

Pathogenic CD4⁺ T cells in patients with asthma

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Asthma encompasses a variety of clinical phenotypes that involve distinct T cell-driven inflammatory processes. Improved understanding of human T-cell biology and the influence of innate cytokines on T-cell responses at the epithelial barrier has led to new asthma paradigms. This review captures recent knowledge on pathogenic CD4⁺ T cells in asthmatic patients by drawing on observations in mouse models and human disease. In patients with allergic asthma, T_H2 cells promote IgE-mediated sensitization, airway hyperreactivity, and eosinophilia. Here we discuss recent discoveries in the myriad molecular pathways that govern the induction of T_H2 differentiation and the critical role of GATA-3 in this process. We elaborate on how cross-talk between epithelial cells, dendritic cells, and innate lymphoid cells translates to T-cell outcomes, with an emphasis on the actions of thymic stromal lymphopoietin, IL-25, and IL-33 at the epithelial barrier. New concepts on how T-cell skewing and epitope specificity are shaped by multiple environmental cues integrated by dendritic cell “hubs” are discussed. We also describe advances in understanding the origins of atypical T_H2 cells in asthmatic patients, the role of T_H1 cells and other non-T_H2 types in asthmatic patients, and the features of T-cell pathogenicity at the single-cell level. Progress in technologies that enable highly multiplexed profiling of markers within a single cell promise to overcome barriers to T-cell discovery in human asthmatic patients that could transform our understanding of disease. These developments, along with novel T cell-based therapies, position us to expand the assortment of molecular targets that could facilitate personalized treatments. (*J Allergy Clin Immunol* 2017;■■■:■■■-■■■.)

Key words: Asthma, allergens, IgE, T_H1, T_H2, T_H17, T_H22, follicular helper T cell, GATA-3, T-bet, thymic stromal lymphopoietin, IL-25, IL-33, epithelial barrier, T-cell epitopes, T-cell plasticity

Asthma now affects approximately 8% of the population in the United States and presents an enormous public health problem for adults and children alike in developed countries.¹ Although milder forms of the disease can typically be managed by currently available therapies, these are not curative, and severe disease is often refractory to treatment. CD4⁺ T cells remain at the forefront of asthma pathogenesis because of their myriad effects on inflammatory responses in the respiratory tract. Consequently, evolving knowledge of the T-cell mechanisms involved will be essential to developing novel strategies that mitigate disease onset and progression.

CONTRASTING ASTHMA IN MICE AND MAN

Much of what we have learned about the immunology of asthma derives from clues provided by allergic mouse models. T_H2 responses are fundamental to IgE-mediated sensitization, airway hyperreactivity (AHR), and eosinophil infiltration, which constitute the hallmarks of allergic airways disease. In mice these features can be reproduced by using immunization regimens that involve administration of allergen to induce sensitization, followed by airway allergen challenge to provoke local inflammation. In recent years, there has been a trend to replace ovalbumin (OVA) models that use intraperitoneal sensitization with models that incorporate clinically relevant allergens administered through the respiratory tract to more closely mimic human disease. This latter approach has yielded seminal advances in the field regarding the role of dendritic cells (DCs) in T_H2-driven asthma, the requirement for Toll-like receptors and other signaling pathways, and knowledge of the cell networks involved. However, we should be cautious when extending findings to human subjects for several reasons. First, the relatively high dose and accelerated mode of allergen administration in experimental models does not portray the chronic low-dose exposure that occurs in asthmatic patients. Second, the complex interplay between microbes and allergen in early life is difficult to reproduce in animal models. Third, observations in genetically identical mice are homogeneous and do not reflect the variability of human disease at the clinical or immunologic levels.

Barriers to studying the immunopathology of asthma in human subjects include the relative inaccessibility of inflamed tissues and the confounding local effects of corticosteroids and other immune modulators. Nonetheless, significant strides have been made regarding the relationship between different clinical phenotypes and their underlying pathologies. Such endotypes encompass “type 2-high” and “type 2-low” asthma, which are

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Abbreviations used

AD:	Atopic dermatitis
AHR:	Airway hyperreactivity
APC:	Antigen-presenting cell
BAL:	Bronchoalveolar lavage
CRTH2:	Chemoattractant receptor-homologous molecule expressed on T _H 2 lymphocytes
DC:	Dendritic cell
Foxp3:	Forkhead box p3
HRV:	Human rhinovirus
ILC:	Innate lymphoid cell
ILC2:	Type 2 innate lymphoid cell
IL-4R:	IL-4 receptor
IRF:	Interferon regulatory factor
MHCII:	MHC class II
miRNA:	MicroRNA
NLRP3:	NLR family pyrin domain containing 3
OVA:	Ovalbumin
OX40L:	OX40 ligand
SLIT:	Sublingual immunotherapy
STAT:	Signal transducer and activator of transcription
T _{CM} :	Central memory T
TCR:	T-cell receptor
T _{FH} :	Follicular helper T
TJ:	Tight junction
Treg:	Regulatory T
TSLP:	Thymic stromal lymphopoietin

distinguishable by their abundance or paucity of T_H2-associated markers, respectively. This review aims to provide a comprehensive overview of the biology of T_H2 cells in the context of asthma and details the broader repertoire of pathogenic CD4⁺ T cells implicated in disease.

T_H2 DIFFERENTIATION AND T-CELL TRAFFICKING IN ASTHMATIC PATIENTS

T_H2 licensing of DCs

Initiation of T_H2 responses in mouse models of allergic asthma requires presentation of antigen mediated by conventional type 2 myeloid DCs. These cells act as a hub to integrate diverse signals from environmental stimuli and inflammatory mediators and translate these cues to T cells engaged through their T-cell receptor (TCR; **Figs 1, A, and 2**). Type 2 DCs display a signature shared by mice and human subjects (CD11c⁺CD26⁺XCR1^{lo}CD172a^{hi}IRF4^{hi}).² A variety of transcription factors endow their T_H2-inducing properties, including interferon regulatory factor (IRF) 3, IRF4, Kruppel-like factor 4 (KLF4), and signal transducer and activator of transcription (STAT) 5.³⁻⁶ In mouse models of allergic asthma, IRF4 expression in DCs is necessary for T_H2-driven lung inflammation.⁷ This molecule, which is modulated KLF4, directly targets the gene encoding the T_H2-promoting cytokine IL-33 (*Il33*) while inhibiting production of the T_H1-differentiating cytokine IL-12 (**Fig 1, A**).^{7,8} The ability for house dust mite extract to upregulate IL-33 in mouse DCs through signaling through the C-type lectin receptor Dectin-2 suggests that allergen can trigger a similar process.⁹ Additional T_H2-licensing stimuli include innate cytokines secreted by epithelial cells (eg, thymic stromal lymphopoietin [TSLP], IL-25, and IL-33), as well as IL-6 and the Notch ligand Jagged1, depending on the context.^{3,10}

Beyond T_H2 initiation, DCs also limit the magnitude of T_H2 responses. A negative feedback loop involving the receptor tyrosine kinase TYRO3 in cDCs and its agonist PROS1 in T cells provides one such example (**Fig 2**).¹¹ Notably, the localization of an asthma-associated single nucleotide polymorphism within putative transcription factor binding sites of *TYRO3* led to this discovery. Transcriptomic and proteomic analysis of human DCs treated with a cocktail of T_H2-licensing mediators revealed overexpression of multiple genes/proteins beyond those known to be T_H2 linked (eg, OX40 ligand [*OX40L*] and *GATA3*).¹² These findings point to the operation of complex pathways in DCs that orchestrate T_H2 responses in a highly coordinated and T_H2-specific fashion.

T_H2 differentiation

T_H2 cells express the canonical type 2 cytokines IL-4, IL-5, and IL-13. In asthmatic patients T_H2 cytokines exert pleiotropic and overlapping effects but are most well characterized in relation to their capacity to induce antibody isotype switching in B cells (IL-4), eosinophilia (IL-5), and AHR and mucus production (IL-13).¹³ Although T_H2 cells are not the sole source of these cytokines, numerous studies have established their requirement for the hallmark features of allergic airway disease. T_H2 cytokines also act on epithelial cells to enhance their production of innate cytokines and to augment the secretion of proinflammatory mediators by mast cells and basophils.

Considerable knowledge has been gained regarding the molecular mechanisms of T_H2 differentiation since the first report of distinct T_H subsets in mice 30 years ago.¹⁴ T_H2 cells express the lineage-specific transcription factor GATA-3 and display a characteristic surface-molecule signature (CCR4⁺CXCR3⁻CRTH2⁺; **Figs 1, B, and 2**). Recent work involving gene expression profiling and epigenomic analysis highlights the complex series of transcriptional events that culminate in this phenotype under T_H2-polarizing conditions.¹⁵

Since its first description related to mouse T_H2 cells almost 20 years ago,¹⁶ GATA-3 is still appreciated to be a dominant player in directing T_H2 differentiation in both mice and human subjects.^{17,18} This molecule specifies the production of T_H2 cytokine genes by binding to their promoter elements and mediating chromatin changes at these loci (**Fig 1, B**). GATA-3 is also crucial for T-cell maintenance and proliferation.¹⁹ The induction of GATA-3 has been extensively studied in mouse systems in response to IL-4 stimulation. Although this occurs in a STAT6-dependent fashion, the precise mechanisms involved remain enigmatic. Additionally, T_H2 differentiation can occur independently of STAT6 either by engagement of Notch ligands on DCs with upregulation of *Gata3* gene expression by intracellular Notch, or else by IL-2 receptor signaling through STAT5A.²⁰

Intriguing new evidence supports a role for NLR family pyrin domain containing 3 (NLRP3) acting downstream of IL-2 receptor/STAT5A in T_H2 differentiation. In this scenario NLRP3, which is best known for its role in activating the inflammasome, acts as a transcription factor, along with IRF4, to promote IL-4 production in T_H2 cells (**Fig 1, B**).²¹

