CD8$^+$ Tc2 cells: underappreciated contributors to severe asthma

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Alongside Th2 and ILC2 cells, CD8$^+$ T-cells are a cellular source of type 2 cytokines. We review recent findings and insights into the pathologic effector functions of type 2 CD8$^+$ T-cells in eosinophilic asthma, especially steroid-resistant disease. http://bit.ly/2KbVGL2

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ABSTRACT The complexity of asthma is underscored by the number of cell types and mediators implicated in the pathogenesis of this heterogeneous syndrome. Type 2 CD4$^+$ T-cells (Th2) and more recently, type 2 innate lymphoid cells dominate current descriptions of asthma pathogenesis. However, another important source of these type 2 cytokines, especially interleukin (IL)-5 and IL-13, are CD8$^+$ T-cells, which are increasingly proposed to play an important role in asthma pathogenesis, because they are abundant and are comparatively insensitive to corticosteroids. Many common triggers of asthma exacerbations are mediated *via* corticosteroid-resistant pathways involving neutrophils and CD8$^+$ T-cells. Extensive murine data reveal the plasticity of CD8$^+$ T-cells and their capacity to enhance airway inflammation and airway dysfunction. In humans, Tc2 cells are predominant in fatal asthma, while in stable state, severe eosinophilic asthma is associated with greater numbers of Tc2 than Th2 cells in blood, bronchoalveolar lavage fluid and bronchial biopsies. Tc2 cells strongly express CRTH2, the receptor for prostaglandin D2, the cysteinyl leukotriene receptor 1 and the leukotriene B4 receptor. When activated, these elicit Tc2 cell chemotaxis and production of chemokines and type 2 and other cytokines, resulting directly or indirectly in eosinophil recruitment and survival. These factors position CD8$^+$ Tc2 cells as important and underappreciated effector cells contributing to asthma pathogenesis. Here, we review recent advances and new insights in understanding the pro-asthmatic functions of CD8$^+$ T-cells in eosinophilic asthma, especially corticosteroid-resistant asthma, and the molecular mechanisms underlying their pathologic effector function.

Introduction

The complex heterogeneity of asthma is reflected in the number of cell types, mediators and pathways described in the pathogenesis of this syndrome [1]. The clinical variability between patients and even in the same patient at different stages of their disease suggest different mechanistic pathways [2, 3]. T-lymphocytes play a major role in the pathophysiology of asthma, but that alone does not address involvement of the many different subsets of T-cells. Moreover, the pathogenesis of asthma has been linked to the production of type 2 cytokines, which can be expressed by several cell types in the lung. Type 2 CD4$^+$ T-cells (Th2) and their capacity to secrete pro-allergic cytokines, interleukin (IL)-4, IL-5 and IL-13 dominate descriptions of asthma pathogenesis [4], particularly eosinophilic and corticosteroid-responsive asthma. Type 2 innate lymphoid cells (ILC2), which are lineage-negative and lack

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a T-cell receptor (TCR), have received attention based on their early presence and capacity for similar type 2 cytokine production [5]. Th9 cells act *via* IL-9 to enhance pro-allergic cytokine expression, airway mast cell recruitment, eosinophilia, airway hyperresponsiveness (AHR) and mucus cell metaplasia in murine models [6]. Th17 cells and their release of IL-17 are proposed to trigger a neutrophilic response in the airways leading to corticosteroid-refractory asthma [7], although, to date, blockade of IL-9 [8] and IL-17 [9] have not proved effective in clinical trials. Invariant natural killer T-cells were implicated in promoting AHR in mice [10], although these cells proved to be rare in human airways [11], in contrast to the abundant mucosal associated invariant T-cell subset [12]. Countering these pro-allergic lymphocyte responses are regulatory T-cells, natural and inducible, attenuating asthma responses through secretion of IL-10 and transforming growth factor-β [13–15]. In contrast to $CD4^+$ T-cells, the role of $CD8^+$ T-cells was suggested to balance the responses of $CD4^+$ T-cells through the secretion of interferon (IFN)-γ [16]. However, until recently few studies have directly examined the presence or role of $CD8^+$ T-cells in asthma pathogenesis. Nonetheless, in experimental models and where they have been examined in human asthma, $CD8^+$ T-cells were proposed to play an important role in asthma pathogenesis, in large part because they are insensitive to corticosteroids compared with $CD4^+$ T-cells and because they have the capacity to undergo transcriptional reprogramming from IFN-γ-secreting cells (Tc1) to type 2 cytokine secreting cells (Tc2), especially producing IL-13 and IL-5 [17]. These two factors position $CD8^+$ Tc2 cells as potentially important effector cells in asthma pathogenesis, especially in severe, corticosteroid-resistant asthma.

