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## Functions of T cells in asthma: more than just T<sub>H</sub>2 cells

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### Abstract

Asthma has been considered a T helper 2 (T<sub>H</sub>2) cell-associated inflammatory disease, and T<sub>H</sub>2-type cytokines, such as interleukin-4 (IL-4), IL-5 and IL-13, are thought to drive the disease pathology in patients. Although atopic asthma has a substantial T<sub>H</sub>2 cell component, the disease is notoriously heterogeneous, and recent evidence has suggested that other T cells also contribute to the development of asthma. Here, we discuss the roles of different T cell subsets in the allergic lung, consider how each subset can contribute to the development of allergic pathology and evaluate how we might manipulate these cells for new asthma therapies.

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Asthma is classically thought to be a T helper 2 (T<sub>H</sub>2)-cell driven inflammatory disease, characterized by eosinophilic inflammation, T<sub>H</sub>2-cell associated cytokine production and airway hyperresponsiveness (AHR). Although many animal models have suggested that T<sub>H</sub>2 cells promote allergic airway inflammation, these studies have not led to new therapies for the increasing number of patients with asthma. Recent advances indicate that asthma can no longer be considered solely as a disease of the adaptive immune system, but that crosstalk between the innate and adaptive immune system is crucial for the initiation and propagation of the allergic immune response. Indeed, the typical clinical heterogeneity of asthma (BOX 1) might reflect the varied interactions that occur among distinct populations of stromal cells, epithelial cells and leukocytes in the allergic lung, as well as the contribution of T cell subsets.

In this Review, we discuss the roles of different T cell subsets in allergic asthma that have led to the view that asthma is more than just a T<sub>H</sub>2-type disease. Although the classical T<sub>H</sub>1–T<sub>H</sub>2 paradigm remains a useful frame-work for understanding T cell heterogeneity, it has been extended by the discovery of several of other T cell subsets, such as T<sub>H</sub>17 cells, T<sub>H</sub>9 cells and regulatory T (T<sub>Reg</sub>) cells (FIG. 1). New data suggest that T cells do not become fully committed to a specific subset following encounter with antigen but may retain plasticity and are influenced by the surrounding microenvironment. These new concepts will be discussed in the context of the unique immunological milieu of the allergic lung (BOX 2).

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**FURTHER INFORMATION** Clare M. Lloyd's homepage: <http://www1.imperial.ac.uk/medicine/about/divisions/nhli/respiration/leukocyte>

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## Effector CD4<sup>+</sup> T cells in allergic inflammation

### T<sub>H</sub>1 and T<sub>H</sub>2 cells

The division of CD4<sup>+</sup> T cells into two distinct functional subsets, namely the T<sub>H</sub>1 and T<sub>H</sub>2 cell subsets, prompted much research into the roles of both subsets in disease<sup>1</sup>, and the increased presence of T<sub>H</sub>2 cells in the airways of patients with asthma categorized asthma as a T<sub>H</sub>2 cell-driven disease<sup>2,3</sup>. Subsequently, a large body of data from both humans and animal models (BOX 3) provided evidence that T<sub>H</sub>2 cells, through the production of cytokines such as interleukin-4 (IL-4), IL-5 and IL-13, can initiate and maintain key pathophysiological features of the disease<sup>4,5</sup>. Whereas IL-4 is important for allergic sensitization and IgE production, and IL-5 is crucial for eosinophil survival, IL-13 has pleiotropic effects in the lungs, including a central role in the development of AHR and tissue remodelling<sup>6</sup>.

In an attempt to inhibit the unwanted effects of a T<sub>H</sub>2-type response, much effort has been invested in elucidating the factors that are involved in T<sub>H</sub>2 cell development. Although IL-4 has long been recognized as an important cytokine in the development of T<sub>H</sub>2 cells, recent progress demonstrates that IL-25, IL-33 and thymic stromal lymphopoeitin (TSLP) can induce many of the key features of an allergen or helminth-induced T<sub>H</sub>2-type response, including eosinophilia and the production of IL-4, IL-5, IL-13 and IgE antibodies. Recent reports show that the cellular sources of these T<sub>H</sub>2-type cytokines include innate lymphoid cells that do not express surface markers for any known leukocyte lineage and have been termed 'innate helper' cells<sup>7-9</sup>. In mice, these cells are found in many different tissues in the absence of immune challenge, and they are particularly prevalent in the mesenteric lymph nodes, spleen, gut and liver, as well as in the lungs<sup>10</sup>. A functional role for these innate helper cells in pulmonary immune responses is inferred from the observation that T<sub>H</sub>2-type disease still develops in mice in the absence of T and B cells<sup>11</sup> (FIG. 2). The fact that a T<sub>H</sub>2-type response can develop in the absence of T<sub>H</sub>2 cells raises the question of what the potential role of these innate helper cells may be during allergic immune responses.

Allergen challenge in patients with asthma provokes the influx of activated T<sub>H</sub>2 cells into the airways and this is accompanied by an increase in the levels of T<sub>H</sub>2-type cytokines and recruitment of eosinophils (reviewed in REF. 12). Notably, the number of T<sub>H</sub>2 cells present in the airways positively correlates with disease severity (reviewed in REF. 12). During asthma exacerbation, both T<sub>H</sub>2 and T<sub>H</sub>1 cells increase in number in blood and induced sputum<sup>13</sup>. T<sub>H</sub>1 cells could perhaps be expected to have an inhibitory role in asthma as they can directly inhibit the development of T<sub>H</sub>2 cells<sup>11</sup>. In support of this, deletion of the T<sub>H</sub>1 cell master transcription factor T-bet in mice results in the development of spontaneous AHR and IL-13-dependent eosinophilia<sup>14</sup>. However, when the signature T<sub>H</sub>1-type cytokine, interferon- $\gamma$  (IFN  $\gamma$ ), is administered to the airways of patients with asthma, there is no improvement of disease symptoms<sup>15</sup>. The correlation between T<sub>H</sub>2 cell numbers and disease severity suggests an essential role for T<sub>H</sub>2 cells in human asthma. However, recent cluster analysis of asthma clinical phenotypes shows that eosinophilic and noneosinophilic subtypes exist, suggesting that mechanisms other than T<sub>H</sub>2 cells may be important for pathology in some patients<sup>16</sup>.

### T<sub>H</sub>17 cells

T<sub>H</sub>17 cells are a distinct lineage of CD4<sup>+</sup> effector T cells that express IL-17 and the key transcription factors retinoic acid receptor-related orphan receptor- $\gamma$  (ROR  $\gamma$ ) and ROR  $\gamma$ <sup>17,18</sup>. Although transforming growth factor- $\beta$  (TGF  $\beta$ ) and the inflammatory cytokines IL-6, IL-21 and IL-23 are thought to be crucial for T<sub>H</sub>17 cell development in mice, it is less clear which cytokines are required for T<sub>H</sub>17 development in humans<sup>19-21</sup>. IL-17A is

upregulated in the lungs of patients with asthma, and levels of IL-17 correlate with disease severity, particularly in patients with neutrophilic, steroid-resistant asthma<sup>22,23</sup>. Furthermore, tissue-infiltrating CD4<sup>+</sup>IL-17<sup>+</sup> T cells have been documented in the lungs of patients with asthma<sup>24</sup>.