New mechanisms that regulate T_H2 differentiation continue to be described. In a mouse system, T-cell factor 1 and its cofactor, β -catenin, which act downstream in the Wnt signaling pathway, increased levels of GATA-3 protein by binding to the *Gata3*

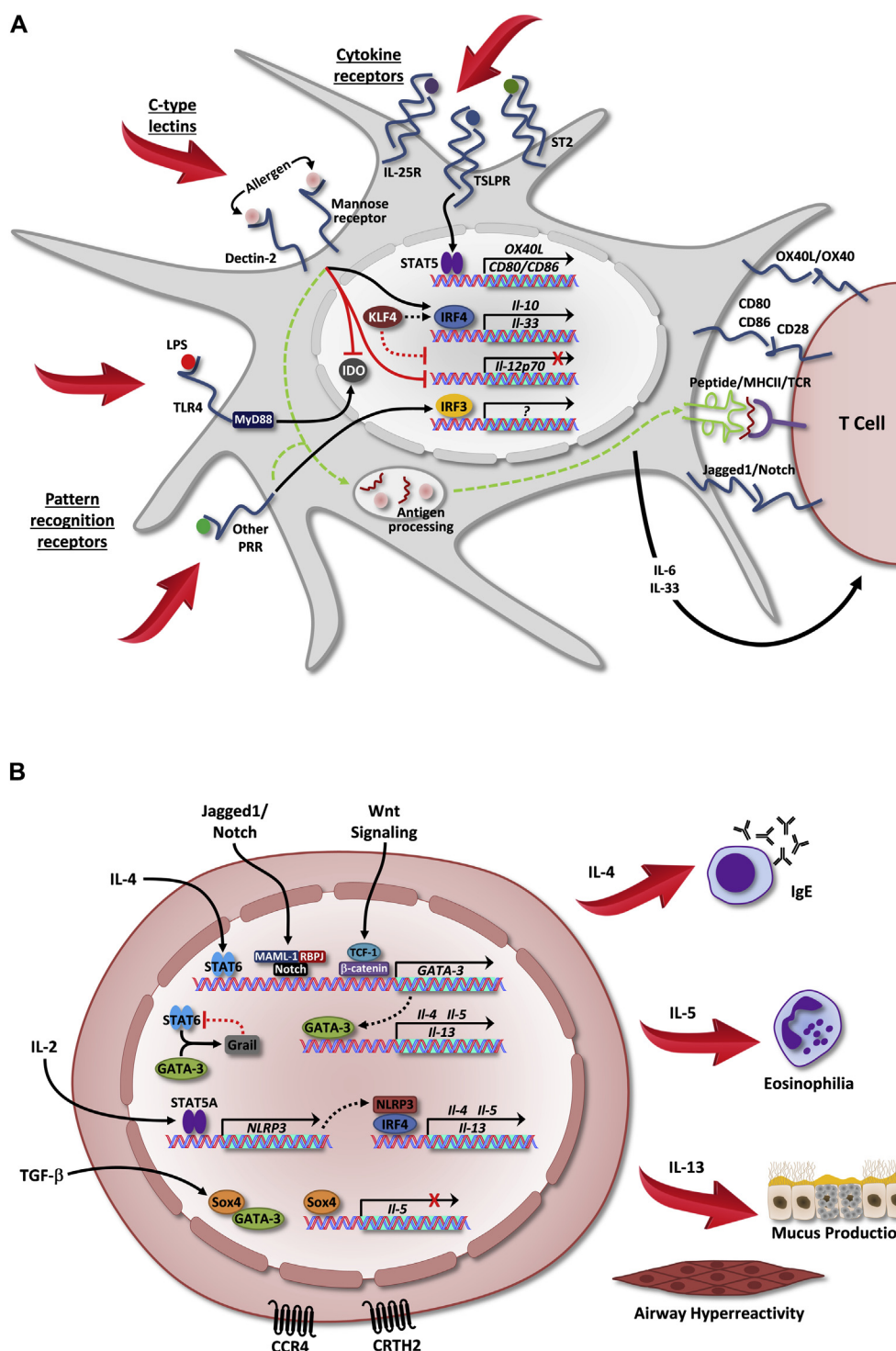


FIG 1. Molecular events in T_H2 differentiation. **A**, DCs act as a hub for T_H2 licensing. T_H2 differentiation is orchestrated by integration of diverse external cues that generate a T_H2 -permissive cytokine milieu in conjunction with an array of receptor/ligand interactions at the T-cell surface. *IDO*, Indoleamine 2,3-dioxygenase; *IL-25R*, IL-25 receptor; *KLF4*, Kruppel-like factor 4; *PRR*, pattern recognition receptor; *TLR4*, Toll-like receptor 4; *TSLPR*, TSLP receptor. **B**, Within T_H2 cells, differentiation is orchestrated by the IL-4-induced GATA-3 pathway, as well as other pathways, that coordinate increased accessibility of T_H2 cytokine gene loci and promote their transcription. *MAML1*, Mastermind-like protein 1; *RBPJ*, recombination signal binding protein for immunoglobulin kappa J region; *Sox4*, Sry-related high-mobility-group box 4; *TCF-1*, T-cell factor 1.

promoter.²² In other work, transcription of *Il4* by GATA-3 was increased by the enhancer element HS2, which is located within the *Il4* locus.²³ More recently, epigenetic analysis with genome-

wide histone modification pinpointed active enhancers associated with T_H2 development in human T cells that were enriched for asthma-associated single nucleotide polymorphisms and

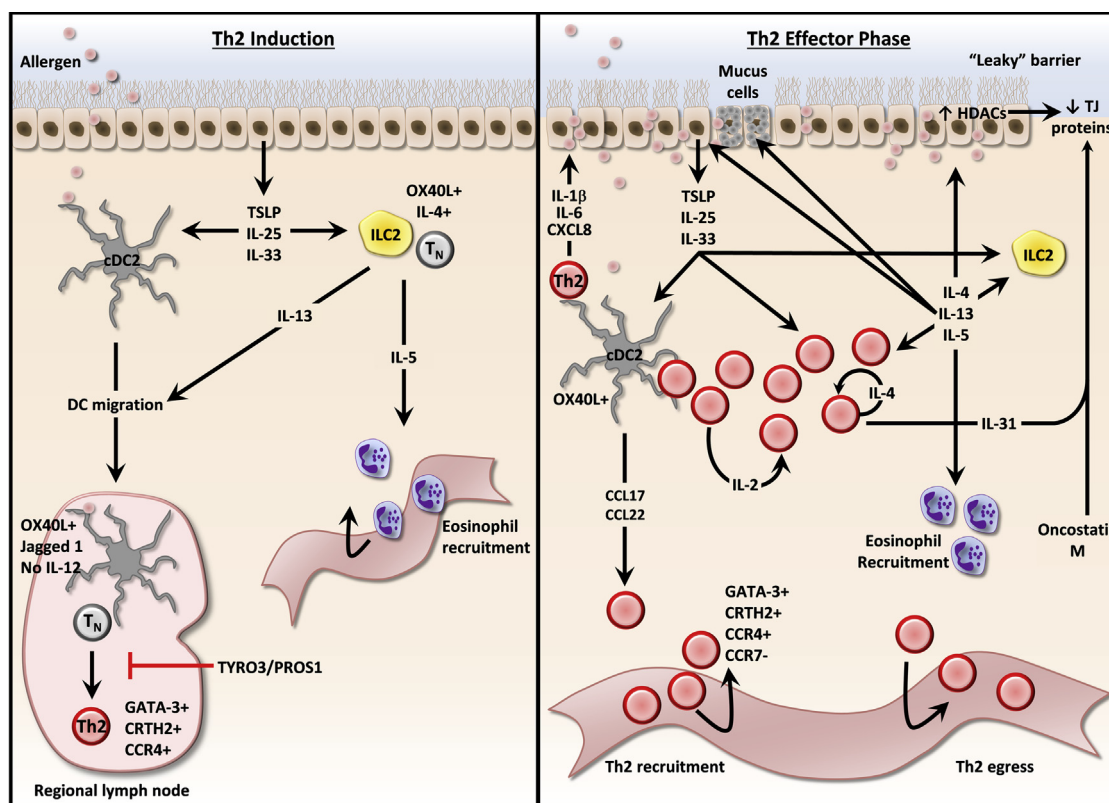


FIG 2. T_H2 -promoting cytokine networks at the epithelial barrier. *Left panel*, Innate cytokines triggered by environmental stimuli induce T_H2 licensing of DCs and their migration to the draining lymph nodes. Rapid activation of ILC2 cells promotes eosinophil recruitment and DC migration. *Right panel*, Antigen-experienced effector ($CCR7^-$) T_H2 cells are recruited to inflamed sites through CCR4 ligands (CCL17 and CCL22) secreted by TSLP-activated DCs, where they respond to direct and indirect effects of innate and T_H2 cytokines. Inflammation is perpetuated by mechanisms that promote a “leaky” barrier through modulation of structural proteins. Egress or “spillover” of T_H2 cells from inflamed sites results in their recirculation and possible reversion to central memory status ($CCR7^+$). *cDC2*, Conventional type 2 myeloid dendritic cell; *HDAC*, histone deacetylase.

contained GATA-3 binding elements.²⁴ Conversely, the transcription factor Sox4 binds both GATA-3 protein and the *Ils* gene promoter region, thereby preventing GATA-3 binding to consensus DNA sequences (Fig 1, B).²⁵

Targeted degradation of signaling molecules involved in T_H2 differentiation provides another mode of T_H2 regulation. As an example, the E3 ubiquitin ligase Grail, expression of which is dependent on STAT6 and GATA-3, targets STAT6 for ubiquitination and degradation, thereby providing a negative feedback loop for T_H2 development.²⁶ Increased susceptibility to asthma observed in Grail-deficient mice aligns with these findings.