Corticosteroid-resistant asthma

Corticosteroids have multiple mechanisms of action, so it is likely that their effects in asthma occur *via* several mechanisms, acting on corticosteroid-sensitive cells which undergo apoptosis or in attenuating susceptible pathways and functional programmes. Although corticosteroids remain "first-line" therapy in asthma, increasingly there is a recognition that some patients experience severe asthma with persistent symptoms despite high-dose inhaled or oral corticosteroids [18]. It is likely that other cell types and pathways, which are corticosteroid-resistant, underlie the immunopathology in these individuals, labelled as corticosteroid-resistant asthmatics. Furthermore, corticosteroids have incomplete efficacy in preventing or treating exacerbations [19, 20], supporting this notion that the diverse triggers for these events (table 1) involve distinct cell types which are more intrinsically corticosteroid-resistant, including neutrophils, Th17 cells [21] and potentially $CD8^+$ T-cells [17]. It is for this group of severe asthmatics that many of the newer biologic agents have been targeted.

$CD8^+$ Tc2 cells in experimental allergic airways disease

In addition to $CD4^+$ Th2 cells and type 2 ILCs, the role of type 2 cytokine-secreting $CD8^+$ Tc2 cells in allergic airways disease has been demonstrated extensively in models of experimental asthma. A number of studies in mice reported that $CD8^+$ T-cells play a protective role in allergic disease [22–26]. Regulatory or suppressive effects on lung allergic responses were associated with secretion of type 1 cytokines such as IL-12 and IFN-γ [16, 25, 26]. In contrast, the plasticity of $CD8^+$ T-cells and their pro-asthmatic activities have now been confirmed in many studies. Depletion of $CD8^+$ T-cells attenuated AHR and airway inflammation in mice exposed to allergen exclusively *via* the airways, in the absence of systemic sensitisation [27]. The responses were restored following reconstitution of $CD8^+$ T-cells, but not IL-13-deficient $CD8^+$ T-cells. $CD8^+$ Tc2 cells have been shown to be prime sources of type 2 cytokines [28–32]. Mice deficient in CD8 develop significantly lower AHR, eosinophilic inflammation and IL-13 levels in bronchoalveolar lavage (BAL) fluid compared with wild-type mice, and these responses are restored by adoptive transfer of antigen-primed $CD8^+$ T-cells [24, 28, 33]. The capacity of $CD8^+$ T-cells to

TABLE 1 Common triggers for asthma exacerbations are mediated by different pathways, frequently involving steroid-unresponsive neutrophils and CD8 cells

	Allergen exposure	Viral infection	Ozone/diesel exhaust	Cigarette smoke	Bacterial infection
Predominant pathway	Th2 cells, IgE	Th1 cells, leukotrienes	Th1 cells, leukotrienes	Th1 cells, leukotrienes	Th17 cells, IL-1β, IL-6, TNF
Predominant effector cells	Eosinophils, Mast-cells, CD4 T-cells	Neutrophils, CD8 T-cells	Neutrophils, CD8 T-cells	Neutrophils, CD8 T-cells	Neutrophils
Steroid responsiveness	High	Low	Low	Low	Low

Th1/2 cells: type 1/2 $CD4^+$ T-cells; IL: interleukin; TNF: tumour necrosis factor.

produce type 2 cytokines in mice was dependent on interactions between $CD4^+$ and $CD8^+$ T-cells with allergen-sensitised $CD4^+$ T-cells providing the critical differentiation factor, IL-4 [28].

Transcriptional reprogramming of $CD8^+$ T-cells from a Tc1 to a Tc2 phenotype

$CD8^+$ T-cells, similar to other T-cell subsets, exhibit plasticity and can undergo transcriptional reprogramming, redirecting their functional activities. Antigen-specific $CD8^+$ T-cells committed to IFN-γ production, when exposed to IL-4 *in vitro* or in an atopic environment, transit through distinct differentiation stages characterised by changes in transcription and translation, resulting in IL-13-producing $CD8^+$ T-cells [34] (figure 1). In $CD8^+$ T-cells, IL-4 resulted in the epigenetic poising of the *Il13* locus through the gain of permissive and loss of repressive histone modifications, which were co-regulated with recruitment of RNA polymerase II. In addition, IL-4 was required for *Gata3* expression in $CD8^+$ T-cells and IL-4-dependent recruitment of GATA3 protein to the Il-13 promoter. Thus, in an allergic inflammatory lung microenvironment containing IL-4, eosinophilic asthma resulted from $CD8^+$ T-cells epigenetically poised for Tc2 conversion *via* differential histone modifications at lineage-specific promoter regions [34].