Careful studies of CD4<sup>+</sup>IL-17<sup>+</sup> T cells have revealed an important role for T<sub>H</sub>17 cells in allergic inflammation. Epicutaneous or airway sensitization with allergen primes modest T<sub>H</sub>2 cell responses, but it also primes strong T<sub>H</sub>17 cell responses that promote airway neutrophilia and acute AHR<sup>25,26</sup>. T<sub>H</sub>17 cells also exacerbate T<sub>H</sub>2 cell-mediated eosinophilic airway inflammation<sup>27</sup>. Interestingly, transfer of antigen-specific T<sub>H</sub>17 cells promotes steroid-resistant airway inflammation and AHR in mice<sup>28</sup>, further strengthening the association between T<sub>H</sub>17 cells and severe asthma.

However, blocking studies *in vivo* have revealed a complex role for IL-17 in the allergic lung. Although abrogation of IL-17 function during allergic sensitization is protective in mouse models of asthma, delivery of exogenous IL-17A during allergen challenge decreases airway inflammation, suggesting that there is also a regulatory role for IL-17 in the allergic lung<sup>29-31</sup>.

### T<sub>H</sub>9 cells

The complexity surrounding T cell subsets increased further with the recent description of a discrete population of IL-9-secreting CD4<sup>+</sup> T cells that depend on TGF $\beta$  for their development<sup>32</sup>. Generation of these T<sub>H</sub>9 cells requires expression of the transcription factor Pu.1, and secretion of IL-9 from T<sub>H</sub>9 cells is upregulated by IL-25 (REFS 33,34). There is a wealth of evidence to suggest that IL-9 is involved in the development of allergic inflammation in the lungs. IL-9 is detected in biopsies from patients with asthma and localizes to CD4<sup>+</sup> T cells, although there are several other cellular sources of IL-9, including mast cells and eosinophils<sup>35-37</sup>. Although IL-9-transgenic mice exhibit baseline pulmonary eosinophilia and AHR in the absence of allergen exposure, IL-9-deficient mice have produced mixed results, indicating a degree of redundancy in the functions of IL-9 (REFS 38,39).

Manipulation of T<sub>H</sub>9 cell functions *in vivo* changes the course of allergic inflammation. For example, compared with wild-type mice, mice with Pu.1-deficient T cells developed normal T<sub>H</sub>2 cell responses after allergen exposure *in vivo*, but they showed reduced allergic pulmonary inflammation, which was associated with decreased IL-9 expression<sup>33</sup>. The discovery of a potentially distinct lineage of IL-9-producing CD4<sup>+</sup> T cells may well clarify this situation; however, direct *in vivo* evidence for the existence of T<sub>H</sub>9 cells in the allergic lung of mice or humans is still lacking. It is also not completely clear whether T<sub>H</sub>9 cells are anti- or pro-inflammatory *in vivo*. Further analysis of the function of these cells in the allergic lung is warranted.

### CD8<sup>+</sup> T cells in asthma

The presence of CD8<sup>+</sup> T cells in asthmatic airways is well documented; bronchial biopsies from patients with atopic and non-atopic asthma contain CD8<sup>+</sup> T cells that produce IL-4 and IL-5 (REF. 40), and spontaneous IL-4, IL-5 and IFN $\gamma$  production by sputum-derived CD8<sup>+</sup> T cells is higher than in healthy subjects<sup>41</sup>. It is now clear that, particularly in patients with asthma, the behaviour of peripheral blood-derived CD8<sup>+</sup> T cells does not resemble that of airway CD8<sup>+</sup> T cells<sup>41</sup>. This may reflect preferential homing or an increased or altered activation state induced by the local airway environment. CD8<sup>+</sup> T cells are particularly well represented among intraepithelial T cells and are present in increased proportions in patients with asthma<sup>42</sup>.

In the past decade, many animal models have shown that the actions of CD8<sup>+</sup> T cells in asthmatic airways can be beneficial or deleterious (reviewed in REF. 43). Although this body of data has provided a stepwise improvement in our understanding of the potential roles of CD8<sup>+</sup> T cells in asthma, our understanding of the role (or roles) these cells actually have in the airways of patients with asthma remains limited. Depletion of CD8<sup>+</sup> cells (using  $\alpha$ X-8 antibody) in previously sensitized rats leads to enhancement of airway inflammation<sup>44</sup>, an increase in the late-phase response<sup>45</sup> (BOX 4) and enhancement of various airway remodelling parameters, including airway smooth muscle mass, epithelial cell proliferation and mucus production<sup>46</sup>, which all suggest a protective role for CD8<sup>+</sup> T cells. However, in addition to T cells, several other cell types can express CD8<sup>+</sup>, including macrophages, dendritic cell (DC) subsets, natural killer (NK) cells, and invariant NKT (iNKT) cells, leaving these depletion studies open for several potential interpretations.

By contrast, a variety of cell transfer studies using CD8<sup>+</sup> T cells show that these cells worsen asthma disease symptoms, either directly<sup>47</sup> or, as the majority of studies indicate, in concert with sensitized CD4<sup>+</sup> T cells<sup>48-51</sup>. CD8-deficient mice are less susceptible to allergic airway inflammation, but the transfer of *in vitro*-generated antigen-primed effector memory CD8<sup>+</sup> T cells into sensitized CD8-deficient mice increased AHR, eosinophilic inflammation and the levels of IL-13 in bronchoalveolar lavage (BAL) fluid. Interestingly, this does not occur when the transferred CD8<sup>+</sup> T cells are derived from IL-13-deficient mice or in the absence of subsequent allergen challenge<sup>49,50</sup>, indicating that these deleterious CD8<sup>+</sup> T cells are activated within the airways and produce IL-13 locally.

In the presence of T<sub>H</sub>2 cells, virus-specific CD8<sup>+</sup> T cells become potent IL-5 producers; they switch off their IFN $\gamma$  production and contribute to increased airway eosinophilia in mice<sup>52</sup>. This observation may in part explain how viral infections contribute to asthma exacerbation. In turn, impaired secretion of IFN $\gamma$  and a non-cytolytic CD8<sup>+</sup> T cell phenotype may prolong the resolution of viral infection, an event that has been documented in rhinovirus-infected patients with asthma. Compared with healthy counterparts, patients with asthma have an enhanced frequency, severity and duration of their rhinovirus infections<sup>53</sup>. Intriguingly, the annual decline in forced expiratory volume in 1 second (FEV1) in patients with asthma can be predicted by the numbers of CD8<sup>+</sup> T cells in the bronchial infiltrate<sup>54</sup>, and cytokine production by sputum CD8<sup>+</sup> T cells correlates with asthma severity<sup>41</sup>. Also, patients who die from asthma have a larger proportion of activated bronchial CD8<sup>+</sup> T cells than patients with asthma who die from other causes<sup>55</sup>; this suggests a detrimental role for CD8<sup>+</sup> T cells in the pathogenesis of human asthma. However, in spite of these existing correlations, the question remains whether CD8<sup>+</sup> cells are non-participating bystanders or acquire a more active role in driving more chronic and severe disease.