Finally, there is emerging evidence to support a role for microRNAs (miRNAs) in regulating T_H2 differentiation. These endogenously expressed strands of nucleotides act to repress protein translation by shepherding the formation of a miRNA-induced silencing complex that binds to a specific mRNA target through complementary base pairing. Under T_H2 -polarizing conditions, specific miRNAs (miR-24 and miR-27) limit IL-4 and GATA-3 protein levels by targeting their respective genes.^{27,28} In mouse models of asthma, miR-155 has been implicated in both the regulation of T_H2 cell differentiation and T_H2 cytokine production and might also play a part in mediating the effects of glucocorticoids.²⁹

T_H2 routing in asthma

In mouse models of allergic asthma, differentiation of allergen-specific T_H2 cells from naive T cells occurs in regional draining lymph nodes (Fig 2). These T cells subsequently traffic to the respiratory tract.³⁰ Such effector memory T cells are distinguished from their central memory T (T_{CM}) cell counterparts by lack of expression of $CCR7$ ³¹ and their ability to home to inflamed sites, where they rapidly release their cytokine payload. In asthma models the dose of inhaled allergen influences the numbers of effector T cells in the lungs, as well as expansion of T_{CM} cells in the draining nodes. Specifically, when inhaled allergen is limiting, the enlistment of B cells as antigen-presenting cells (APCs), in addition to DCs, contributes to optimal expansion of these populations and concomitant asthma.³²

An array of chemokine receptors have been implicated in routing of T cells to the respiratory tract, including $CCR4$, $CCR5$, $CXCR3$, $CCR6$, and $CCR8$.³³ $CCR4$ is arguably the most consistent marker reported in mice and human subjects in relation to circulating allergen-specific T_H2 cells. This receptor aids in T_H2 recruitment through binding of its chemokine ligands CCL17 (often referred to as thymus and activation-regulated chemokine [TARC]) and CCL22, which are overexpressed in inflamed airways, with DCs serving as a principal source.³⁴ IL-2 provides migrational cues to T_H2 cells by modulating chemokine

receptor profiles. Moreover, IL-2 signaling is required for lung homing and retention of long-lived allergen-specific memory T_H2 cells that do not recirculate (Fig 2). In a dust mite model such lung-resident memory cells are sufficient to induce AHR *in situ* and persist for up to 100 days.³⁵ In other work, IL-4 receptor (IL-4R) α -responsive T_H2 cells were essential for sustaining AHR but not for inducing acute disease, thereby implying a key role for IL-4/IL-4R α signaling in perpetuating inflammation in the tissues.³⁶

INNATE CYTOKINES THAT PROMOTE T_H2 -DRIVEN ASTHMA AT THE EPITHELIAL INTERFACE

In allergic subjects, bronchial epithelial cells overproduce a broad array of cytokines in response to an array of environmental triggers, including allergens, microbes, and pollutants. These include the T_H2 -promoting cytokines TSLP, IL-25, and IL-33, as well as other proinflammatory cytokines, including IL-1 α/β , IL-6, IL-8, and TNF- α . This process occurs rapidly and reflects cell-intrinsic and extrinsic pathways governed by complex gene-environment interactions. Mediator release fosters extensive cross-talk between a variety of innate immune cells and T cells at the epithelial interface, which serves to perpetuate T_H2 responses. In new work, the concerted effort of innate mediators in this process was elegantly demonstrated by the requirement for multiple cytokines in terminal differentiation of effector T_H2 cells in the lungs but not for T_H2 priming in regional lymph nodes.³⁷ Thus, exploitation of this “tissue checkpoint” might prove to be a useful therapeutic strategy for simultaneous blockade of innate and adaptive arms of T_H2 responses. This section focuses on cytokine networks operating at the epithelial barrier in patients with T_H2 -driven asthma.

Dysfunction of the epithelial barrier

Disruption of the architecture of the epithelial barrier with consequent increased accessibility of immune stimuli drives T_H2 responses. Recent studies highlight the role of cytokines in undermining the structural integrity and responsiveness of the epithelial barrier in the respiratory tract. Reduced expression of genes encoding proteins involved in tight junctions (TJs) contributes to a “leaky” barrier in patients with asthma, as well as those with allergic rhinitis.^{38,39} In air-liquid interface cultures containing bronchial epithelial cells obtained from asthmatic patients, IL-4 and IL-13 decreased TJ integrity by enhancing the production of enzymes that suppress gene transcription through histone modification (histone deacetylases, Fig 2).⁴⁰ Moreover, inhibition of histone deacetylase restored barrier integrity, thereby confirming the ability for a T_H2 -rich milieu to modify the epithelial barrier through epigenetic effects. In other work, the IL-6 family member oncostatin M, levels of which are increased in bronchoalveolar lavage (BAL) fluid of allergic asthmatic patients, reduced airway epithelial integrity by disrupting TJ assembly but did not influence the production of TJ components.⁴¹ The related cytokine, IL-31, expression of which is also increased in the airways of asthmatic patients with severe disease,⁴² has likewise been implicated in barrier dysfunction (Fig 2). Secretion of this novel cytokine by T_H2 cells requires autocrine IL-4.⁴³ Akin to IL-4, IL-31 downregulates genes involved in barrier function in the skin⁴⁴; however, its effects in the lungs remain to be elucidated.

TSLP

TSLP has garnered much attention for its T_H2 -differentiating ability mediated through DCs.⁴⁵ This solved the conundrum posed in animal systems by the occurrence of T_H2 differentiation despite a lack of IL-4. TSLP, an IL-7-related cytokine, is overexpressed in the asthmatic lung and, most strikingly, in skin lesions of patients with atopic dermatitis (AD), indicating a key role in allergic inflammation.^{45,46} Experimental work in mice and human subjects supports a coordinated effect of TSLP and allergen on DC-mediated induction, expansion, and maintenance of T_H2 cells. In animal models, both TSLP and antigen are required for T_H2 -driven lung inflammation through TSLP signaling in DCs.^{5,47} In human subjects with established allergic disease and thus pre-existing memory T_H2 cells, TSLP alone is a weak inducer of DC-mediated T_H2 responses, whereas TSLP and allergen act synergistically.⁴⁸ Similar findings on the coordinated effect of TSLP and allergen have since been described in the context of allergic rhinitis.⁴⁹ The TSLP responsiveness of DCs in allergic patients might be linked to the propensity to increase surface TSLP receptor levels in response to Fc receptor signaling triggered by allergen binding.⁴⁸ TSLP binds to a heterodimeric receptor comprising a ligand-binding subunit, TSLP receptor, and a signal-transducing subunit, IL-7 receptor α .¹³ Signaling through the TSLP receptor induces phosphorylation of the transcription factor STAT5, which is critical for T_H2 induction by TSLP-activated DCs in mice.⁵ In human subjects there is some evidence that downstream induction of OX40L is required for TSLP-activated DCs to promote T_H2 responses through ligation of OX40 on T cells (Fig 1, A).⁵⁰ Recent work in allergic asthmatic patients identified myeloid DCs in the airways with the molecular machinery for TSLP responsiveness; however, although TSLP polarized CD4⁺ T cells toward both T_H2 and T_H9 (IL-9⁺) phenotypes, only OX40L was required for T_H2 induction, suggesting a T_H2 -specific role.⁵¹

In addition to licensing DCs for T_H2 differentiation and expansion, TSLP induces their migration and secretion of CCL17/TARC, which binds CCR4 on T_H2 cells.^{45,49,52} TSLP also acts directly on T_H2 cells (Fig 2). TSLP receptor is preferentially expressed on dividing human T_H2 cells isolated from allergic subjects⁵³ and on skin-homing T_H2 cells (CLA⁺CCR4⁺CXCR3⁺) in patients with AD.⁵⁴ Moreover, skin-homing T cells increase their expression of TSLP receptor in response to IL-4 and enhance their secretion of IL-4 in response to TSLP, forming a positive feed-forward loop. T_H2 cells also positively feedback on epithelial cells in the allergic host to enhance their production of TSLP.⁵² Interestingly, these effects are principally mediated by IL-1 β , IL-6, and CXCL8 as opposed to T_H2 cytokines themselves.

Other innate cytokines

A variety of other cytokines secreted by epithelial cells influence T_H2 responses, including the IL-17 family member IL-25 (IL-17E), the IL-1 family member IL-33, and GM-CSF (Fig 2). A detailed account of these cytokines is beyond the scope of the present article, and excellent reviews can be found elsewhere.^{3,55} Instead, a few concepts relevant to human disease are presented here.

IL-25 and IL-33 have each been implicated in pathogenic processes in the lungs of asthmatic patients and in skin lesions of patients with AD.^{56–58} In mice, both IL-25 and IL-33 induce

OX40L on DCs.^{59,60} By contrast, recent work in human subjects showed that IL-33, which mediates its effects through its receptor, ST2, did not induce OX40L on human CD11c⁺ DCs.⁵⁶ Nonetheless, IL-33 increased T_H2 polarization by TSLP-primed human DCs or by OX40L alone, suggesting an ability to augment the TSLP/OX40L axis. In the same study IL-33 also acted directly on naive T cells to promote T_H2-like cells expressing IL-5, IL-9, and IL-13. In mice, IL-33 also acts directly through ST2 on memory T_H2 cells *in vivo* to selectively enhance *Il5* gene expression and protein secretion through the downstream mitogen-activated protein (MAP) kinase p38.⁶¹ The ability of IL-33 to enhance IL-5 and IL-13 production in memory T_H2 cells isolated from nasal polyps of patients with eosinophilic chronic rhinosinusitis suggests a similar pathway operates in human subjects.⁶² Together, these findings indicate that the IL-33–ST2–p38 pathway operates independently of TCR ligation to promote T_H2 pathogenicity. A similar mechanism might contribute to asthma exacerbations induced by human rhinovirus (HRV).⁶³ However, work in human subjects also links IL-33 to steroid-resistant severe asthma, suggesting a role beyond T_H2-driven asthma.⁵⁷

IL-25 mediates its effects by binding to IL-25 receptor (IL-17RB). Levels of this receptor are increased on the surfaces of both myeloid and plasmacytoid DCs in the airways of patients with mild asthma after allergen challenge.⁶⁴ IL-25 coordinates with TSLP-activated human DCs to induce T_H2 polarization and augment T_H2 responses by enhancing expression of genes encoding T_H2-associated transcription factors (*GATA3*, *c-MAF*, and *JUNB*), increasing expression of T_H2 signature surface markers (CCR4 and IL-4R) and maintaining chemoattractant receptor–homologous molecule expressed on T_H2 lymphocytes (CRTH2; Figs 1, B, and 2). IL-25 also sustains expression of transcription factors and augments production of IL-5 and IL-13 in an IL-4–independent manner through direct T-cell effects.⁶⁵ In addition to amplifying established T_H2 responses, IL-25 acts directly on naive human CD4⁺ T cells to induce T_H2 differentiation, although not to the same extent as the prototypical T_H2-differentiating cytokine IL-4.⁶⁶ IL-25 also mirrors the direct effects of IL-33 on memory T_H2 cells in the context of chronic rhinosinusitis.⁶² These actions, coupled with an association between IL-25 and T_H2-high asthma,⁶⁷ support a pathogenic role for IL-25 in T_H2-driven airway disease resembling that of IL-33.

