced by the thymus and exert their effects primarily in a contact-dependent manner. By contrast, adaptive Tregs are induced in the periphery and mediate their suppressor effects through TGF-β or IL-10, although cell contact may also be a requirement.[70] Studies in mice indicate a role for TCR engagement in induction of adaptive Tregs. Another type of Treg is the T regulatory 1 cell, which lacks Foxp3 expression and is antigen-specific. These cells can be induced *in vitro* using a variety of factors, and mediate their effects largely through IL-10. Differences in the properties of murine and human Tregs pose a major challenge when defining the role of human Tregs in allergic disease. For example, Foxp3 was originally reported to be selectively expressed in $CD4^+CD25^+$ Tregs. However, in contrast with mice, Foxp3 expression can be induced *in vitro* in activated $CD25^-Foxp3^-$ human cells. This suggests that some $Foxp3^+$ cells isolated from patients with inflammatory diseases represent effector T cells.[70] Consistent with this theory, we have isolated a subset of $CD4^+CD25^+$ cells from patients with severe AD that express Foxp3 but secrete T_H2 cytokines and fail to suppress T_H2 cytokine production ex vivo.[71] Key questions that remain to be addressed include the following: (1) What is the antigen specificity of Tregs? (2) Is the type of Treg induced dependent on the route of allergen exposure (eg, injected or

inhaled)? (3) Are different types of Tregs induced simultaneously? (4) Do the nature and dose of the allergen determine the type of Treg induced?

ALLERGEN-SPECIFIC T-CELL RESPONSES IN HUMAN BEINGS

Responses to whole allergen do not fit a T_H2-restricted profile

Numerous studies have attempted to identify characteristics of allergen-specific T cells in patients with allergy that distinguish them from those in nonallergic subjects. Although allergen-specific T-cell clones with a T_H2 phenotype can be isolated from patients with allergy,[72] the cytokine-secreting patterns do not strictly adhere to the T_H2 definition. Indeed, in subjects sensitized to food (ovomucoid) and wasp venom (Ves v 5) allergens, allergen-specific T-cell clones are predominantly T_H1-like or T_H0-like.[73,74] Thus, exposure to allergens through the gastrointestinal tract or systemically may favor parallel priming of distinct T_H subsets, which nevertheless drive allergic disease.

It is clear that PBMCs can be activated *in vitro* to proliferate and secrete cytokines in the presence of allergen regardless of allergic status of the donor. In bulk culture systems, the dichotomy between T_H2 and T_H1 responses in subjects with and without allergy, respectively, is often blurred. This is not surprising given that allergen-specific T cells are heterogeneous at the single cell level. Moreover, a full compliment of memory T cells with diverse antigenic specificities is present in PBMC cultures, and allergen stimulation likely results in bystander activation of nonallergen-specific T cells. That being said, enhanced production of T_H2 cytokines in cultures from donors with allergy has been widely reported. Interestingly, recent observations in murine asthma models indicate that T_H1 cells may actually be required to generate an efficient T_H2 response and the associated inflammatory sequelae.[75,76] Thus, a mixed T-cell landscape likely contributes to the pathogenesis of asthma and is evident at all stages of the disease process.

Epitope mapping: What have we learned?

In the last decade, elucidation of the primary amino acid sequence of many allergens has facilitated analysis of T-cell epitopes. Typically, such studies use synthetic peptides (15 to 20 amino acids in length) spanning the length of the molecule to map regions targeted by T cells. A major challenge has been to identify allergy-related or protective epitopes. Epitope mapping is inherently tricky using PBMC cultures on the basis of the low precursor frequency of allergen-specific cells within the $CD4^+$ T-cell compartment. The challenges and caveats associated with these types of studies are reviewed elsewhere.[77] Alternative approaches have used T-cell lines or clones from atopic individuals to screen allergen-derived peptides. This may be used as a first-line approach before testing the T-cell–activating potential of specific epitopes in a larger number of subjects. Because there is no gold standard for epitope mapping, the variety of approaches used has resulted in a dizzying array of epitopes for certain allergens. Table I highlights the lack of uniformity between multiple studies designed to identify T-cell epitopes of 2 major allergens, Der p 1 and Fel d 1.[63,78-86] Although some allergens (eg, Fel d 1) contain broad immunodominant regions encompassing multiple nested epitopes, other allergens (eg, Can f 1) contain many epitopes scattered throughout the molecule.[63,84,87] On the other hand, for some inhalant allergens (eg, mugwort), T-cell responses may be dominated by a single epitope.[88,89] In those cases, mapping of epitopes to conserved regions of the molecule that cross-react with related food allergens may preferentially drive T-cell recognition of these epitopes.