### T<sub>Reg</sub> cells in asthma

Reduced or altered function of T<sub>Reg</sub> cell populations provides a possible explanation for the inappropriate immune response to allergens observed in patients with asthma. Several T<sub>Reg</sub> cell subsets have been described, including naturally occurring forkhead box P3 (FoxP3)<sup>+</sup>CD4<sup>+</sup>CD25<sup>+</sup> cells and inducible T<sub>Reg</sub> cells, which develop *in vitro* or *in vivo* following antigen stimulation (reviewed in REF. 56). Transfer of CD4<sup>+</sup>CD25<sup>+</sup> T<sub>Reg</sub> cells ameliorates the development of airway inflammation and AHR and prevents the allergen-induced activation of DCs in the airways<sup>57-61</sup>. T<sub>Reg</sub> cells exert their inhibitory action through direct and indirect mechanisms: they produce anti-inflammatory cytokines, such as IL-10 and TGF $\beta$ , express inhibitory molecules, such as cytotoxic T lymphocyte antigen 4 (CTLA4)<sup>57,58</sup> and induce the downregulation of mHC class II and the co-stimulatory molecules CD80 and CD86 by antigen-presenting cells<sup>62</sup>. Interestingly, T<sub>Reg</sub> cells do not necessarily produce IL-10 themselves but, instead, can induce IL-10 production from bystander CD4<sup>+</sup> T cells, which then mediate the suppressive effect<sup>58</sup>. T<sub>Reg</sub> cell-mediated

suppression of DCs is dependent on direct cell contact: T<sub>Reg</sub> cells form lymphocyte function-associated antigen 1 (LFA1)-dependent aggregates with DCs, which leads to downregulation of DC co-stimulatory molecules in response to T<sub>Reg</sub> cell-expressed CTLA4 (REF. 63). Consistent with this, higher frequencies of airway DCs with increased levels of co-stimulatory molecules and an improved stimulatory capacity are detected in T<sub>Reg</sub> cell-depleted mice<sup>64</sup>.

Both mouse and human data show that T<sub>Reg</sub> cells need to be maintained by chronic local antigen exposure. If not, the suppressive effect diminishes and airway responses to allergen return<sup>61,65</sup>. Waxing and waning of a T<sub>Reg</sub> cell response owing to discontinuous allergen exposure may contribute to the intermittent pattern of exacerbations observed in patients with asthma. The presence and functional status of T<sub>Reg</sub> cells has been investigated in human blood and airway compartments. Depletion of CD4<sup>+</sup>CD25<sup>+</sup> T cells from peripheral blood cultures of non-atopic, non-allergic control mice shows that the remaining CD4<sup>+</sup>CD25<sup>-</sup> T cells are fully capable of proliferating in response to allergen<sup>66</sup>. CD4<sup>+</sup>CD25<sup>+</sup> T cells suppress this response: the highest levels of suppression occur in non-atopic controls, but suppression is reduced in atopic patients. Suppression is lowest when CD4<sup>+</sup>CD25<sup>+</sup> T cells come from atopic individuals during the active pollen season<sup>66-68</sup>. Similar observations have been made in both adult and paediatric patients<sup>66-68</sup>. In the airways of children with asthma, the percentage of CD4<sup>+</sup>CD25<sup>+</sup> T cells is lower than in healthy children, which might be explained by the reduced response to chemokines of peripheral blood CD4<sup>+</sup>CD25<sup>+</sup> T cells noted in patients with asthma<sup>71</sup>. The presence of CD4<sup>+</sup>CD25<sup>+</sup> T cells in the airways is positively correlated with FEV1 and, interestingly, corticosteroid treatment increases the number of CD4<sup>+</sup>CD25<sup>+</sup> T cells in the airways of patients with asthma<sup>70</sup>.

Several immunotherapy regimens have been shown to increase T<sub>Reg</sub> cell responses (reviewed in REF.56). The combination of corticosteroid and vitamin D induces IL-10-producing T<sub>Reg</sub> cells *in vitro* (reviewed in REF. 72), and vitamin D by itself restores the defective *in vitro* production of IL-10 that is seen in T cells from patients with corticosteroid-resistant asthma<sup>73</sup>. Interestingly, in a small clinical study, administration of calcitriol (the active form of vitamin D) to corticosteroid-resistant asthmatics for one week restored the *ex vivo* IL-10 response of T cells from these patients<sup>74</sup>. Clinical research designed to evaluate T<sub>Reg</sub> cell-inducing therapies will greatly benefit from specific surface markers that allow definitive identification of T<sub>Reg</sub> cells. A recent genome-wide screen of human FoXP3 target genes showed that 739 target genes are differentially expressed in FoXP3<sup>+</sup> T<sub>Reg</sub> cells and that peptidase inhibitor 16, in conjunction with CD25, may serve as a useful marker for T<sub>Reg</sub> cells (these molecules are expressed by >80% of T<sub>Reg</sub> cells)<sup>75</sup>. An intriguing observation is that T<sub>Reg</sub> cells often have several transcriptional control elements in common with the cells they regulate, which may ensure expression of the same homing receptors and facilitate homing of both the 'regulator' and the 'regulatee' to the same organ<sup>76</sup>.

## Innate-like T cells in asthma

### NKT cells

NKT cells express an invariant T cell receptor (TCR): V<sub>14</sub> in mice and V<sub>24</sub> in humans. This TCR recognizes glycolipids, instead of conventional peptide antigens, in the context of the mHC class I-like molecule CD1d, and it is highly conserved between species, suggesting that it behaves like a pattern-recognition receptor and that NKT cells contribute to innate immune responses. Activation of the invariant TCR of NKT cells leads to the rapid production of a range of inflammatory cytokines, including IL-4 and IFN $\gamma$ , providing early support to drive adaptive immune responses. Another population of NKT cells, referred to as

non-classical or type II NKT cells, also recognize CD1d-associated antigens, but do not express the invariant TCR.