Mouse models of allergic asthma imply a key role for GM-CSF in driving T_H2 immunity. Its secretion by epithelial cells in response to a variety of environmental triggers orchestrates the maturation and proliferation of DCs and equips them to induce T_H2 responses along with the hallmark features of allergic asthma. GM-CSF can also condition the airways to respond to lower doses of allergen through a pathway involving IL-33 secretion by alveolar type II cells.⁶⁸ In asthmatic patients, bronchial epithelial cells overexpress GM-CSF.⁶⁹ However, GM-CSF has also been linked to neutrophilic asthma, which lacks a prominent T_H2 signature, thereby questioning its role in T_H2-driven disease in human subjects.⁷⁰

Influence of type 2 innate lymphoid cells on T_H2 cells

Innate lymphoid cells (ILCs) provide an early source of type 2 cytokines at the epithelial interface in asthmatic airway.⁷¹ Similar to T_H2 cells, type 2 innate lymphoid cells (ILC2s) secrete IL-4,

IL-5, and IL-13 in response to TSLP, IL-25, and IL-33. ILCs originate from a common lymphoid precursor but lack T-cell lineage markers (CD3, CD4, and TCR). Although they share many features of CD4⁺ T cells, there are key differences. Work in mice shows that ILCs are poised to respond rapidly because of increased accessibility of chromatin in proximity to effector genes. Interestingly, chromatin remodeling occurs during ILC development and before their activation. This contrasts with T_H2 cells, which undergo chromatin remodeling at similar gene loci during the differentiation process (ie, after TCR activation).¹⁵ Additionally, ILCs exist as mature cells in the periphery and, unlike T_H2 cells, do not require STATs for their development.

On the other hand, there are striking similarities between ILC2s and T_H2 cells that point to shared “immune modules.” For example, STAT6, which is required for T_H2 differentiation, is also required for ILC2 effector functions, a requirement that is specific to the ILC2 type.⁷²

In mouse models of asthma, ILC2s are required for T_H2 responses to allergens at the epithelial barrier,^{73,74} and both their secreted cytokines and surface proteins contribute to this process. Indeed, similar to DCs, ILC2s express costimulatory molecules and can present peptide antigen in the context of MHC class II (MHCII), although not as efficiently as professional APCs.⁷⁵ These direct effects of ILC2s on CD4⁺ T cells appear to require IL-4 and OX40/OX40L interactions (Fig 2).⁷⁶ ILC2s also promote migration of DCs to regional lymph nodes and goblet cell hyperplasia through their secretion of IL-13, and are principal instigators of eosinophil recruitment through IL-5 in mouse models of allergic asthma.^{73,77} ILC2s act in concert with other cell types to amplify T_H2-driven allergic inflammation during the effector phase.⁷⁸ Their interaction with CD4⁺ T cells is bidirectional, wherein each aids the other in the production of cytokines (Fig 2).⁷⁵ T_H2 cells can also enhance IL-13 production by ILC2s in response to IL-25.⁷⁹ Conversely, ILC2s are also subject to the suppressive effects of induced regulatory T (Treg) cells through ICOS–ICOS ligand cell contact. This interaction was found to control AHR and diminish lung eosinophil counts in a humanized ILC2 mouse model of asthma induced by using the fungal allergen *Alternaria alternata*.⁸⁰ It remains unclear whether ILC2s are essential for the development and/or persistence of “type 2–high” asthma in human subjects.

T_H2 responses to allergens and asthma

Allergen-reactive T_H2 cells are pivotal to inflammatory responses in patients with allergic asthma. Much of our insight into their mode of induction and pathogenicity comes from mouse models, which allow direct sampling of lung and lymphoid tissues. By contrast, in human subjects we typically rely on sampling and *in vitro* expansion of circulating T cells obtained from patients with established allergic disease to study allergen-reactive T cells.⁸¹ This section focuses on new insights into allergen-specific T cells and their cognate ligands.

Influence of allergen structure on T_H2 skewing

The reasons as to why allergens are such strong T_H2 inducers remain unclear. Early work in an OVA mouse model of asthma found that “contamination” of allergen with low-dose LPS favored T_H2 induction.⁸² Subsequent identification of Der p 2 as a structural and functional homolog of MD-2, the lipid-binding

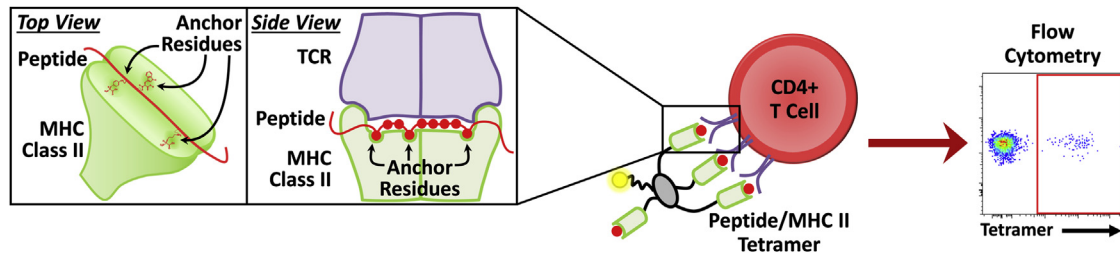


FIG 3. Schematic of an MHCII tetramer. Peptide epitopes bind to MHCII molecules through interactions between side chains of anchor residues and MHC pockets of the peptide-binding groove. Synthetic multimeric peptide/MHCII complexes bind to antigen-specific CD4⁺ T cells with high avidity.

component of the Toll-like receptor 4 pathway, demonstrated that allergens could possess intrinsic T_H2 adjuvanticity.⁸³ Allergens bind a variety of pattern recognition receptors, including C-type lectin receptors (eg, mannose receptor and Dectin-2) and dendritic cell-specific intercellular adhesion molecule 3–grabbing nonintegrin (DC-SIGN). These interactions increase the propensity for T_H2 skewing, primarily by enhancing the release of T_H2-promoting cytokines by epithelial cells and licensing DCs. Binding of heavily mannoseylated allergens (eg, from house dust mite and cockroach) to mannose receptor on human DCs attenuates Toll-like receptor 4–induced expression of the T cell-suppressive enzyme indoleamine 2,3-dioxygenase (IDO) and decreases production of the T_H1-differentiating cytokine IL-12p70 (Fig 1, A).^{84,85} The stability of protein folding can also modulate T_H2 responses. Recent work with mutant molecules of the major birch allergen Bet v 1 suggests that increased stability might favor delivery of allergen to the late endosomal compartment for efficient processing and consequent increased epitope density at the DC surface.⁸⁶ This concept merits further study.

Specificity of allergen-reactive T cells

Epitope mapping of allergens offers a refined approach to studying allergen-reactive T cells, as well as the promise of new peptide-based immunotherapies that avoid the clinical sequelae arising from IgE cross-linking using intact allergens. CD4⁺ T cells recognize protein antigens presented as linear peptides bound to MHCII molecules on the surfaces of APCs (Figs 1, A, and 3). These peptide/MHCII complexes engage the TCR in a manner that is exquisitely specific. Cognate peptides for CD4⁺ T cells, which typically range in length from 14 to 24 amino acids, contain a core 9-mer epitope that binds to the MHCII cleft, and variable numbers of flanking residues that contribute to stabilization of the peptide/MHCII interaction, TCR binding, and consequent T-cell activation.

The advent of the recombinant DNA era led to elucidation of the amino acid sequence of many allergens and the consequent discovery of T-cell epitopes that induced the strongest T-cell reactivity in a reproducible fashion across HLA-diverse subjects.^{81,87,88} Such immunodominant T-cell epitopes bind to different MHCII molecules in a promiscuous fashion, which might involve multiple binding registers involving different anchor residues. A common epitope-mapping approach uses synthetic overlapping peptides spanning the entire length of the amino acid sequence of the allergen to stimulate T-cell responses *in vitro*. Other approaches include computer prediction algorithms based on known peptide-binding motifs for MHCII

molecules and *in silico* platforms that leverage existing knowledge on known T-cell epitopes (eg, the Immune Epitope Database).^{89,90} Each approach has its strengths and pitfalls, and each allergen presents its own unique challenges.