Corticosteroid-insensitivity of $CD8^+$ T-cells

How might these Tc2 cells contribute to corticosteroid-resistant disease? Corticosteroids effectively suppress inflammatory responses through repression of many immune genes by means of interaction with the glucocorticoid receptor. However, susceptibility to corticosteroids differs among T-cell subpopulations and states of maturity [35]. Administration of corticosteroids to asthmatic patients results in significant decreases in numbers of $CD4^+$ but not $CD8^+$ T-cells in peripheral blood [36]. Activated human [37] and

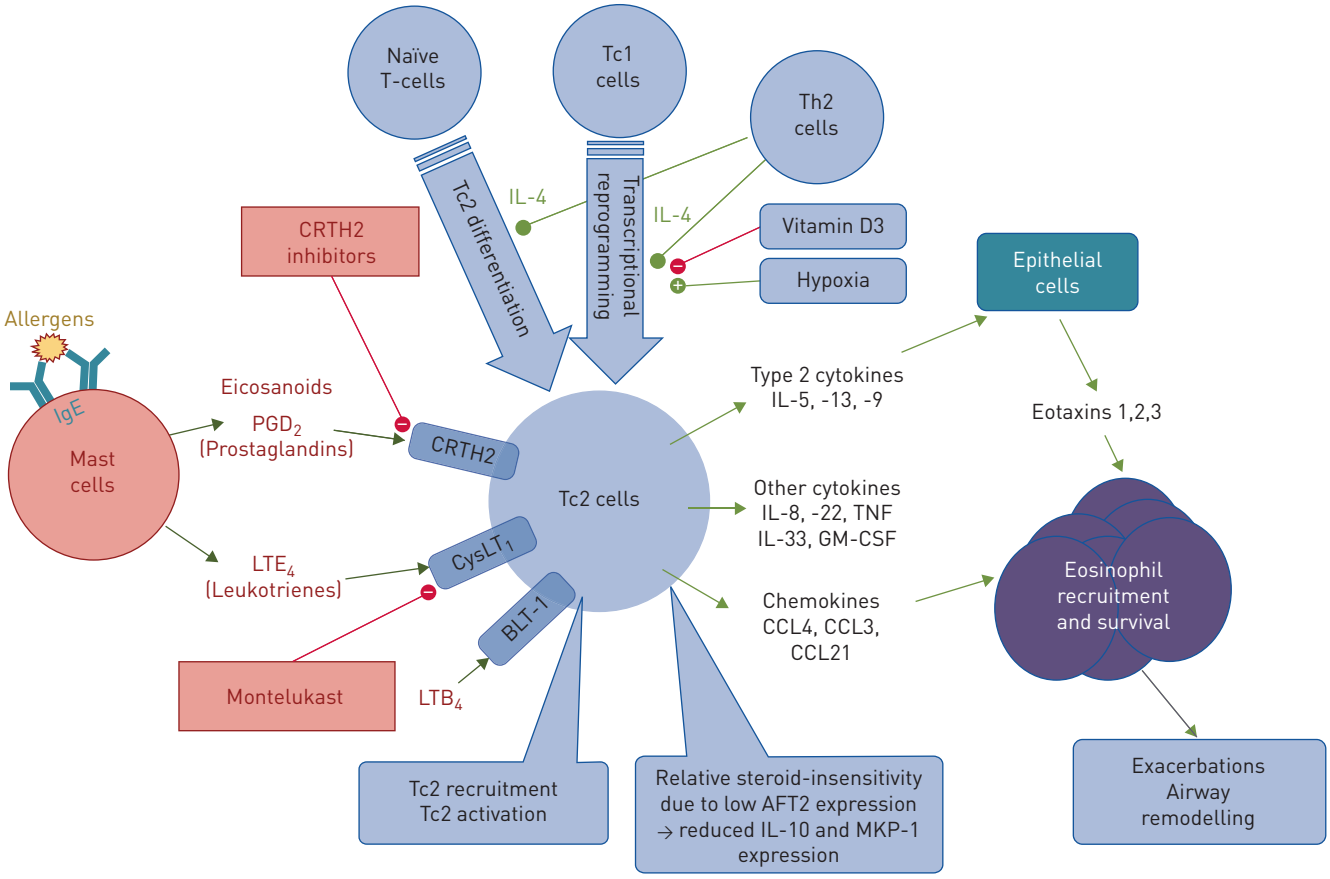

FIGURE 1 Under the influence of interleukin-(IL)-4, type 2 $CD8^+$ (Tc2) cells differentiate from naïve $CD8^+$ T-cells or arise by transcriptional reprograming of Tc1 cells. Tc2 cells highly express chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2), a receptor for prostaglandin D2 (PGD_2). In addition, Tc2 cells express the cysteinyl leukotriene receptor 1 ($CysLT_1$) and leukotriene B4 receptor (BLT-1). Inflammatory stimuli, such as cross-linking of immunoglobulin (Ig)E on mast cells, leads to production of eicosanoids. Through CRTH2, PGD_2 elicits Tc2 cell chemotaxis, activation and production of chemokines, type 2 cytokines and other cytokines, resulting directly or indirectly in eosinophil recruitment and survival. In turn, airway eosinophilia is associated with airway remodelling and exacerbations. ATF2: activating transcription factor-2; CCL: C-C motif chemokine ligand; GM-CSF: granulocyte–macrophage colony-stimulating factor; LT: leukotriene; MKP: mitogen-activated protein kinase phosphatase; Th2 cells: type 2 $CD4^+$ T-cells; TNF: tumour necrosis factor.

mouse [38] $CD8^+$ T-cells are more resistant to corticosteroids than $CD4^+$ T-cells. Therefore, other than $CD4^+$ T-cells, $CD8^+$ T-effector cells are proposed to play an important role in the pathophysiology of inflammatory diseases, especially after initiation of corticosteroid treatment.

Glucocorticoid insensitivity of lymphocytes has also been described in a number of human diseases [39–43] with data demonstrating that human $CD8^+$ T-cells, similar to mouse $CD8^+$ T-cells, are relatively corticosteroid-insensitive compared to $CD4^+$ T-cells [37, 44]. One mechanism which may explain this differential sensitivity to corticosteroids is lower expression of the DNA binding protein and histone acetyltransferase activating transcription factor-2 (ATF2) in $CD8^+$ than $CD4^+$ T-cells [44]. While the inhibitory (transrepression) immunosuppressive effects of corticosteroids on cytokine secretion and cell proliferation are similar in both subsets, as ATF2 is required for corticosteroid-induced transactivation, $CD8^+$ T-cells have reduced corticosteroid-induced transactivation including reduced IL-10 induction [44].