Evidence from animal models strongly implicates NKT cells in the development of allergic airway disease. Mice that are deficient in NKT cells (*J 18<sup>-/-</sup>* or *CD1d<sup>-/-</sup>* mice) retain some degree of eosinophilic inflammation, but fail to develop AHR<sup>77</sup>. Conversely, pulmonary instillation of the NKT cell TCR ligand glycolipid  $\alpha$ -galactosylceramide ( $\alpha$ -GalCer) promotes NKT cell production of IL-13 and TSLP and promotes AHR<sup>78</sup>. Moreover, exposure of mice to  $\alpha$ -GalCer in the presence of antigen promotes NKT cell-dependent allergen sensitization following subsequent allergen exposure<sup>79</sup>. Similar results were obtained following  $\alpha$ -GalCer challenge of non-human primates<sup>80</sup>. However, the role of NKT cells in the development of human asthma has been controversial as the reported incidence of NKT cells in allergic airways varies from 2% to 60% of BAL cells<sup>81,82</sup>. Although these differences probably reflect variances in experimental techniques and patient populations, the fact remains that NKT cells are increased in asthmatic patients following allergen challenge<sup>83,84</sup>. NKT cells may amplify allergic responses in the presence of T<sub>H</sub>2 cells, but may also drive AHR in the absence of adaptive immune responses, particularly during asthma associated with viral infections, pollution or neutrophils<sup>85</sup>.

### $\gamma\delta$ T cells

A local population of  $\gamma\delta$  T cells resides in close association with the lung epithelium, where they have an immunosurveillance function, responding to endogenous stress signals and localizing to sites of tissue damage<sup>86,87</sup>. The proportion of  $\gamma\delta$  T cells in BAL is higher in patients with asthma than in healthy controls. Moreover,  $\gamma\delta$  T cells from patients with asthma, but not from controls, secrete IL-4 and proliferate to specific, but not unrelated allergen<sup>88</sup>. Furthermore, a high proportion of  $\gamma\delta$  T cells in the nasal mucosa of patients with allergic rhinitis express IL-4 but little IFN  $\gamma$ <sup>89</sup>. This correlation was not observed in peripheral blood cells, underscoring the unique milieu of the lungs and the need to assess tissue-resident cells. However,  $\gamma\delta$  T cell function has not yet been studied in the lungs of allergic patients.

Studies in mouse models suggest that  $\gamma\delta$  T cells have dual contrasting roles in the development of allergic inflammation. Analysis of subsets of pulmonary  $\gamma\delta$  T cells shows that there is a pro-inflammatory  $\gamma\delta$  T cell subset that expresses V 1<sup>+</sup> TCR and a suppressive subset that expresses V 4<sup>+</sup> TCR<sup>90,91</sup>. Similarly to T<sub>H</sub>2 cells, V 1<sup>+</sup>  $\gamma\delta$  T cells produce IL-5 and IL-13 in the airways and synergize with NKT cells in mediating AHR. The capacity of V 4<sup>+</sup>  $\gamma\delta$  T cells to suppress established allergic inflammation and AHR depends on their ability to secrete IL-17A<sup>31</sup>. These data suggest that depending on its cellular source (either T<sub>H</sub>17 or  $\gamma\delta$  T cells), IL-17A can have distinct functions in the airways, thereby further emphasizing the site- and context-specific nature of cytokine production within the lung. Interestingly, AHR occurs in non-allergic mice that lack  $\gamma\delta$  T cells, suggesting a role for these cells in the maintenance of normal airway function<sup>92</sup>.

Collectively, these experiments suggest that the tissue localization of  $\gamma\delta$  T cells and their ability to recognize stress or damage-associated antigens allows them to initiate protective immune responses, but signals from the local inflammatory environment may promote regulatory activity during established responses in order to control inflammation and stimulate resolution.

### T cell trafficking in asthma

Similar to their cytokine expression, T cell subsets have distinct chemokine receptor expression patterns (FIG. 3). For example, T<sub>H</sub>2 cells have been delineated by expression of

CC-chemokine receptor 4 (CCR4) and CCR8, and T<sub>H1</sub> cells by expression of CCR5 and CXC-chemokine receptor 3 (CXCR3). However, given the opposing functions of T<sub>H2</sub> cells and T<sub>Reg</sub> cells, it is perhaps surprising that they share expression of CCR4 and CCR8. Recruitment of T<sub>Reg</sub> cells is abrogated in CCR4-deficient mice<sup>93</sup>. Mice with a specific loss of CCR4 in their T<sub>Reg</sub> cell compartment develop lymphocytic infiltration and severe pulmonary inflammatory disease. Analogous expression patterns have been seen in human T<sub>Reg</sub> cells<sup>56</sup>. A similar shared pattern of chemokine receptor expression is observed between T<sub>H17</sub> and T cells: both populations express CCR6 and migrate towards CC-chemokine ligand 20 (CCL20) *in vitro* and *in vivo*<sup>94–97</sup>. Whether this shared receptor expression is another facet of flexibility is not clear but, again, it is likely to be determined by the local cytokine milieu. The chemokine receptor repertoire of T<sub>H9</sub> cells has yet to be determined.

It is generally accepted that T cell responses are induced following the uptake of antigen by local DCs and the transport of this antigen to the draining lymph node for presentation to T cells. However, there is evidence that effector T cells constantly recirculate through the lungs and have an immunosurveillance role. Moreover, resident pulmonary antigen-presenting cells can present allergen long after cessation of allergen exposure and have been shown to promote T<sub>H2</sub> cell differentiation *in situ*, indicating that migration to secondary lymphoid organs is not crucial for T cell priming within the respiratory tract<sup>98,99</sup>. With the growing acceptance of T cell plasticity and flexibility, it is possible that T cells can de-differentiate or be reprogrammed locally. The subsequent change in cytokine secretion pattern probably evokes a change in chemokine receptor expression to allow correct migration within the surrounding airways.

## Flexibility of T cell subsets in the lung

The discovery of several potential new T cell lineages suggests that the fate of CD4<sup>+</sup> T cell subsets may be more flexible than previously thought. Immunological dogma dictates that, following antigen stimulation and several rounds of division, T<sub>H1</sub> and T<sub>H2</sub> cells become irreversibly committed to these lineages. However, the finding that TGF $\beta$  can subvert T<sub>H2</sub> cells to the T<sub>H9</sub> cell lineage has led to the understanding that effector CD4<sup>+</sup> T cell populations might be more plastic than originally thought<sup>32,100</sup>. Recent studies have also shown that T<sub>Reg</sub> and T<sub>H17</sub> cells are not stable populations and have the capacity for re-differentiation<sup>101</sup>. Given that most of the original evidence for the commitment of these cells stems from *in vitro* analysis, it is perhaps not surprising that the situation is different *in vivo*.

Flexibility of cytokine secretion potentially enables T cells to respond efficiently and effectively to the many immunological insults that they encounter. Thus the influence of molecular mediators produced by the local microenvironment is probably the crucial cue for change; this fact is particularly pertinent to the lungs, as the respiratory environment is a uniquely specialized site that is constantly exposed to the external atmosphere. The lung needs to limit immunological responses to airborne antigens, most of which do not represent a threat to the host. The mechanisms that discriminate these allergens from pathogens involve both innate and adaptive immune responses, including activation and differentiation of T cell subsets. It is clear that flexibility of T cell subsets within the lung would permit rapid reaction to changing circumstances. In addition, although cooperation among all of the different T cell subsets present in the asthmatic lung is easily envisaged, evidence for these interactions is not clearly documented in the literature. In the future, it will be vital to study these local T cell networks and their interactions.