Many allergic patients with asthma are sensitized to multiple inhalant allergens, and T cells appear to contribute to this phenomenon. A new approach to epitope analysis that analyzed the entire transcriptome (totaling 50,000 transcripts) of 9 representative pollens from trees, grasses, and weeds revealed a link between conserved T-cell epitopes of timothy grass and polysensitization to other outdoor allergens.⁹¹ However, because of the increase in food allergies, attention has largely shifted away from epitope mapping of inhalant allergens to focus on food allergens that pose a serious risk of anaphylaxis.⁹²⁻⁹⁷ Unfortunately, little is known about the relationship between T-cell epitopes of food allergens and asthma development, although this aspect is worth exploring, particularly among polysensitized children. As of 2010, only approximately 17% of all allergens listed by the International Union of Immunological Societies database had been mapped, indicating much work remains to be done.⁹⁸

Tetramers as a tool for studying allergen-reactive T cells in asthma

Culture systems that expand allergen-reactive T cells isolated from the blood by using whole protein or peptides remain the mainstay for characterizing these cells. Not surprisingly, T-cell responses in allergic subjects are highly variable, despite classification according to disease type, symptoms, and IgE status. Moreover, stimulation assays that use “bulk” cell preparations, such as PBMCs, yield a range of T-cell phenotypes comprising a minor allergen-specific subset admixed with a much larger number of bystander cells with undefined specificities.⁹⁹ Although generation of T-cell clones circumvents this problem, prolonged culture is required, and the resulting cells might not reflect the allergen-specific T-cell repertoire as a whole. Fortunately, these pitfalls can be avoided by using synthetic MHCII tetramers. These fluorochrome-tagged reagents, which contain multiple copies of the same HLA molecule displaying a single peptide epitope, bind allergen-specific T cells with high avidity through their cognate TCR, thereby providing a precise and sensitive tool for their detection (Fig 3). Moreover, because T cells can be identified directly *ex vivo* with minimal manipulation, those markers expressed *in vivo* are faithfully preserved. Labeling of allergen-specific T cells with MHCII tetramers also allows their isolation to high purity by means of cell sorting for functional studies.

Over the last decade, MHCII tetramers have been used to map CD4⁺ T-cell epitopes of several allergens in the context of common HLA-DR molecules, and the resulting tetramer panels have proved useful for studying mechanisms of disease and protection.^{94,96,99-101} Work by Campbell et al¹⁰² in an HLA-DR1 transgenic mouse model of cat-induced allergic asthma provided an elegant precedent in this regard. After injection of a peptide vaccine containing selected epitopes of Fel d 1, tetramer analysis of Fel d 1-responsive cells isolated from the lungs revealed much lower numbers of tetramer-positive T cells specific for the injected epitopes compared with tetramer-negative IL-10⁺ T cells. These findings supported the ability of a small set of injected peptide epitopes to induce IL-10-secreting Treg cells directed against different epitopes of Fel d 1, presumably through linked epitope suppression.

More recently, work in an OVA asthma model used MHCII tetramers to verify the presence of allergen-specific IL-13⁺CD4⁺ cells in the lungs, suggesting that CD4 expression is downregulated at inflamed sites.¹⁰³ Interestingly, these T_H2-like cells were dependent on atypical IL-4⁺ Treg cells that were activated at the time of allergen sensitization, thereby questioning the paradigm of Treg cells as endogenous suppressors of asthma.

In addition to mechanistic insights, tetramer work has confirmed higher numbers of allergen-specific T cells in patients who are allergic and have more severe disease versus nonallergic subjects.^{104,105} As predicted, such T cells predominantly display the T_H2-associated marker CCR4 but lack the T_H1-associated marker CXCR3; however, surface expression of the alternative T_H2 marker CCR2 differs according to allergen specificity.^{94,106} Moreover, cytokine profiles are often heterogeneous, reflecting T_H1, T_H2/T_H17, T_H1/T_H2, and T_H17-like profiles in addition to a classical T_H2 signature, which points to varying degrees of T_H2 skewing depending on the allergen.^{94,96,106-108} Extending this notion further, tetramer-positive T cells contain different proportions of T_{CM} cells (CCR7⁺) and effector memory T cells (CCR7⁻) for seasonal and perennial allergens, although this feature does not seem to relate to current exposure status.¹⁰⁷ Together, these patterns might yield insight into the hierarchy of allergens and its relationship to the pathogenicity of T cells, including their ability to induce T_H2-dependent antibodies.¹⁰⁸ For example, recent tetramer work linked the numbers of Fel d 1-specific T_H2 cells to cat-specific IgG₄ levels among allergic subjects living with a cat who were exposed to higher levels of allergen.¹⁰⁶ The ability for tetramer-sorted Fel d 1-specific T cells to support IgG₄ production by memory B cells from allergic subjects confirmed their participation in antibody production.

HETEROGENEITY OF T CELLS AND INFLUENCE OF THE TISSUE MICROENVIRONMENT

Analyzing human T cells in the blood as a proxy for those mediating pathogenic effects in tissues is not ideal because mediators in inflamed tissues provide cues to T cells that alter their form and function. Here we outline concepts regarding the attributes of T_H subsets in the asthmatic lung.

T-cell plasticity and influence of the cytokine milieu

Discrete T_H subsets are characterized by their expression of T-cell lineage-specifying transcription factors, as well as their profiles of cytokines and chemokine receptors (Fig 4). Major T_H types include T_H1, T_H2, T_H9, T_H17, and T_H22 subsets. Although

GATA-3 is the dominant transcription factor determining T_H2 fate, the generation of other T_H subsets requires alternative transcription factors that include T-bet (T_H1), PU.1 (T_H9), retinoic acid-related orphan receptor γ (T_H17), and aryl hydrocarbon receptor (T_H22). In turn, these transcription factors dictate secretion of the following principal T_H cytokines that distinguish the major T_H types from each other: IFN- γ (T_H1); IL-4, IL-5, and IL-13 (T_H2); IL-9 (T_H9); IL-17 (T_H17); and IL-22 (T_H22). Transcription factors also act to repress the acquisition of opposing T_H fates, thereby safeguarding T-cell commitment.

In addition to their hallmark cytokines, T_H types secrete a broad array of cytokines that are shared among conventional T_H types depending on the context (eg, TNF- α). Moreover, novel T-cell subsets with “mixed/intermediate” phenotypes continue to be identified, which appear to arise from reprogramming of polarized T cells. Such plasticity is highly relevant to asthma because of the rich milieu of cytokines expressed in the inflamed lung. These mediators act as the main instigators of T-cell plasticity. As an example, if we consider the “classical” scenario wherein T_H1 and T_H2 cells differentiate from naive CD4⁺ T cells in response to IL-12 and IL-4, respectively, it is now known that each T-cell type can repolarize toward the other when treated with the opposing cytokine.^{109,110} This might explain the high numbers of IL-4⁺IFN- γ ⁺ T cells displaying a mixed chemokine receptor signature in allergen-stimulated cultures from allergic subjects (Fig 4). This notion of plasticity extends to the recently discovered T_H9 subset. These cells can arise from T_H2 cells in response to TGF- β .¹⁰⁹ Similarly, T_H17 cells can acquire the capacity to secrete IFN- γ in response to IL-12 or IL-23 (Fig 4).¹¹¹ These T-cell events are governed by Janus kinase/STAT pathways triggered by cytokine receptor engagement. Similarly, follicular helper T (T_{FH}) cells that provide help to B cells for antibody production through IL-21 can acquire the capacity to secrete IL-4, IFN- γ , or IL-17 in the presence of specific cytokines. Other contributing factors include signaling strength provided by TCR engagement and the costimulatory molecules CTLA4 and CD28.¹⁰⁹

As we would expect, the type of APC is also an important determinant. For example, antigen-primed B cells induce T_H1-like T_{FH} cells (IFN- γ ⁺/IL-21⁺) through IL-6 but not IL-12.¹¹² Functional miRNAs and epigenetic mechanisms also contribute to the machinery of T-cell plasticity through precise regulation of gene expression.^{113,114}

T-cell plasticity might also affect Treg cells and their functional properties in patients with diseases. These cells, which are reviewed elsewhere, typically act to suppress inflammatory processes mediated by effector T cells.¹¹⁵ Those Treg cells, which derive from the thymus, express the transcription factor forkhead box p3 (Foxp3) and mediate central tolerance. By contrast, those induced in the periphery generally lack Foxp3 expression and exert their suppressive effects through high expression of IL-10. However, human Treg cells can also be coaxed to express T_H cytokines, including IFN- γ or IL-17, under conditions that might or might not attenuate Foxp3 expression.¹¹⁶⁻¹¹⁸ Treg cells can also undergo reprogramming toward a T_H2-like phenotype based on coexpression of Foxp3 and IL-4 in response to proinflammatory cytokines akin to those expressed in inflamed tissues.^{119,120} At the level of gene transcription, miRNAs control the ability for Treg cells to acquire effector functions. This is illustrated by the ability for miR-17 to repress the Treg cell-stabilizing transcription factor Eos while derepressing genes encoding effector