CYP11A1 activation is required for $CD8^+$ Tc2 differentiation

Activation of the steroidogenic enzyme, CYP11A1 is an essential component in the development of Tc2-mediated experimental asthma. This mitochondrial P450 cytochrome is the first and rate-limiting enzyme in steroidogenesis converting cholesterol to pregnanolone. In the presence of IL-4, CYP11A1 enzymatic activation was a critical regulator of Tc2 conversion, resulting in increased IL-13 and decreased IFN-γ production [34, 45]. Of interest, vitamin D3 is a key modulator of the functional conversion of $CD8^+$ T-cells from an IFN-γ- to an IL-13-producing cell [45]. This appears to be, at least in part, through the regulation of CYP11A1 enzymatic activation, an effect driven by vitamin D3-mediated changes in the recruitment of vitamin D receptor (VDR) transcription factors to the promoter region of *CYP11A1*. This was parallelled by changes in the enzymatic activation of CYP11A1 and the prevention of lung allergic responses. Of note, in humans, an epistatic effect between genetic variants in *CYP11A1* and VDR was shown with protective effects on the development of asthma in children [45].

Hypoxia enhances $CD8^+$Tc2 differentiation

Another feature distinguishing $CD8^+$ T-cells from $CD4^+$ T-cells is the response to tissue hypoxia, which occurs in many pathological conditions including asthma and promotes expression of genes through post-translational modifications and stabilisation of the α-subunits of hypoxia-inducible factors (HIF-1α and HIF-2α) [46]. Although hypoxia can inhibit T-lymphocyte function, robust T-cell responses occur at many hypoxic inflammatory sites [47, 48] and hypoxic exposure significantly amplified $CD8^+$ Tc2 differentiation, with increased release of IL-13 *in vitro* and augmentation of lung allergic responses *in vivo* [49]. The IL-4-dependent increases in Tc2 differentiation under hypoxia were dependent on HIF-1α. This combination of corticosteroid insensitivity and activation of the hypoxia response pathway and HIF-1α upregulation resulting in increased IL-13 production in Tc2 cells may thus contribute to maintenance of corticosteroid-resistant asthma.