## Environmental influences on airway T cells

Several factors are likely to influence the development of T cell responses to allergen, including age, gender, obesity, infection history, atopic status, allergen exposure levels,

antibiotic use, environmental factors (such as exposure to diesel) and nutrition (for example, vitamin levels and hormone exposure) (FIG. 3), and these factors probably contribute to the heterogeneity of clinical disease (BOX 1). Although asthma was once thought to be primarily a disease of aberrant T<sub>H</sub>2-cell directed immune responses, clinical studies now indicate that individuals with asthma can be phenotyped according to their symptoms and that these clinical phenotypes are closely associated with environmental factors<sup>102,103</sup>. Moreover, interaction between the epithelium and the immune system is crucial in promoting disease, and the genetic signature of airway epithelial cells may influence clinical phenotype.

### Epithelial cells

Evidence that epithelial cell function affects the degree of T<sub>H</sub>2-type inflammation has come from studies of the epithelium from patients with asthma. The T<sub>H</sub>2-type cytokine induced genes *POSTN* (encoding periostin), *SERPINB2* and *CLCA1* have previously been identified as epithelial cell-expressed genes that are specifically induced in patients with asthma; these genes are proposed to represent an asthma-specific mRNA signature for epithelial cells<sup>104</sup>. At least two distinct molecular phenotypes of asthma are defined by the degree of T<sub>H</sub>2-type inflammation<sup>105</sup>. Direct experimental evidence linking epithelial gene expression to the development of T<sub>H</sub>2-type immunity comes from mice with a specific deletion of *Foxa2*. The loss of this transcription factor from the pulmonary epithelium leads to spontaneous eosinophilic inflammation and mucous metaplasia<sup>106</sup>. Interestingly, *Foxa2* mice show reduced levels of both IL-33 and CCL20, both of which are important for immune responses against inhaled allergens. These findings may explain, to some extent, the clinical heterogeneity of asthma, as the epithelial gene signature of an individual may influence the strength of T<sub>H</sub>2-type inflammatory responses to allergen.

### Age and diet

Age is also a confounding factor in development of asthma with early onset and late onset disease showing apparently different phenotypes<sup>102</sup>. The majority of patients with asthma are diagnosed by the age of 6, and atopic disease and viral wheezing illness in infancy are increased risk factors for, and predictive of, childhood asthma<sup>107,108</sup>. These factors suggest that early life events influence the development of protective regulatory mechanisms that are required for the suppression of inappropriate immune responses to innocuous particles such as allergens. In support of this, T<sub>Reg</sub> cell function is already impaired in cord blood in neonates that have a hereditary risk for allergy<sup>109</sup>. Interestingly, cluster analysis defines a group of older, mostly female, obese asthmatics with adult-onset asthma and less atopy, comprising about 10% of patients with severe asthma<sup>102</sup>. These studies strengthen the growing belief that diet and metabolism may affect the degree of severity of asthma.

Nutrients such as vitamin D have notable effects on the immune system, with a vitamin D-deficient diet being associated with decreased regulatory responses to allergen<sup>110,111</sup>. Importantly, these effects occur even prenatally<sup>112</sup>. The apparent divergent phenotypic characteristics observed in all of these studies suggest different pathophysiological processes that may determine responses to therapy responses and thus affect asthma control. In the future it will be important to determine whether these clusters of patients are representative of different T cell responses in their lungs.

### Infection

Infection history influences the shaping of pulmonary immune responses from the very early stages of life. Recurrent wheeze during the first 3 years of life is considered a major risk factor for asthma. As the majority of lower respiratory tract wheeze in childhood is caused by viruses, it has been postulated that early viral exposure has long-term effects on airway



immunity<sup>113</sup>. By contrast, the ‘hygiene hypothesis’ proposes that early childhood infections inhibit development of allergic disease<sup>114</sup>, and early infection is thus protective. However, most of the evidence relates to protection against atopy and atopic diseases rather than asthma itself. It has been shown that prenatal exposure to infections influences the development of the innate immune system. Maternal exposure to an environment rich in microbial compounds during pregnancy is associated with higher levels of Toll-like receptor 2 (TLR2), TLR4 and CD14 expression in children<sup>115</sup>. This suggests that early exposure might prevent allergic sensitization of children owing to the development of a transient local T<sub>H</sub>1 response. More directly, farm exposure during pregnancy is associated with increased number and function of T<sub>Reg</sub> cells within cord blood, as well as reduced production of T<sub>H</sub>2-type cytokines and decreased lymphocyte proliferation following innate restimulation<sup>116</sup>. Studies in mice have shown that exposure of mothers to endotoxin from the cowshed-derived bacterium *Acinetobacter lwoffii* F78 prevents subsequent allergen-induced sensitization and airway inflammation in the pups<sup>117</sup>. Whether infection is beneficial or harmful, it is clear that both the timing and nature of the infection have crucial implications for the development of T cell responses in the lung.

## Potential of new therapies

### Limitations of existing therapies

The discovery of new T cell subsets and of alternative ways to induce a T<sub>H</sub>2-type response, together with a deeper understanding of the lung microenvironment, has provided us with new insights into the pathology of asthma, and thus with opportunities for innovation in therapy. One important area of unmet need in asthma is curative therapies for patients with mild to moderate asthma (discussed in REF. 118), which would be a welcome replacement for the symptomatic treatment that most patients need to take for many consecutive years. The second area of unmet need in asthma is symptom control in patients with severe asthma (discussed in REF. 118). Current management of asthma is by inhaled corticosteroids with or without long-acting  $\beta_2$ -agonists. Although steroids are relatively safe, inexpensive to manufacture and highly effective in clinical trial settings, their use in the asthma population at large leaves over half of patients with poorly controlled disease<sup>119</sup>. Poor adherence to therapy seems to be an important factor, particularly in patients with difficult-to-treat asthma<sup>120</sup>.

### Targeting cytokines

The effectiveness of steroids in a clinical trial setting sets a high bar for any new therapeutic or treatment regimens that seek to improve or replace them. In spite of recent progress, the increase in our understanding of T<sub>H</sub>2-type responses and new T cell subsets may not easily lead to a new ‘blockbuster’ therapy; however, there is a growing realization in the field that improvement in therapy may be achievable when approaches become more tailored to distinct clinical phenotypes that are only just being recognized<sup>16,102</sup>. Moreover, treatments targeting a single mediator are less likely to be effective in the general asthma population, and may be of greater value in specific subgroups of patients or as an add-on to current therapy. This may be particularly true for anti-cytokine treatment approaches (Supplementary information S1 (table)).