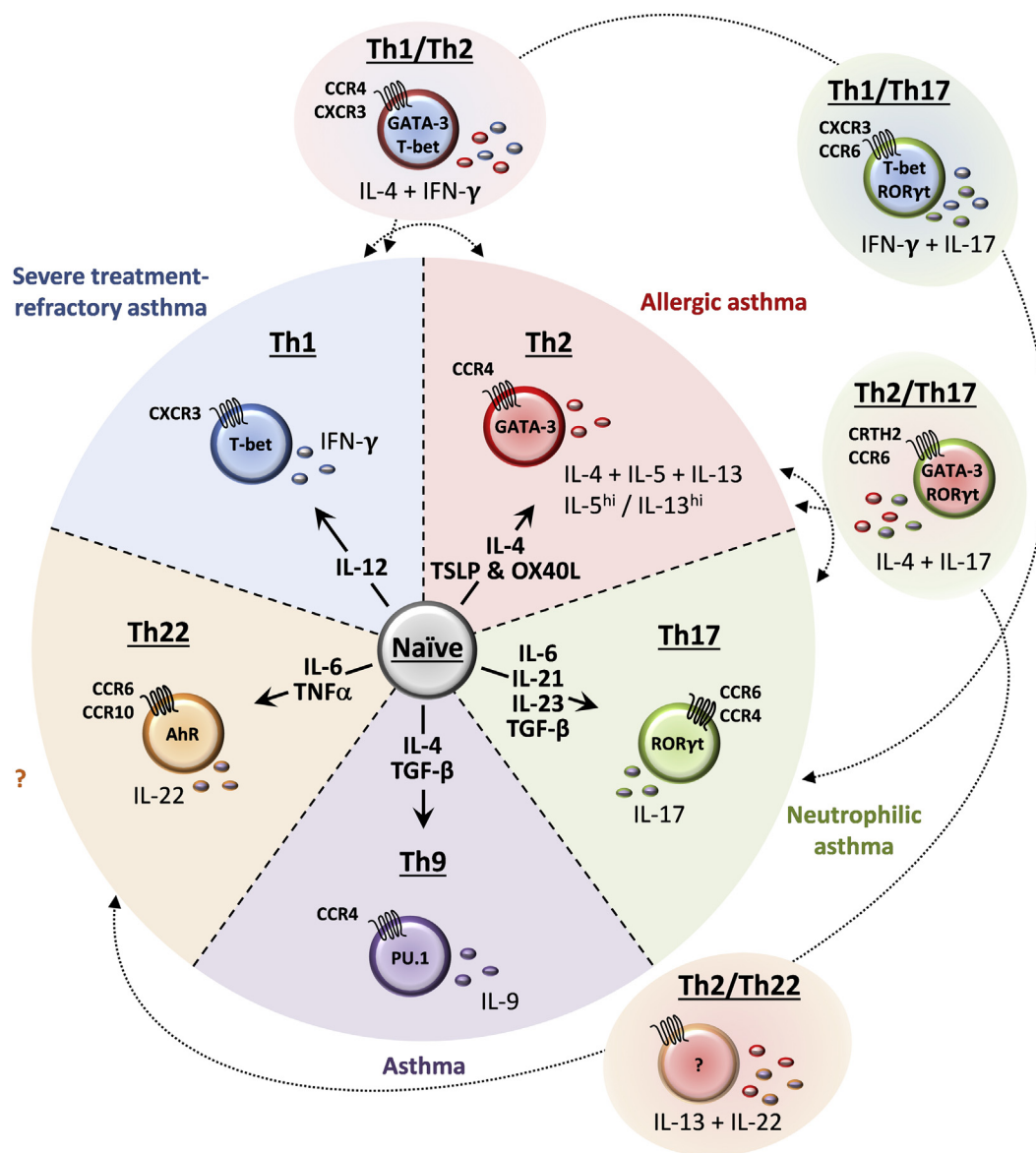


FIG 4. CD4⁺ T_H types linked to asthma. Distinct T_H subsets differentiate from naïve CD4⁺ T cells in response to different inductive cytokines. Each T_H subset bears a characteristic molecular signature defined by lineage-specifying transcription factors, surface chemokine receptors, and secreted cytokines. Atypical T_H types are also shown. The transcription factor profile of T_H2/T_H22 cells is unknown. AhR, Aryl hydrocarbon receptor; ROR γ t, retinoic acid–related orphan receptor γ t.

cytokines.^{121,122} Whether such processes contribute to persistent inflammation in the asthmatic lung is unknown.

T_H2 heterogeneity in asthma

Building on the notion of atypical T_H2 cells, there is evidence that variations in T_H2 cells exist in distinct allergic diseases that might reflect differentiation, conditioning, or both at different anatomic sites. These include different T_H2 cytokine profiles, different levels of specific cytokines within individual cells, or alterations in the proportions of T_H2 subtypes within the allergen-reactive T_H2 pool.^{108,123–125} In patients with severe asthma, IL-4⁺IL-17⁺ T cells in BAL fluid were found to express higher IL-4 levels compared with single-positive IL-4⁺ T cells that were linked to milder airway disease, supporting

a pathogenic role for IL-4^{hi} cells.¹²⁵ IL-4⁺IL-17⁺ cells coexpressed GATA-3 and the T_H17 lineage–specifying transcription factor retinoic acid–related orphan receptor γ t (Fig 4). Notably, in contrast to IL-4⁺IL-17[−] cells, these T cells were resistant to dexamethasone-induced cell death and linked to more severe airway obstruction. These observations, coupled with evidence of reciprocal regulation of T_H2 and T_H17 inflammatory pathways in asthmatic patients, suggest the ability to switch between T_H2/T_H17 phenotypes in response to changing contexts.¹²⁶

There is also provocative new evidence to support T-cell plasticity within T_{FEH} cells in mouse models of allergic asthma. Specifically, IL-4–committed T_{FEH} cells that were confined to mediastinal lymph nodes were a precursor for IL-4⁺IL-13⁺ T_H2 cells that accumulated in the lungs and recruited

eosinophils after allergen challenge.¹²⁷ Interestingly, the development of IL-4-committed T_{EH} cells during the sensitization phase appeared to require B cells, highlighting a new role for B cells in patients with allergic inflammation. This work advanced an earlier observation of a role for T_{EH} cells in eosinophil recruitment to the airways.¹²⁸

It is likely that additional flavors of T_{H2} cells remain to be characterized in asthmatic patients that define different disease endotypes. Examples that have been described in patients with other types of allergic disease include IL-5^{hi} T_{H2} cells and IL-13⁺/IL-22⁺ T_{H2} cells.^{123,129} As already mentioned, IL-22 is the signature cytokine of T_{H22} cells, which lack IL-4, IL-17, and IFN- γ and were previously thought to represent a stable T-cell lineage.¹³⁰ In addition to their altered cytokine signature, the enhanced tissue-trafficking potential of atypical T_{H2} cells, coupled with their link to more severe forms of allergic disease, further implicates these cells in disease pathogenesis (Fig 4).^{124,129,131} Nonetheless, the origins of these cells and, in particular, the transcription factors driving their fate remain enigmatic.

The T_{H9} subset, which was already mentioned, can be induced from polarized T_{H2} cells and differentiates from naive T cells in response to IL-4 with TGF- β (Fig 4).¹³² The signature cytokine IL-9 is expressed in the absence of hallmark cytokines of other T_H types. Initial work established the ability for IL-9 to augment IL-4-mediated IgE production in B cells and to act as a growth factor for mast cells.¹³³ Similar to T_{H2} cells, these cells require GATA-3 for their induction, as well as other transcription factors, including STAT6, IRF4, and PU.1. The tyrosine kinase Itk, which acts downstream in TCR signaling, is also required. In a papain model of asthma that is known to induce a strong T_{H9} response, mice deficient in Itk were found to have decreased lung pathology.¹³⁴ In models of allergic asthma, T_{H9} cells often coexist with T_{H2} cells in the lungs. Thus their relative contributions to disease are unclear.

Interestingly, T_{H2} and T_{H9} cells appear to be coregulated, as evidenced by decreases in both T_H types concomitant with an increase in T_{H17} cell counts in asthmatic mice that are IL-6 deficient.¹³⁵ Nonetheless, T_{H9} cells can exert distinct effector functions. For example, in an OVA model, enhanced mast cell accumulation followed adoptive transfer of T_{H9} cells but not T_{H2} cells, and this effect was dependent on T cell–derived IL-9.¹³⁶ Analysis of T_{H9} cells in relation to asthma phenotypes in human subjects could shed more light on their role.

T_{H1} cells: Friend or foe?

T_{H1} cells are polarized by the transcription factor T-bet to secrete IFN- γ , which counterregulates IL-4. For a long time, the T_{H1}/T_{H2} paradigm provided a simple framework for defining the “balance” between protective and pathogenic responses in patients with allergic disease. This model has been corroborated by the decreased prevalence of allergic sensitization and asthma among European children living on farms who were exposed to high environmental endotoxin levels.¹³⁷ Subsequent studies of allergic asthma in mice provided a T-cell basis for the protective effects of endotoxin. Notably, coadministration of allergen with high-dose LPS favored T_{H1}-skewed responses to allergen,⁸² whereas exposure to high-dose endotoxin before allergen sensitization suppressed T_{H2} immunity.¹³⁸ Despite this, strong evidence of protective T_{H1} responses to allergens in human subjects is lacking at the cellular level. In polyclonal T-cell

systems IFN- γ is readily detected on allergen stimulation, irrespective of whether cells derive from allergic or nonallergic subjects. However, this phenomenon is often ignored, with scientists instead choosing to focus on the T_{H2} component of the response, which is augmented in those subjects sensitized to the test allergen.

Work with MHCII tetramers has confirmed IFN- γ expression in allergen-specific CD4⁺ T cells; however, T cells targeting non-allergens are also likely contributors to T_{H1} responses *in vivo*. To understand these facets, it is worth considering the evolution of asthma from early childhood. The role of respiratory viruses, such as respiratory syncytial virus and HRV, in the development of early-onset wheeze is well established.¹³⁹ These viruses trigger many of the same innate cytokines as allergens, including TSLP and IL-33,^{63,140,141} yet are potent inducers of T_{H1} responses. HRV, the major cause of the common cold, induces acute wheezing episodes in allergic asthmatic patients. Thus, studying this phenomenon could yield insight into the pathogenicity of T_{H1} cells in asthmatic patients. After experimental infection of healthy subjects with HRV-A16, circulating HRV-specific CD4⁺ T cells identified by means of tetramer staining that expanded after infection were predominantly IFN- γ ⁺.¹⁴² Moreover, IFN- γ ⁺CD4⁺ T cells are readily detected in BAL fluid from allergic asthmatic patients experimentally infected with HRV.¹⁴³ Increases in levels of T_{H1}-associated chemokines in the bronchial mucosal lining fluid of patients with varying degrees of asthma control have also been reported after experimental HRV infection.¹⁴⁴ Additionally, a pathogenic role for T_{H1} cells in patients with severe asthma is now emerging, as evidenced by a T_{H1} predominance in the lungs of adults with treatment-refractory severe asthma.¹⁴⁵

Bacteria linked to severe asthma (eg *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Chlamydia pneumonia*) produce an array of potent T_{H1} adjuvants, including cyclic-di-GMP.^{146–150} Recent work in mice implicates this second messenger molecule in the development of severe asthma through dysregulation of an axis involving IFN- γ and the asthma-suppressive factor SLP1.¹⁴⁵

Among patients with established asthma, there is a clear interplay between allergic sensitization and acute wheezing episodes. This relationship is exemplified by the increased incidence of risk for HRV-associated wheezing among asthmatic children who have high levels of serum IgE to house dust mite.¹⁵¹ Although it is tempting to speculate that IgE contributes to this phenomenon through T_{H2}-dependent processes, it might be necessary to reconfigure this paradigm to consider a role for IgE in promoting pathogenic T_{H1} responses. Indeed, in certain contexts IgE binding to antigen can actually enhance secretion of the type I interferon IFN- α by plasmacytoid DCs, which might be predicted to augment T_{H1} responses.¹⁵² Conversely, there is intriguing new evidence to suggest that the blockade of IgE *in vivo* might enhance IFN- α responses in asthmatic children.¹⁵³ Thus further studies are warranted to explore the role of the IgE/IFN- α axis in asthmatic patients.