In general, most individuals have T cells that react *in vitro* to multiple epitopes within the same allergen, irrespective of allergic status; however, each subject's T-cell reactivity profile is unique and typically dominated by 1 or 2 epitopes. Few studies have identified clear-cut differences in the patterns of T-cell epitope recognition between subjects with and without allergy. The dermatophyte allergen derived from *Trichophyton rubrum*, Tri r 2, is an exception.[90] Specifically, comparison of T-cell reactivity to overlapping peptides of Tri r 2 among subjects with immediate hypersensitivity and delayed type hypersensitivity skin tests to this protein revealed striking differences in proliferation to a single peptide located within the amino-terminal region of the molecule. However, most other allergens do not elicit delayed skin tests in subjects without allergy. Thus, similar T-cell epitope reactivity in subjects with contrasting allergic status may indicate a lack of dichotomy in T_H immune responses, pointing instead to more subtle differences at the T-cell level. As an example, although the same epitope may elicit T-cell reactivity in subjects with and without allergy, the frequency of recognition may be increased in subjects with allergy. This may be explained by the presence of an HLA-restricting element within the epitope, which is expressed at high frequency in sensitized subjects.[91] Alternatively, qualitative differences (ie, cytokine induction or proliferation) in the T-cell response directed against the same epitope may be observed. Such differences may be revealed only when attention is paid to the HLA binding potential of the epitope in question and the HLA type of subjects studied.[63]

Are responses in the periphery reflective of the local inflammatory response?

Most studies of T-cell responses to allergens have focused on cells from peripheral blood. An ongoing debate surrounds the relevance of T cells in the periphery to immune events occurring at sites of allergic inflammation (eg, respiratory tract and skin). Recently, increased circulating IL-4–producing $CD4^+$ T cells have been correlated with markers of airway inflammation.[92] However, few studies have examined changes in circulating allergen-specific T cells that are associated with asthma exacerbations. Moreover, because no definitive lung-homing

TABLE I. Summary of T-cell epitope mapping studies of major allergens from house dust mite (Der p 1) and cat (Fel d 1)

Allergen	T-cell epitopes	Patients	Molecular tool	Experimental system	Readout	Reference
Der p 1	aa45-67, 94-104, 117-143	Atopic* (n = 2)	Recombinant deletion proteins (>45aa) fused to GST and synthetic peptides	T-cell clones	T_H2 cytokines	78
	aa1-56	Atopic* (n = 35)	Overlapping recombinant fragments (39-114aa)	PBMCs	Proliferation	79
	aa107-119, 110-119, 110-131	Atopic* (n = 1)	Recombinant peptide fragments	T-cell lines and clones	Proliferation	80
	aa105-133	Atopic* (n = 14) Nonatopic (n = 11)	Pooled overlapping synthetic peptides (19aa)	PBMCs†	Proliferation and IL-5	81
	aa110-128A or 110-128V‡	Atopic* (n = 15) Nonatopic (n = 13)	Polymorphic synthetic peptides (19aa)	PBMCs	Proliferation	82
Fel d 1	Chain 1: aa39-52, 53-66 Chain 2: aa9-21, 22-35, 57-70	Atopic* (T-cell lines, n = 4; T-cell clones, n = 1)	Overlapping synthetic peptides of Fel d 1 chains 1 and 2 (12-16aa)	T-cell lines and clones	Proliferation	83
	Chain 1: aa9-55, 56-69 Chain 2: aa74-92	Atopic* (n = 53)	Overlapping synthetic peptides of Fel d 1 chains 1 and 2 (14-26aa)	T-cell lines	Proliferation	84
	Chain 1: aa29-42: allergic Chain 1: aa37-55: nonallergic	Allergic* (n = 42) Nonallergic (n = 16)	Overlapping synthetic peptides of Fel d 1 chains 1 and 2 (14-26aa)	T-cell lines	Proliferation	85
	Chain 1: aa29-69: ↑ IL-5:IFN-γ ratio in cat-allergic asthmatics *No difference in proliferation between groups*	Cat-allergic asthmatics* (n = 20) Non–cat-allergic asthmatics (n = 10) Cat-allergic patients with rhinitis* (n = 10) Normal controls (n = 10)	Overlapping synthetic peptides of Fel d 1 chain 1 (17-18aa)	PBMCs	Proliferation and IL-5/IFN-γ	86
	Chain 1: aa8-24, aa36-52 Chain 2: aa1-17: ↑IL-10 in $DR7^+$ modified T_H2 aa8-24: IFN-γ -inducing	Cat-allergic* (n = 14) Modified T_H2 (n = 12) Control (n = 11)	Overlapping synthetic peptides of Fel d 1 chains 1 and 2 (17aa)	PBMCs	Proliferation and IL-5/IL-10/ IL-13/IFN-γ	63