A relevant example of this is the experience with the IL-5-specific antibodies mepolizumab (Bosatria; GlaxoSmithKline) and reslizumab (Ception Therapeutics). Initial clinical trials in patients with both mild and severe asthma showed that these antibodies effectively reduce the number of eosinophils in blood and sputum, but failed to improve other disease symptoms<sup>121–123</sup>. However, in these initial trials with IL-5-specific antibodies, a trend was noted for improved efficacy of this therapy in patients with asthma exacerbations.

Subsequent trials focused on a more specific clinical phenotype and patients were pre-selected for high sputum eosinophilia. Mepoluzimab significantly reduced the number of blood and sputum eosinophils and allowed the use of lower doses of the steroid prednisone in these patients<sup>124,125</sup>.

Another new approach to asthma therapy is the development of antibodies targeting IL-9. Expression of IL-9 is detected in biopsies from patients with asthma and localizes to CD4<sup>+</sup> T cells, mast cells and eosinophils, and several observations point to a role for IL-9 in the development of allergic inflammation<sup>35</sup>. Efficacy trials with IL-9-specific antibodies are currently ongoing, and these studies should identify the precise role of IL-9 in asthma patients and reveal whether targeting IL-9 may perhaps be especially beneficial to asthma patients with certain clinical phenotypes ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00968669) Identifier: NCT00968669).

As IL-17A and IL-17F production is upregulated in patients with neutrophilic, steroid-resistant asthma and correlates with disease severity, IL-17 family members may be an interesting therapeutic target for severe asthma<sup>22,23</sup>. Data from animal models, however, show that IL-17 can have both beneficial and detrimental effects in the allergic airway, and it may be necessary to specifically inhibit IL-17 production by CD4<sup>+</sup> T cells to obtain any advantage. Antibody approaches targeting IL-17 are currently in the preclinical development stages (discussed in REF. 118).

### Promoting T<sub>Reg</sub> cell responses

When contemplating new therapeutic directions for patients with asthma, great emphasis ought to be placed on new therapies that could potentially result in disease modification. In search of this 'Holy Grail' of disease modifiers for asthma, it may be desirable to stimulate regulatory pathways or mechanisms that are already present in the airways, in particular those that have the potential to induce long-term suppression or lasting changes in T cell populations. Examples may include induction of T<sub>Reg</sub> cells and stimulation of various TLR pathways. It is suggested that allergen-specific immunotherapy regimens increase the numbers of both CD25<sup>+</sup> T<sub>Reg</sub> cells and IL-10-producing T<sub>Reg</sub> cells<sup>126,127</sup>. Although long-term inhibition is induced by immunotherapy, ultimately symptoms do return<sup>128</sup>. As T<sub>Reg</sub> cells are maintained by continued antigen exposure, frequent repetition of immunotherapy regimens may be required<sup>61,65</sup>. Further understanding of the mechanisms responsible for long-term inhibition will be an important aspect of improving immunotherapy for asthma.

Interestingly, corticosteroid treatment *in vivo* enhances CD4<sup>+</sup>CD25<sup>+</sup> T<sub>Reg</sub> cell function<sup>70</sup> and can, in combination with vitamin D, also induce IL-10 producing T<sub>Reg</sub> cells *in vitro*. of note is the finding that 2-agonists enhance the production of IL-10 by these cells<sup>129</sup>. of course, it is well known that the beneficial effects of corticosteroids quickly dissipate when treatment stops. Vitamin D treatment itself may be an approach that is particularly promising for the treatment of patients with severe corticosteroid-resistant asthma. Treatment with calcitriol (the active form of vitamin D) restores the *ex vivo* steroid responsiveness in T cells from patients with steroid-resistant asthma, and a trial investigating the efficacy of calcitriol in patients with severe asthma is underway ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00712205) Identifier: NCT00712205).

### Indirect targeting of T cell response

An immediate and effective way to silence allergen-induced activation of T<sub>H</sub>2 cells and the downstream T<sub>H</sub>2-type response in the airways is through stimulation of TLR7 or TLR9 pathways<sup>130–132</sup>. A large number of experiments in animal models have shown successful inhibition of both acute and existing asthma responses following TLR targeting, along with long-lasting duration of effects<sup>131–132</sup>. Stimulation of the TLR9 pathway leads to

downregulation of co-stimulatory molecules from airway antigen-presenting cells and subsequent silencing of T<sub>H</sub>2 cell function<sup>131</sup>. An initial 4-week clinical trial with weekly dosing of the TLR9 agonist 1018 ISS failed to show clinical benefit<sup>133</sup>; however, exploration of this pathway in humans is being continued with second-generation compounds and longer treatment regimens. Encouraging data were observed with the TLR9 agonist 1018 ISS conjugated to the ragweed protein Amb a1; patients with ragweed allergic rhinitis treated before the ragweed season showed substantial benefit during the next ragweed season 1 year later<sup>134</sup>. Interestingly, treatment induces a significant increase in the number of CD4<sup>+</sup>CD25<sup>+</sup> T cells in the nasal mucosa of these patients when compared with placebo<sup>135</sup>. Further studies are needed to determine whether similar beneficial effects would be observed in patients with asthma.

The discovery of alternative ways to induce a T<sub>H</sub>2-type response (FIG. 2) is a large step forward in the field, and it may provide an explanation for why therapies solely aimed at T cell depletion have shown limited success in patients with asthma. Initially, these new pathways were observed mostly in the gut mucosa; however, experiments in recombination-activating gene 2 (RAG2)-knockout mice indirectly indicate that the same phenomena also occur in the lungs<sup>10,136,137</sup>. It will be exciting to test the contribution of the newly described innate helper cells in the airways of patients with asthma, and this effort has already started with the development of antibody approaches targeting IL-25 and IL-33, which are currently in preclinical stages (discussed in REF 118). In addition, a clinical study aims to characterize IL-33 expression in different clinical phenotypes by investigating emergency room patients with breathing difficulties ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00707811) Identifier: NCT00707811).

The fact that asthma is a chronic disease makes the therapeutic application of microRNA of particular interest. MicroRNAs are involved in the fine-tuning of expression levels of networks involving hundreds of proteins, and inhibiting a particular microRNA is therefore the antithesis of a ‘single mediator’ approach<sup>138</sup>. Expression profiling of microRNAs in both mouse and human airways has identified several microRNAs that may be of interest in asthma. Recent studies in which miRNA-126 and Let-7a were inhibited reported beneficial effects: that is, T<sub>H</sub>2 cell numbers and T<sub>H</sub>2-type responses were decreased in animal models of asthma<sup>139,140</sup>. However, the extent to which these observations translate to humans or whether this approach will have long-term benefits remains to be determined.