Other T_H types

Beyond those T_H types already mentioned, a variety of other permutations exist. In addition to its coexpression with T_{H2} cytokines, IL-17 coexpresses with IFN- γ in human-based allergen culture systems.¹⁰⁸ Similarly, depending on the context, IL-22 is coexpressed by a variety of different T_H subsets; however, it

is often described in relation to T_H17 cells because of its link to IL-23, which induces expansion and maintenance of both T_H17 and T_H22 types.¹⁵⁴ Thinking beyond conventional T_H types, it is worth mentioning the contributions of T cells that recognize nonprotein antigens presented in the context of nonclassical MHC molecules, such as CD1. CD1a-reactive T cells are a normal component of the $\alpha\beta$ T-cell repertoire present in both CD4⁺ and CD8⁺ T-cell subsets. These T cells, which can secrete both IL-13 and IFN- γ in allergic patients, can be activated by the generation of novel lipid antigens by naturally occurring enzymes found in various allergen sources, as was recently described for bee venom.^{155,156} Given the abundance of lipid antigens in allergens and microbes, a role for CD1a-reactive T cells in asthmatic patients is entirely plausible.

FUTURE DIRECTIONS AND CLINICAL IMPLICATIONS

Knowledge gaps and new research tools

Much remains to be learned about the assortment of CD4⁺ T cells involved in patients with asthma. Analyzing T cells in highly characterized patients is essential given the complexity of clinical phenotypes that continue to emerge and the disparate underlying T-cell mechanisms. Particular attention should be given to the dynamic T-cell processes that occur in infancy and early childhood during developmental windows, when cells can be particularly susceptible to reprogramming. The same holds true for chronic diseases that provide sustained proinflammatory cues to T cells. Thus, isolating T cells from inflamed tissues will be important. Related to this point, although we have learned a lot about allergen specificity at the T-cell epitope level, little is known about the specificity of the vast majority of infiltrating T cells in the asthmatic lung. The contributions of microbial antigens, as well as less known autoantigens, warrant further study. Recent work indicates that autoantigens can cross-react with environmental antigens or else arise *de novo* through fusion of components that colocalize within intracellular compartments.^{157,158}

The application of state-of-the-art experimental tools and technologies promises to transform our understanding of human T cells by yielding data that are unprecedented in its scope, both at the single-cell level and from a systems biology perspective (Fig 5). Many of these new platforms require much lower numbers of cells for study compared with conventional tools, thereby overcoming many of the barriers that have hindered our understanding of T cells at inflamed sites and in very young children. Depending on the system, minimal *in vitro* manipulation of cells might be required, making it feasible to construct a T-cell portrait resembling that existing *in vivo*.

Gene expression profiling of individual antigen-specific T cells identified by using MHCII multimers now provides a powerful tool for interrogating their heterogeneity and for tracking their response over time in interventional models.¹⁵⁹ A major strength is that it allows the assessment of approximately 100 gene transcripts within a single cell by selecting specific genes for study or else many thousands of transcripts by using genome-wide analyses with single-cell RNA sequencing.¹⁶⁰ Other single-cell platforms now enable functional assays of a single cell *in vitro*.¹⁶¹

Conventional flow cytometric methods use fluorescent-based platforms that typically allow parallel multicolor analysis of 16 to 20 markers on a single cell. By contrast, mass cytometry analyzes up to approximately 45 markers.¹⁶² This enhanced capability

arises from the use of antibodies labeled with metal isotopes that discriminate markers based on discrete masses of metal ions, thereby avoiding the problems with spectral overlap that limit fluorescent-based methods. This technology yields high-dimensional data that provide a systems biology viewpoint of multiple cell types¹⁶³ and reveal complex molecular signatures, including signaling networks and functional responses, at the single-cell level.^{164,165} Moreover, because data are analyzed in an unsupervised fashion using new computational tools,^{166,167} it maximizes the opportunity for discovery of novel cell populations and biomarkers of disease. Similar to transcriptome profiling, mass cytometry promises to prove particularly valuable in interventional models using immunotherapy or experimental infection. In this context, parallel analysis of those cell types known to modulate T_H responses in asthmatic patients (eg, DCs, ILC2s, Treg cells, and regulatory B cells¹⁶⁸) could inform their relative contributions. Looking to the future, the greatest challenge will be to harness the quantities of data generated by using these new technologies and to integrate these with other disease measures to identify T-cell targets for mechanistic studies and new treatments (Fig 5).

Clinical implications

There is a strong rationale for T cell–based therapies in patients with asthma and allergic disease given the critical role T cells play in both initiation and persistence of disease, as already outlined. One of the mainstays of existing guidelines-based therapy¹⁶⁹ is inhaled corticosteroids used alone or in combination with β -agonists, antileukotriene agents, or both. Glucocorticoids have pluripotent effects, among which is the induction of expression of the potent anti-inflammatory cytokine IL-10 by T cells.¹⁷⁰

Many of the currently available T cell–based treatments for patients with severe or treatment-resistant asthma target patients with a type 2–high endotype characterized clinically by the presence of eosinophilia, production of allergen-specific IgE, and disease exacerbations driven by allergen exposure. Perhaps the most well-known and enduring example of this is allergen immunotherapy, which relies on the plasticity of T cells and/or modulation of T_H2 numbers to induce a shift away from a T_H2 to a T_H1/Treg cell phenotype.¹⁷¹

Conventional strategies that use subcutaneous injection of intact allergens capable of cross-linking IgE are fraught with risks to the patient that warrant a cautious up dosing strategy administered over an extended period of time. Unfortunately, the development of accelerated regimens has proved challenging. In a recent study of grass pollen immunotherapy, repeated intradermal injections of allergen over a 12-week period before the grass pollen season using doses 1000 times lower than those typically used for conventional immunotherapy actually resulted in increased serum IgE levels and worse asthma symptoms.¹⁷² In the same study analysis of skin biopsy specimens harvested after intradermal challenge at the end of the pollen season revealed increased expression of the T_H2 marker CCR4 on CD4⁺ T cells. These findings were unexpected, given that the same regimen was previously shown to attenuate late-phase responses in the skin, a phenomenon largely mediated by infiltrating T cells.¹⁷³ Thus, it appears that systemic T_H2 responses can be augmented by intradermal allergen, despite local suppression.

The side effects associated with administering allergen through the skin might be reduced by using sublingual

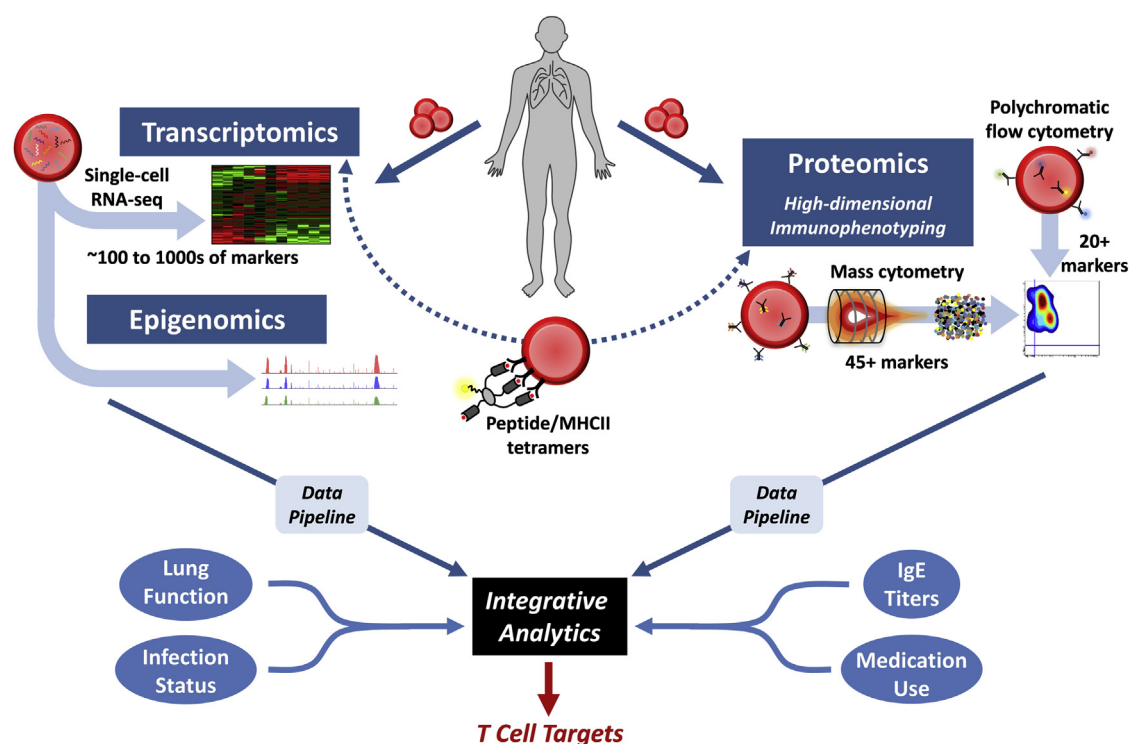


FIG 5. Tools for T-cell discovery in asthma. New technologies enable highly multiplexed analysis of selected T-cell populations or single cells. These “omics” approaches capture large amounts of data at the genetic/epigenetic, gene transcript, and protein levels, which are then deciphered by using bioinformatic pipelines. Emerging computational tools will integrate these data with other clinical and immune parameters to inform new research directions and treatments. *RNA-seq*, RNA sequencing.

immunotherapy (SLIT). Although the protective mechanisms for this strategy remain elusive, they likely involve priming of CD4⁺ T cells by mucosal APCs, among which DCs, Langerhans cells, and macrophages have each been implicated.^{174,175} Dust mite allergen provides an attractive target for this therapeutic modality, given the high prevalence of sensitization among allergic asthmatic patients. Indeed, the efficacy of dust mite SLIT in reducing rhinitis symptoms was recently established in an environmental exposure chamber study.¹⁷⁶ As an extension of this concept, in a randomized trial of asthmatic patients with dust mite allergy whose symptoms were poorly controlled with inhaled corticosteroids, the addition of SLIT to maintenance medications modified the occurrence of asthma exacerbations during a period when inhaled corticosteroids were reduced.¹⁷⁷

Nonetheless, a major hurdle for any immunotherapy strategy that relies on intact allergen is how to efficiently target T cells (preferably at the lowest allergen dose possible) while steering the response toward protective as opposed to pathogenic T-cell types. There is evidence that this can be achieved based on increased markers of T-cell tolerance in patients injected intralymphatically with low-dose Fel d 1 in a form engineered to enhance APC delivery.¹⁷⁸ However, it remains to be seen how practical such a strategy might be in patients.