aa, Amino acids; *GST*, glutathione S-transferase.
*On the basis of perennial rhinitis, serum IgE, or positive skin test.
†Cells cultured in serum-free medium.
‡Peptide incorporating Ala/Val substitution at amino acid position 124.

marker has been identified to date, tracking T cells in the periphery that contribute to inflammatory events in the respiratory tract has not been feasible. By contrast, expression of CLA is a hallmark of T cells with skin-homing potential. In patients with AD, induction of T-cell reactivity against allergens occurs predominantly within the CLA^+ subset.[93] Interestingly, increased circulating $CD4^+CD25^+$ T cells are present in patients with AD, and a significant proportion of these cells express CLA.[71,94] This may reflect the large surface area of skin that is affected in AD and trafficking of Tregs and/or activated effector cells through the circulation. In an attempt to link T cells in the periphery with local events, several studies have characterized T cells present in

bronchoalveolar lavage fluid. Although this has defined the expression profile of cytokine/chemokine receptors on lung cells, it is not clear how these cells relate to their phenotypic counterparts in the periphery. In addition, it is important to consider that when sampling the lung, allergen-specific T cells are likely to be only a minor component of the T-cell inflammatory infiltrate.

Analysis of TCR Vβ usage has been used to identify allergen-specific T cells on the basis of the premise that allergen drives clonal expansion of T cells expressing specific TCR Vβ chains. A higher frequency of $V\beta18^+$ and $V\alpha2.3^+$ T cells has been reported in the blood of atopic subjects, and these clonotypes were expanded after *in vitro* stimulation with dust mite allergen.[95] Similar observations have been reported for birch and cat allergens. Expansion of allergen-specific clonotypes has also been reported after allergen challenge in the lung.[96] However, few studies have attempted to correlate these changes with those driven by allergen in the peripheral T-cell repertoire. In one study, T-cell clonotypes present in PBMCs stimulated with dust mite allergen did not reflect clonotypes in skin lesions of patients with AD.[97] Thus, given the existing data, it is important not to overinterpret relationships between allergen-specific T cells at distinct anatomic sites. What is not in dispute is that human studies using T cells from the blood have yielded important insight into the nature of allergen-specific T-cell responses.

Work by Larche's group[98] supports the capacity for allergen-specific T cells to traffic from the periphery to the lung. In those studies, intradermal administration of Fel d 1 peptides induced a marked reduction in airway function in a subset of cat-allergic asthmatics within hours. Importantly, specific peptides present in the injected peptide mixture induced IL-5 production by T cells *in vitro* when presented in the context of HLA-DR molecules expressed in patients with altered lung function. Moreover, the short peptides used lacked the ability to cross-link IgE. These findings imply that allergen-specific T cells in the periphery not only traffic to the lung but also mediate changes in airway function independent of IgE.