## Concluding remarks

In summary, T cell subsets and the cytokines that they secrete are undoubtedly central to the pathogenesis and regulation of immune responses to allergen, and the new T cell subsets that have emerged extend the existing paradigm of asthma as a T<sub>H</sub>2-type disease. The need for tight control of immune responses in the airways makes the lung a specialized environment where recognition of pathogens elicits appropriate inflammatory responses to limit infection while discriminating from harmless inhaled particles, a process that becomes dysregulated in patients with asthma. Factors that damage the pulmonary epithelium — including pathogens, air pollution and allergen protease activity — result in the production of cytokines such as IL-33, IL-25 and TSLP, which are able to promote the development of T<sub>H</sub>2-type responses in the airways through activation of specific effector cells that can produce large amounts of IL-4, IL-5 and IL-13. The presence of these alternative T<sub>H</sub>2-type response pathways may contribute to asthma being a heterogeneous collection of clinical phenotypes. The influence of environmental factors and the genetic makeup of an individual determine the specific clinical phenotype in each patient. More research is needed to understand the molecular mechanisms underlying phenotypic clusters, along with the development of validated biomarkers and clinical read-outs to efficiently identify the various phenotypes in the clinic. In the future, asthma therapy may need a more sophisticated approach with new treatments

that are more tailored to specific patient groups in order to achieve improvement over standard of care.

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## Glossary

<b>Airway hyperresponsiveness (AHR)</b>	A hyperreactivity of the airways, initiated by exposure to a defined stimulus that is usually tolerated by normal individuals; it causes bronchoconstriction and inflammatory-cell infiltration in allergic individuals. This is a defining physiological characteristic of asthma
<b>Forced expiratory volume in 1 second (FEV1)</b>	A primary indicator of lung function
<b>Hygiene hypothesis</b>	This hypothesis originally proposed that the increased incidence of atopic diseases in westernized countries was a consequence of living in an overly clean environment, resulting in an under-stimulated immune system that responded inappropriately to harmless antigens. More recently it has been proposed that an absence of exposure to pathogens, in particular helminths, may predispose to both increased allergy and autoimmune disease.
<b>2-agonist</b>	A class of medication aimed at dilating the airways by the agonism of the $\beta$ -adrenoreceptor pathway.
<b>Allergen-specific immunotherapy</b>	Allergen immunotherapy was introduced in the early 1900s. In general, it involves subcutaneous injection of increasing doses of specific allergen into the patient. This is carried out under carefully controlled clinical conditions because of the potential for life-threatening adverse reactions. On average, it results in ~50% reduction of clinical symptoms and medication usage, and it also results in beneficial modifications of the patient's immune response to allergen. Following the initial course of injections (either conventional or rushed), patients receive maintenance injections (less frequently) of allergen for optimal clinical benefit.
<b>MicroRNA</b>	Small, non-coding RNA molecules that regulate the expression of several genes by binding to the 3' untranslated regions of specific mRNAs.

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**Box 1****Asthma heterogeneity**

Asthma is recognized to be a heterogeneous disease with a number of distinct clinical phenotypes. The most common form of asthma is allergic asthma, which results from an inappropriate immune response to common inhaled proteins (or allergens) in genetically susceptible individuals. These individuals are termed atopic asthmatics and exhibit IgE reactivity to specific antigens. By contrast, non-atopic asthmatic patients have disease that is not driven by a specific allergen. Although most individuals present with eosinophilic diseases, a number of patients with particularly severe asthma show a substantial neutrophil component to airway infiltrates. These patients often have resistance to treatment with steroids. More recently, detailed statistical cluster analysis has revealed defined phenotypes of patients that differ on the basis of lung function, age of asthma onset, disease duration, atopy, gender, symptoms, medication use and health care usage. A better understanding of these clinical phenotypes will provide the opportunity to develop tailored therapies aimed specifically at each phenotype.

**Box 2****The lung represents a unique mucosal environment**

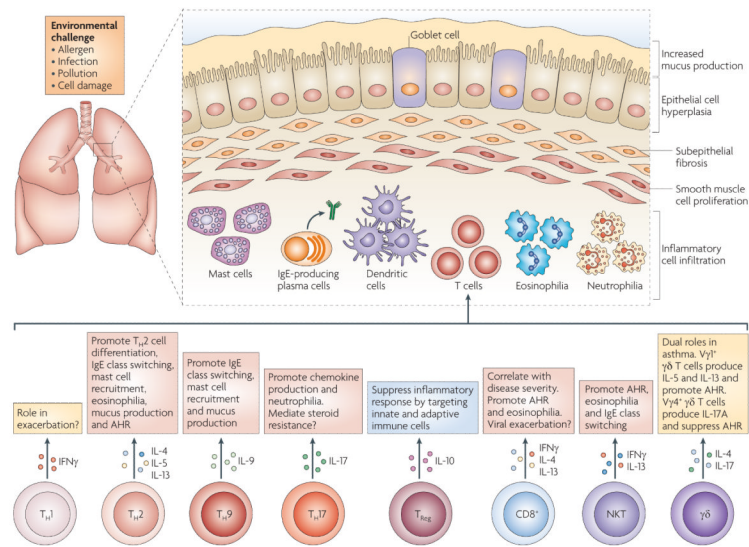
The primary function of the lung is gaseous exchange. There are a number of mechanisms in place to ensure that this function is not compromised. The large surface area of the lung is constantly exposed to the external environment, which contains airborne particles that, although immunogenic, do not generally represent a threat to the host — that is, allergens. A number of specialized control mechanisms ensure that *de novo* immune responses are not continually instigated. This is of vital importance as repeated stimulation of the airway immune system would generate effector memory cells that promote chronic inflammation and damage to the epithelial barrier, thus compromising gaseous exchange. An absolute requirement of these control mechanisms is the ability to discriminate between harmless airborne particles and infectious agents.

**Box 3****Animal models of asthma**

The majority of animal models of asthma are initiated by one or more intraperitoneal injections of a protein allergen (most commonly ovalbumin) in conjunction with the T helper 2 (T<sub>H</sub>2)-type skewing adjuvant aluminium hydroxide (alum). This sensitization phase is followed 7–14 days later by direct administration of allergen to the lung either in a soluble form via the intranasal route, or by aerosolization. This protocol results in airway hyperresponsiveness (AHR), eosinophilic inflammation in the lung, serum IgE production and pulmonary production of T<sub>H</sub>2-type cytokines, including interleukin-4 (IL-4), IL-5 and IL-13. These models have focused almost exclusively on the role of T<sub>H</sub>2-type inflammation and do not adequately characterize asthma that is not driven by T<sub>H</sub>2 cell responses. However, more recently, models have been developed whereby environmental allergen, such as house dust mite extract, is administered directly to the airways in the absence of systemic sensitization with allergen. This model also results in AHR and eosinophilia, but the pathology is less T<sub>H</sub>2-skewed, and neutrophils are also present in the lung. Disease mechanisms are likely to be different in this inhaled model as the first contact of allergen with the immune system is through pulmonary, rather than peritoneal, dendritic cells, and also depends on contribution from the respiratory epithelium. Other models have used neonatal mice to take age into account, and others have combined allergen exposure with viral challenge or particulate matter.