Short synthetic peptides containing immunodominant T-cell epitopes of allergens provide another opportunity for T-cell modulation. These peptides can induce Treg cell-mediated suppression without IgE cross-linking and its troublesome local and/or systemic sequelae (Fig 6, A). In this regard T-cell epitope mapping of Fel d 1 set an important precedent that resulted in the

design of peptide-based therapies for cat allergy that might in theory prove beneficial for asthmatic patients. Notably, intradermal administration of a peptide cocktail provided durable clinical benefit and a greatly simplified treatment regimen compared with conventional immunotherapy.¹⁷⁹⁻¹⁸¹ Trials of other peptide therapies related to house dust mite, grass pollen, and ragweed are ongoing.^{182,183} Nonetheless, the challenges are considerable in terms of optimizing dosing and selecting those peptide combinations that might prove efficacious within the general population. Indeed, early studies of cat peptides demonstrated their ability to induce a marked decrease in FEV₁ in asthmatic patients with cat allergy several hours after injection. This not only corroborated the pivotal role of allergen-specific memory T cells in late-phase responses in the asthmatic lung but also the potential side effects of peptide therapies.¹⁸⁴ Moreover, the development of “pooled” peptides to treat patients with multiple allergic sensitizations is in its infancy, and thus traditional immunotherapy will remain the mainstay of therapy for these patients for the foreseeable future.

There is now a growing list of mAbs that inhibit T_H2 pathways,¹³ including those that block IL-5/IL-5 receptor α in eosinophilic asthma (eg, mepolizumab, reslizumab, and benralizumab) and IL-4/IL-13 in patients with high periostin levels (eg, dupilumab; Fig 6, B).¹⁸⁵ Biologics that block the IgE pathway (eg, omalizumab)¹⁸⁶ can also exert an indirect effect on T cells by dampening proinflammatory cues and modifying IgE-mediated allergen uptake by DCs, thereby reducing their T_H2-licensing capabilities. Although these approaches have shown promise in reducing asthma exacerbations in patients with a type 2–high endotype, the type 2–low group, which is more clinically

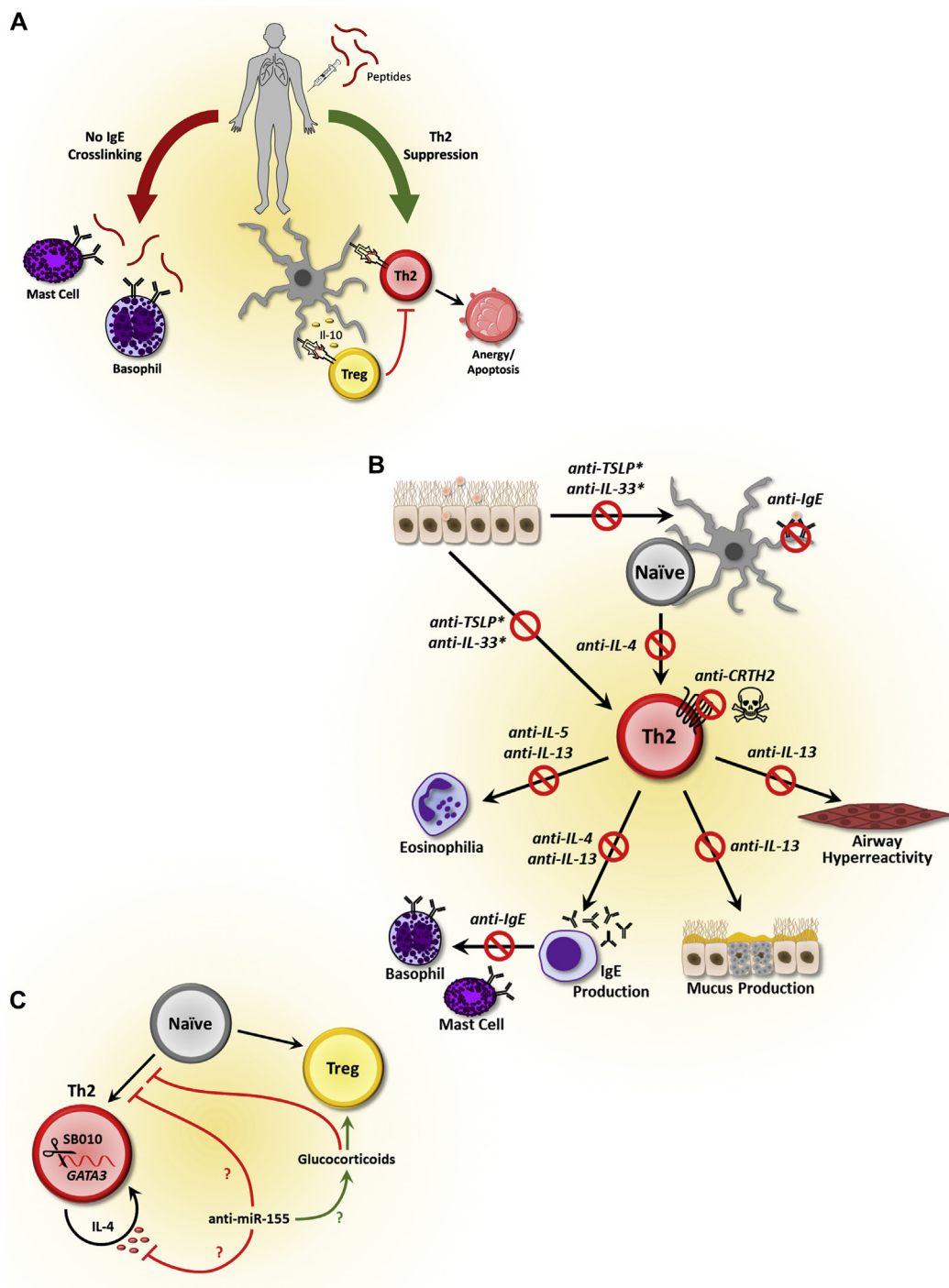


FIG 6. T cell-based therapeutic strategies. **A**, Peptide-based immunotherapy suppresses T_H2 responses without inducing adverse sequelae mediated by IgE receptor cross-linking. **B**, Effect of mAb therapies on T_H2 effector functions. *Clinical trials in progress. **C**, Emerging molecular therapeutics for allergic asthma.

heterogeneous, has not demonstrated a clear benefit. In addition, evidence for a persistent disease-modifying effect of these therapies is currently lacking. Thus there is a desire for therapies that target a broader patient population or else inhibit earlier stages in disease pathogenesis (eg, T-cell priming) to produce more sustained benefit. Obvious upstream targets include epithelial cell-derived cytokines. Indeed, neutralization of TSLP in a mouse model of allergic asthma showed diminished T_H2 responses and

attenuated hallmarks of disease.¹⁸⁷ Extending this to patients, treatment of mild asthma with the anti-TSLP mAb AMG 157 (tezepelumab) reduced allergen-induced bronchoconstriction and decreased airway inflammation.¹⁸⁸ Neutralization of the IL-33/ST2 pathway in asthmatic patients might be predicted to have similar benefit, and its translation to patients is underway.¹⁸⁹ Also being explored are CRTH2 antagonists¹⁹⁰ and CRTH2 depleting mAbs,¹⁹¹ which target memory T_H2 cells, as well as

CRTH2⁺ innate immune cells (eosinophils, basophils, and ILC2s) that have homed to the lungs.

Several molecules engineered to inhibit discrete stages in the T_H2 differentiation process are currently being tested in preclinical and early clinical trials (Fig 6, C). An inhaled DNA enzyme (SB010) that cleaves and inactivates *GATA3* mRNA demonstrated the ability in a small, randomized, double-blind, placebo-controlled trial to attenuate the asthmatic response to allergen challenge; however, larger-scale trials are needed to validate this finding.^{192,193} There is also interest in targeting miRNAs, such as miR-155; however, given that these molecules can exert differential effects at discrete stages of the T_H2 pathway, it is difficult to predict outcomes (Fig 6, C).²⁹ Moreover, developing a pharmacologic strategy by which to deliver an anti-miRNA agent into cells in a specific stable manner will be a significant challenge.

The failure of other T cell–targeted therapies to demonstrate clinical benefit, including OX40L blockade,¹⁹⁴ provides a cautionary tale and highlights the great complexity of T cells in patients with allergic disease that precludes a “one size fits all” approach. With this in mind, sophisticated computational tools are rapidly emerging that can integrate large data sets with a view to linking specific T-cell types with clinical phenotypes.¹⁹⁵ Together, the strategies described should provide an important stepping stone on the path to personalized treatments that target pathogenic CD4⁺ T cells in patients with asthma and allergic disease.

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