ALL ALLERGENS ARE NOT CREATED EQUAL: T-CELL MECHANISMS AT THE MOLECULAR LEVEL

Allergens are a diverse array of molecules. Many different proteins can become allergens, but it is increasingly clear that the magnitude of IgE antibody responses varies for allergens from different sources (eg, dust mite and cat).[99] This has led to the view that some allergens are more potent than others. The major dust mite allergen, Der p 1, is a cysteine protease whose enzymatic activity may enhance allergen delivery and augment inflammatory responses.[100] Cleavage of CD25 by Der p 1 enhances T_H2 skewing by inhibiting IFN-γ and increasing induction of the IgE switch factor, IL-4.[101,102] In addition, cleavage of CD23 (the low-affinity receptor for IgE) by Der p 1 has been proposed to upregulate IgE synthesis by disrupting negative feedback signaling through CD23.[100]

By contrast, the major cat allergen, Fel d 1, is a homologue of uteroglobin, which is constitutively expressed in the lung. Interestingly, uteroglobin represses allergen-induced inflammation in mice through blockade of the prostaglandin D_2 receptor.[103] Whether Fel d 1 exhibits similar anti-inflammatory properties is unknown; however, induction of IL-10 production by T cells is a prominent feature of this molecule in cultures from subjects with and without allergy.[63] Although defined regions of the Fel d 1 molecule preferentially induce specific cytokines (Table I), there are insufficient data to assess whether other allergens exhibit similar properties. However, these observations raise important questions related to the intrinsic cytokine-inducing properties of allergen-derived peptides, which are distinct from functional properties of the whole molecule. Extensive studies on altered peptide ligands have led to the view that the quality of the T-cell response, as judged by the type of cytokines induced, can be influenced by changes in the amino acid sequence of the peptide that alter the MHC-peptide/TCR interaction. Enhanced IL-10 production in cultures from HLA-$DR7^+$ subjects after stimulation with an IL-10–inducing peptide derived from Fel d 1 may be explained by increased affinity and/or stability of the peptide/DR7 interaction coupled with increased density of DR7-peptide complexes at the surface of the APC.[63] This could alter T-cell signaling events to favor induction of Tregs. This being said, the view that peptide ligands influence T-cell cytokine production by inducing qualitatively distinctive signals through the TCR remains controversial.

Environmental exposure to airborne cat allergen is much higher than for dust mite allergen. Moreover, as already mentioned, exposure to high-dose cat allergen is associated with high titer allergen-specific IgG Ab without IgE Ab. Several *in vitro* systems have shown that peptide antigens preferentially induce T_H2 development at low dose, and T_H1 cytokines at higher dose. This phenomenon appears to depend on the total number of T cells initially activated (ie, low numbers at low dose and higher numbers at high dose) and subsequent influences on the cytokine milieu, rather than altered signaling through the TCR.[104] With this in mind, it is tempting to speculate that differences in environmental exposure to high-dose allergens (eg, cat) versus low-dose allergens (mite) induce similar events *in vivo*. Further analysis of the effects of allergen peptide dose on cytokine responses, both in bulk cultures and at the single cell level, may be valuable in resolving this issue.

ALLERGENS AND T CELLS: THINKING BEYOND THE PROTEIN

In the last few years, popularity of the hygiene hypothesis provided impetus to studies on the T-cell immunomodulatory properties of nonprotein bacterial adjuvants such as LPS and CpG-containing immunostimulatory