$CD8^+$ T-cells in human asthma

The discovery in the early 1990s of the pathogenic role of type-2 cytokines constituted a key conceptual advance. The seminal work by Robinson *et al.* [50] used *in situ* hybridisation to show elevated type 2 cytokine mRNA for IL-2, -3, -4 and -5 in BAL fluid cytospins from atopic asthmatics compared to healthy controls. Follow-up studies demonstrated activation measured by CD25 upregulation of $CD4^+$ cells, but not $CD8^+$ cells, during allergen challenge [51], and was associated with symptoms and impaired lung function [52]. Many subsequent studies supported the paradigm of pathogenic type 2 secreting $CD4^+$ Th2 cells in asthma pathogenesis, dominating asthma thinking and research [1, 53]. Importantly, many of these studies did not directly examine the presence or function of $CD8^+$ T-lymphocytes. Moreover, in many of the molecular studies identifying type 2 signatures in cells from the airways of asthmatics, the specific cell source of these cytokines was not determined [20], even though numbers of $CD8^+$ T-cells outnumbered $CD4^+$ T-cells [54–57]. It is only more recently that other type 2 cytokine secreting cells in the lung have been identified [57–62] and that $CD8^+$ T-cells have also been recognised as major producers of type 2 cytokines. Indeed, human blood $CD8^+$ T-cells produce more IL-4 than $CD4^+$ T-cells [63]. This was subsequently confirmed by *in situ* hybridisation and immunohistochemistry on bronchial biopsies, showing that IL-4 and IL-5 were produced by both $CD4^+$ and $CD8^+$ T-cells, as well as mast cells and eosinophils within the airways [64]. Moreover, $CD8^+$ cells secreting IL-4, -5 and -13 and specific for the dominant house dust mite aeroallergen Derp 1 are found in human atopic disease and associated with disease severity, with responses believed to be mediated through antigen cross-presentation [32].

$CD8^+$ T-cells in fatal asthma

In human asthma, indirect evidence for the pathogenic involvement of $CD8^+$ T-cells is provided by studies of *post mortem* samples. Fatal asthma is characterised by infiltrates of T-cells, macrophages and activated

eosinophils, which, in contrast to stable asthma, is associated with a $CD8^+$ T-cell predominance, with $CD8^+$ cells outnumbering $CD4^+$ cells 2:1 in the interstitium of distal airways, and 6:1 in the lamina propria of proximal airways [65]; in another study, $CD8^+$ cells were present, but numbers were not increased [66]. In addition, asthma death was associated with activated peribronchial cytotoxic $CD8^+$ T-cells with higher expression of CD25 and a 10-fold higher expression of the cytotoxicity marker perforin [67]. These cells expressed both type-1 (IFN-γ) and type 2 (IL-4) cytokines, but asthma death was associated with an increase of more than double in the ratio of type 2 to type 1 cells, implicating Tc2 cells specifically in the inflammatory process. Similarly, a *post mortem* study from Brazil found higher numbers of granzyme A- and granzyme B-expressing cells in the lamina propria and adventitia in fatal asthma, out of which ∼50% of granzyme-positive cells were $CD8^+$ [68].

$CD8^+$ T-cells in stable asthma

While observations from fatal asthma may not be fully generalisable, a number of clinical studies have observed associations between airway $CD8^+$ T-cell frequencies and stable asthma. $CD8^+$ T-cell frequencies were increased in BAL fluid in asthma; this increase was greater for $CD8^+$ cells than for $CD4^+$ or $CD3^+$ cells [69, 70], and was correlated with AHR [70]. Increased BAL fluid $CD8^+$ cells have been described specifically associated with eosinophilic lung diseases including asthma [71].

In bronchial biopsies, $CD8^+$ T-cells were enriched in the lamina propria in asthma [57, 72], being present despite regular inhaled corticosteroid treatment, and were also associated with AHR measured by methacholine challenge, while AHR did not correlate with $CD4^+$ T-cells.