**Box 4****Phases of asthma**

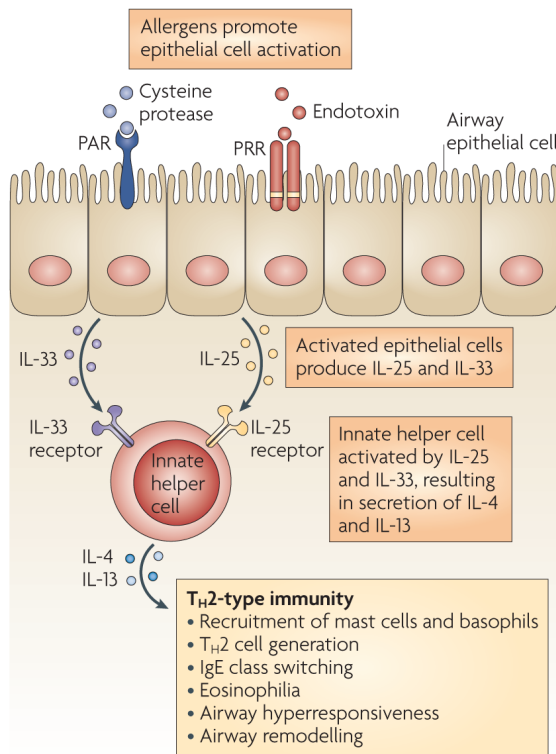
The specific timing and mechanisms of sensitization of patients with asthma to allergens remains unclear; however, various factors influencing the process of sensitization have been discovered, including genetic predisposition, diet, infection history, gender and age. After sensitization, exposure to allergen leads to early-phase reactions in most patients; these reactions involve IgE-mediated degranulation of mast cells and subsequent constriction of the airway smooth muscle. This is followed 4–18 hours later by the late-phase reaction, which is characterized by recruitment of eosinophils and T cells. In a number of patients, this leads to chronic inflammation and airway remodelling that remains difficult to treat.



### Figure 1. T cells involved in the induction of the allergic phenotype

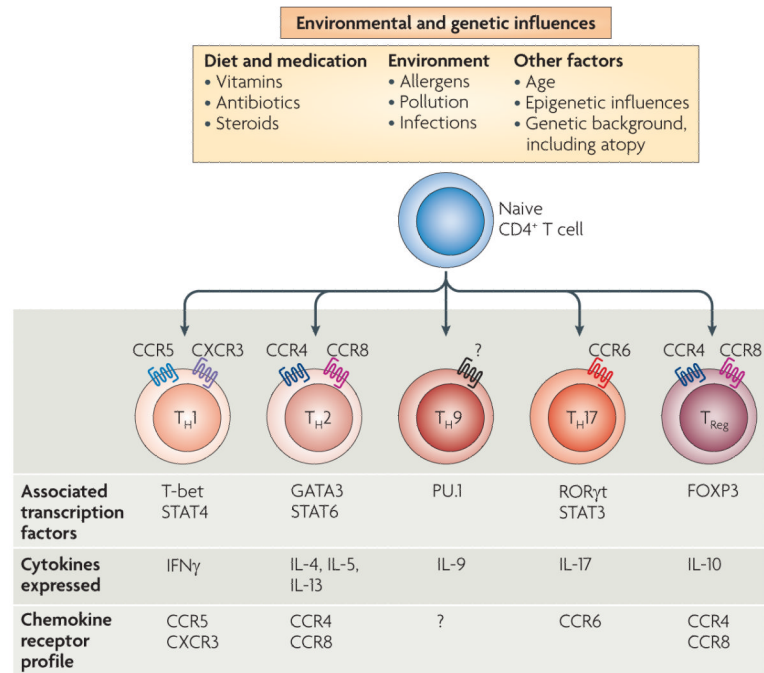
Asthma is a heterogeneous disease that is characterized by airway hyperresponsiveness (AHR), recruitment of inflammatory leukocytes to the lung and tissue remodelling, including mucus production and airway smooth muscle changes. A number of different T cell subsets are thought to influence the nature and magnitude of the allergic immune response by the cytokines that they secrete. T helper 2 (T<sub>H</sub>2) cells are thought to promote eosinophil recruitment, in conjunction with nature killer T (NKT) cells and CD8<sup>+</sup> T cells. By contrast, T<sub>H</sub>1 cells and T<sub>H</sub>17 cells are thought to be associated with severe, steroid-resistant asthma, which is often marked by neutrophilic infiltrates. Regulatory T (T<sub>Reg</sub>) cells and subtypes of T cells are able to downregulate pulmonary immune responses and are thought to be important for maintenance of immune homeostasis in the lungs. The nature and magnitude of allergic inflammation in the lung is influenced by external environmental stimuli, such as exposure to allergens and pollution as well as infection with pathogens. IFN $\gamma$ , interferon- $\gamma$ ; IL, interleukin.





**Figure 2. Alternative pathway to a TH2-type response in the airways**

Cysteine protease activity and endotoxin within allergens can activate lung epithelial cells through protease-activated receptors (PARs) and pattern-recognition receptors (PRRs), such as Toll-like receptors. Recent experimental data indicate that a population of ‘innate helper’ cells can secrete interleukin-4 (IL-4) and IL-13 in response to epithelial cell-derived cytokines, such as IL-33 and IL-25, and promote T helper 2 (TH2)-type immune responses. Thymic stromal lymphopoietin (TSLP) can promote TH2-type responses in the lung, but a direct association with this cytokine and innate helper cells in the lung has not yet been found. Although these innate helper cells have been identified in a number of different tissues, including in the resting lungs, evidence for their involvement in allergic airway inflammation remains indirect. Therefore the model described here is theoretical and remains to be tested *in vivo*.



**Figure 3. T cell subset signatures are affected by a variety of genetic and environmental influences**

T cell subsets can be distinguished by their cytokine secretion patterns, as well as by their chemokine receptor expression. Distinct T cell lineages are determined by the expression of ‘master’ transcription factors. Although once thought to be committed stable lineages, it is becoming apparent that a range of environmental and genetic influences are able to promote flexibility in T cell programmes. Thus, effector subsets can rapidly react to changing environmental circumstances to promote effective immune responses. CCR, CC-chemokine receptor; CXCR, CXC-chemokine receptor; FOXP3, forkhead box P3; GATA3, GATA-binding protein 3; IFN $\gamma$ , interferon- $\gamma$ ; IL, interleukin; ROR $\gamma$ t, retinoic acid receptor-related orphan receptor- $\gamma$ ; STAT, signal transducer and activator of transcription; T<sub>H</sub>, T helper; T<sub>Reg</sub>, regulatory T.