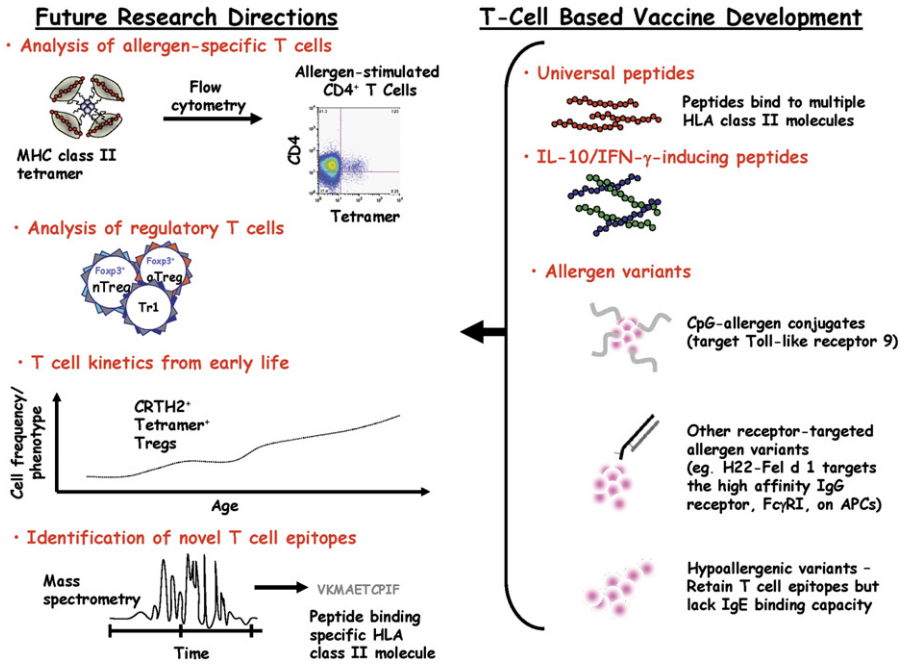

FIG 5. Future research directions and T-cell–based vaccines. Candidate peptides and allergen variants provide useful molecular tools for future research directions as well as for vaccine development.

DNA sequences (ISS). In animal models, immunization with ISS inhibits T_H2-mediated airway responses to allergen.[105] ISS activate Toll-like receptor (TLR)–9 expressed in plasmacytoid DCs and promote the development of T_H1 responses. In the last few years, an immunotherapeutic compound composed of ragweed pollen antigen, Amb a 1, conjugated to ISS has been developed. This conjugate stimulated decreased production of T_H2 cytokines in PBMCs from patients with ragweed allergy, indicating therapeutic potential.[106] A phase 2 clinical trial of this vaccine has recently reported clinical efficacy; however, analysis of T cells showed no difference in cytokine patterns between the treatment group and controls.[107] In mice, inhibition of allergic airway responses by ISS has been attributed to an inability for lung APCs to activate T_H2 cells, but not T_H1 cells.[108] This may occur through TLR9-mediated induction of the inhibitory molecule, programmed death (PD)-1, as well as other costimulatory molecules on APCs. Interestingly, ISS also inhibits IgE-dependent release of T_H2 cytokines from airway basophils and mast cells.[108] This observation is consistent with decreased basophils expressing intracellular IL-4 in patients treated with Amb a 1–ISS conjugate.[107] Thus, whether ISS exert their effects primarily on human mast cells and basophils, which express TLR9, or on T cells via the APC remains to be determined.

Nonprotein components of the allergen molecule itself can influence allergen-specific T-cell responses. For example, glycan structures present on peanut glycoallergens act as T_H2 adjuvants on the basis of increased induction of IL-4^+ and IL-13^+ $CD4^+$ T cells.[109] By contrast, oligosaccharides on the major *Cryptomeria japonica* pollen allergen, Cry j 1, actually inhibit Cry j 1–induced proliferation and IL-4 production.[110] Thus, the oligosaccharides on Cry j 1 do not exhibit T_H2 adjuvanticity, nor are they targeted by T cells. Looking beyond the realm of $CD4^+$ T cells, other types of T cells that recognize nonprotein antigens have been implicated in the immune response to allergens. γδ T cells recognize lipid antigens presented in the context of the nonclassical MHC molecule, CD1d. As an example, the pollen membrane lipid antigen, phosphatidyl-ethanolamine, induces proliferation of cloned γδ T cells, which secrete both T_H1 and T_H2 cytokines.[111] NKT cells, which also recognize nonprotein antigens, have recently been implicated in the induction of airway hyperreactivity via an allergen-independent mechanism.[112] Moreover, NKT cells were reported to make up the majority (60%) of pulmonary $CD4^+CD3^+$ cells in patients with moderate-to-severe persistent asthma.[113] Despite the presence of lipid antigens in pollens, it seems unlikely that such antigens are a major target for T cells in human beings. Consistent with this, phospholipids extracted from cypress grains were shown to stimulate the proliferation of a variety of T-cell types in sensitized subjects, with $CD4^+$ T cells constituting the predominant type and NKT cells responding only rarely.[114] At this point, the role of NKT cells in allergic inflammation is disputed and has not been corroborated.[115] Nevertheless, taken collectively, these findings raise several important questions: (1) How do nonprotein components of allergen molecules influence $CD4^+$ T-cell responses? (2) Does this effect depend on whether the source is mammalian versus nonmammalian? (3) Are the effects dependent on direct linkage or close association of the nonprotein component to the allergen molecule?