More recently, the Unbiased Biomarkers in Prediction of Respiratory Disease Outcomes (U-BIOPRED) study performed a large transcriptomic and immunohistochemical analysis on epithelial brushings and biopsies from 107 patients with moderate–severe asthma and identified two distinct subtypes associated with high type 2 cytokine expression [73]. The group characterised by the highest submucosal eosinophilia, high exhaled nitric oxide fraction, frequent exacerbations and high oral corticosteroid use were marked by significantly increased $CD3^+$ and $CD8^+$ T-cells, but with no differences in $CD4^+$ T-cells, again showing a strong association between $CD8^+$ cells and eosinophilic airways disease. In contrast, in type-2-low, IL-6-high asthma, sputum transcriptomics found lower expression of $CD8^+$ T-cell signatures [74].

Activation of $CD8^+$ T-cells

Significant activation of circulating $CD8^+$ T-cells in peripheral blood has been seen in severe asthma and correlated with downregulation of specific microRNAs (miR-146a/b and miR-28–5p) and differential expression of multiple intronic long noncoding RNAs which might regulate $CD8^+$ T cell activities [75]. Asthma is associated with increased expression in circulating $CD8^+$ T-cells, specifically of mitogen kinase kinase 3, a proinflammatory member of the p38 mitogen activated protein kinase pathway implicated in neutrophil recruitment [76].

Multiple genome wide association studies have identified *ORMDL3* as an important asthma-susceptibility locus associated with childhood asthma [54, 77–79]. A recent epigenetic analysis of human peripheral blood leukocyte subsets found decreased DNA methylation levels in the 5′ untranslated region of *ORMDL3* specifically in $CD8^+$ T-cells, which might explain the increased ORMDL3 mRNA expression observed in $CD8^+$ T-cells compared with other leukocytes [79]. As increased ORMDL3 expression promotes T-cell activation and pro-inflammatory cytokine production, this also associates $CD8^+$ T-cells in the link between this risk-allele and asthma pathogenesis.

Within the airways, $CD8^+$ T-cells may be activated by allergens cross-presented by class I major histocompatibility complex on airway dendritic cells [25], and indeed BAL fluid $CD8^+$ frequencies are increased after segmental allergen challenge [80]. Additionally, activated human $CD8^+$ T-cells express the receptors for the key airway epithelial cell alarmins IL-33 [81] and thymic stromal lymphopoietin (TSLP) and its ligation by TSLP enhances their proliferation [82, 83]. TSLP also acts indirectly *via* pulmonary inflammatory dendritic cells to enhance antiviral $CD8^+$ T cell responses [84] and to induce IL-5/-13-producing $CD4^+$ [85] and $CD8^+$ T-cells [86]. Likewise, IL-33 potently enhances antiviral $CD8^+$ T-cell responses [87]. An association of $CD8^+$ cells with cigarette use [56] suggests that smoke may also activate $CD8^+$ cells, while Tc2 cells in both mice and humans express perforin and granzyme [88] and induction of granzyme A mRNA and protein have been demonstrated in response to diesel fume particulates in human bronchoscopy samples [89].

$CD8^+$ Tc2 conversion during viral infection

Viruses play a key role in acute asthma [90], being responsible for ∼80% of asthma exacerbations [91]. Thus, it might be expected that viral infections contribute to an association between $CD8^+$ cells and

asthma (table 1). Although the mechanisms supporting these associations are incompletely understood, viral infection of epithelial cells can trigger the release of important alarmins such as IL-25 and IL-33 increasing type 2-related airway inflammation and mucin production [92, 93]. In experimental models of asthma, virus-specific $CD8^+$ T-cells can switch to IL-5 production and induce airway eosinophilia [94]. *In vitro* studies showed that IL-4 could switch virus-specific $CD8^+$ T-cells to IL-5 production. Thus, a viral infection which leads to recruitment of virus-specific $CD8^+$ T-cells to the airways, in an atopic asthma environment, the presence of IL-4 could trigger $CD8^+$ Tc2 conversion and release of type 2 cytokines. Severe respiratory syncytial virus (RSV) infection in early life is suspected to play a role in the later development of wheezing and asthma [95]. In contrast to initial RSV infection at a later stage, early infection in mice with RSV followed by reinfection at a later stage resulted in increased AHR, airway eosinophilic inflammation, mucus hyperproduction and IL-13-producing $CD8^+$ T-cells [96, 97]. Inhibition of IL-13 abolished AHR and mucus hyperproduction. As discussed later, a unique population of IL-6Rα-high effector memory $CD8^+$ T-cells was found in peripheral blood of asthmatics with an increased frequency compared to healthy control subjects [98]. These effector memory $CD8^+$ T-cells exclusively produced IL-5 and IL-13 in response to asthma-associated RSV and bacterial superantigens [98]. These data, in contrast to those from an *in vitro* study [99], suggest that Tc2 cell populations arising from polarisation early in life might persist long term *in vivo*.

Longitudinal studies of $CD8^+$ cells are associated with lung function decline

Few longitudinal studies have investigated T-cell subsets. However, one study performed bronchoscopies before and 2 years into a 7.5-year prospective follow-up of a cohort of adults with atopic asthma [100]. Over time, decline in post-bronchodilator forced expiratory volume in 1 s (FEV_1) correlated not with typical markers of eosinophilic inflammation (bronchial biopsy eosinophils) or remodelling (subepithelial reticular membrane thickness), but with bronchial biopsy $CD8^+$ cells measured either at baseline or at the 2-year time point. A strength of this study is that the findings were confirmed by subsequent follow-up of the same patients at 14 years [101]. Decline in FEV_1 was highest in those with high $CD8^+$ numbers at baseline or those with high $CD8^+$ numbers or high granzyme B expression at the 14-year time point; these associations were specific to $CD8^+$ cells, being weaker or not observed with $CD4^+$ cells.

Subsets of peripheral blood $CD8^+$ cells

These human studies describe disease associations with $CD8^+$ cells, but what is known of type 2 cytokine-secreting $CD8^+$ T-cells, specifically? Definitions of Tc2 cells vary between studies and are based either on intracellular expression of a type 2 cytokine (IL-4 [102], IL-5 [64], IL-13 [4, 88]) in $CD8^+$ T-cells or on co-expression of CD8 with a surrogate surface marker such as the prostaglandin D2 receptor 2, CRTH2 [88]).

Resting blood $CD8^+$ cells produce more IL-4 in asthma than in health [103], and several studies reported increases in Tc2 cells in peripheral blood in asthma, while this association was typically stronger than with Th2 cells. In one report, circulating $CD8^+IL\text{-}4^+$ cells were increased in allergic asthma [103], and in another $CD8^+IL\text{-}5^+$ cells were significantly increased in eosinophilic asthma [104]; this was not true for $CD4^+IL\text{-}5^+$ cells [104]. Similarly, peripheral blood $CD8^+IL\text{-}13^+$ Tc2 cells were increased in asthma, and correlated most with severe disease and with the eosinophilic phenotype [88] (figure 2). In the same cohort, significant increases in $CD4^+IL\text{-}13^+$ Th2 cells were observed only in mild disease [4, 88], but not with eosinophilic disease. Tc2 frequencies were associated with nasal polyposis and with current cigarette use. Moreover, a positive correlation was shown between frequencies of peripheral blood $CD8^+IL13^+$ Tc2 cells and type 2 lung inflammation, measured by expression of IL-4 by sputum T-cells, but again there was no such association with blood $CD4^+IL13^+$ Th2 cells [88]. Similarly, we and others have found using CRTH2 as a surface marker for type 2 cells, that $CD4^+CRTH2^+$ Th2 cells were not associated with eosinophilic disease [88, 105], while peripheral blood $CD8^+CRTH2^+$ cells were strongly associated with the severe eosinophilic phenotype [88].

Data regarding peripheral blood IFN-γ-secreting Tc1 cells are less clear-cut. Three studies reported increased frequencies of IFN-γ-secreting $CD8^+$ cells in peripheral blood in asthma *versus* health [106–108]. Conversely, another report found reduced blood Tc1 cells in allergic asthma [109] and we observed no significant differences between asthma and health [110].

$CD8^+IL6\text{-}R\alpha^+$ cells

IL-6Rα expression has been described as a surface marker for effector memory $CD8^+$ T (T_{EM})-cells. IL-6 is a pro-inflammatory cytokine and biomarker of systemic inflammation and metabolic dysfunction, and has been associated with more severe, obesity-related asthma [111], and with frequent exacerbations in poorly controlled, non-type-2 asthma [112]. $CD8^+IL\text{-}6R\alpha^+$ T_{EM}-cells express the type-2-associated nuclear