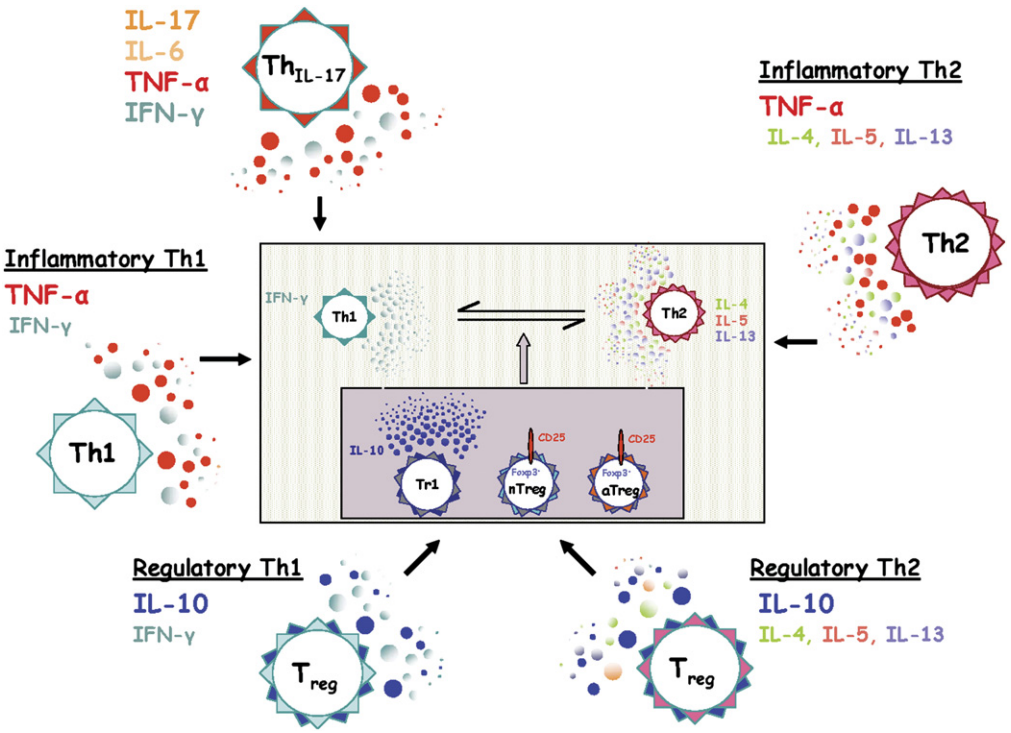

FIG 6. Thinking beyond the T_H1/T_H2/Treg paradigm. This model depicts newly described effector and regulatory T cells that may influence the existing T-cell paradigm.

THERAPEUTIC IMPLICATIONS AND FUTURE DIRECTIONS

The rational design of T-cell–based therapies for allergic disease is dependent on well designed studies of T-cell responses to allergens in human beings (Table II). Rigorous T-cell epitope mapping is warranted to identify peptide candidates for vaccine development. Ideally, these epitopes should induce T-cell reactivity in a genetically diverse population of atopic subjects by binding to multiple HLA class II molecules (Fig 5). Because short allergen-derived peptides lack IgE binding capacity, the risk of adverse events is low compared with conventional immunotherapy. It is tempting to speculate that T-cell epitopes that preferentially induce IL-10 or IFN-γ would be better candidates for therapy than others.[63] To this end, further investigation of the relationship between the amino acid sequence of allergen-derived peptides and their cytokine-inducing properties would be valuable.

Despite what we have learned about T-cell epitopes, peptide vaccines have been relatively slow in coming to fruition, particularly in the United States.[116] Building on knowledge gained from cat peptide vaccine trials in the late 1990s,[117,118] a new vaccine was developed in the United Kingdom that contained shorter peptides that do not bind or cross-link IgE.[119,120] This vaccine, which appears to confer clinical benefit, was associated with changes in allergen-induced T-cell cytokine profiles *in vitro*. Further investigation of T-cell responses to allergens at the epitope level is clearly warranted to hasten the bench-to-bedside transition. In the cancer field, mass spectrometry has proven useful for identifying tumor-associated peptide antigens that have been incorporated into peptide vaccines. Refinements in this technique should make it feasible to identify novel allergen-derived peptides presented in the context of specific HLA molecules (Fig 5). Once new therapies are identified, the efficacy of such vaccines administered in early versus later life will need to be evaluated.

TABLE II. Key points

- Generation of memory ($CD45RA^-CD45RO^+$) T_H2 responses to allergen likely occurs within the first few years of life, rather than within the first few months or *in utero*.
- Allergen-specific memory T_H2 cells are long-lived, express CRTH2, and may be maintained *in vivo* by the IL-7–like cytokine TSLP produced at sites of allergic inflammation.
- T-cell responses to allergens are plastic in both early life and adulthood and can be altered by a change in environmental exposure or immunotherapy.
- T-cell responses to whole allergens are dependent on the unique properties of each allergen and atopic status of the host.
- Enhanced suppression of T_H2 responses by regulatory T cells may favor nonallergic responses; however, little is known about the types of Tregs involved, their antigen specificity, or their mode of action.
- Identification of novel allergen-derived T-cell epitopes is critical to developing new reagents for monitoring allergen-specific T cells *in vitro* and *in vivo*, and is central to the rational design of new vaccines.

Additional future directions for research include analyzing the kinetics of T-cell responses in early life and developing techniques for monitoring the immunomodulatory potential of new vaccines. To this end, further characterization of allergen-specific T cells is critical (Fig 5). Identification of CRTH2 as a marker of allergen-specific memory T_H2 cells is an important step forward. However, more studies on $CRTH2^+CD4^+$ T cells are needed in allergic subjects to clarify their phenotype and function. MHC-peptide tetramers (and the newer generation pentamers and Ultimers [Proimmune Inc., Springfield, Va]) consist of MHC molecules and their associated peptide ligands linked to a central streptavidin or core structure. These fluorochrome-conjugated complexes bind to T cells with defined TCR specificities and are emerging as a useful tool for identifying and enumerating circulating allergen-specific $CD4^+$ T cells by flow cytometry (Fig 5).[121,122] However, to date only a limited panel of MHC class II molecules is available as tetramers, and because these reagents can only be used in subjects expressing specific HLA class II alleles, it is necessary to screen many patients to identify sufficient subjects for a study.

Regulatory T cells remain a hot research topic, and targeting these cells is an attractive possibility. However, further studies on the influence of conventional immunotherapy and allergen variants (eg, CpG conjugates or other receptor-targeted allergens[123]) on the Treg compartment are necessary. Finally, researchers should not be constrained by existing T-cell paradigms, especially in light of recent discoveries. It has been suggested that T_H1 and T_H2 cells be recategorized as inflammatory, on the basis of a capacity to secrete TNF-α, or regulatory, on the basis of secretion of IL-10.[46] In addition, a new lineage of effector T cells (T_H17) has recently been identified that is distinct from T_H1 or T_H2 cells.[124] These emerging T-cell subsets may have a considerable impact on our current understanding of T-cell responses to allergens (Fig 6). Broadening our T-cell horizons could identify new therapeutic targets and pave the way for exciting new developments in the treatment of allergic disease from the T-cell perspective.

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