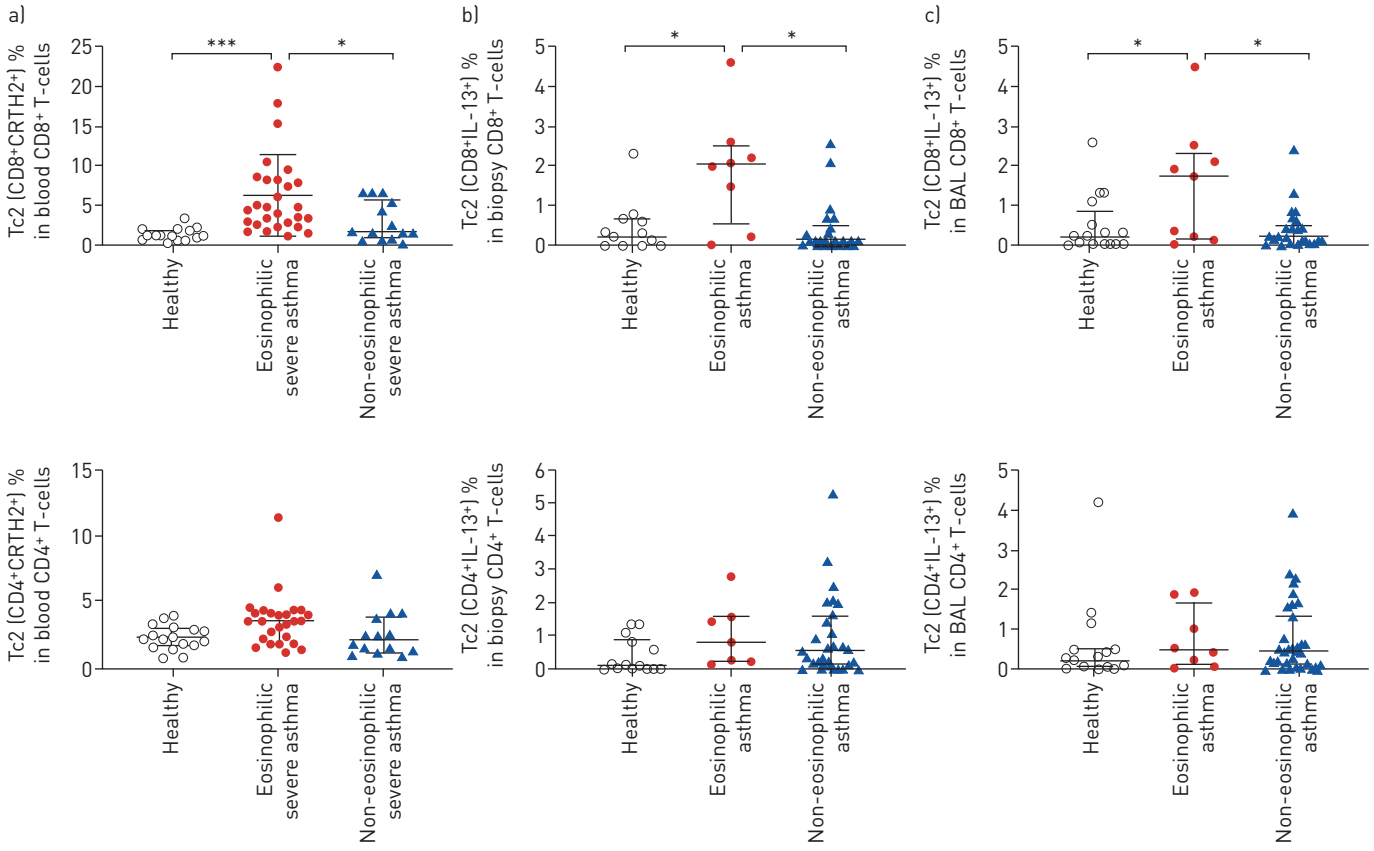

FIGURE 2 Tc2 cells are enriched in eosinophilic asthma. a) Frequencies of $CD3^+CD8^+CRTH2^+$ Tc2 and $CD3^+CD4^+CRTH2^+$ Th2 cells in $CD4^+$ or $CD8^+$ T-cells in total peripheral blood leucocytes compared between healthy controls and severe asthma patient groups (eosinophilic and non-eosinophilic) in a cohort from Oxford, UK by flow cytometry. b,c) Tc2 cells are increased in lung in eosinophilic asthma in a cohort from Southampton, UK. Frequencies of $CD3^+CD8^+IL\text{-}13^+$ and $CD3^+CD4^+IL\text{-}13^+$ T-cells in b) bronchial biopsy and c) bronchoalveolar lavage (BAL) compared between healthy controls and asthma groups (eosinophilic and non-eosinophilic) by intracellular cytokine staining. *: $p<0.05$, ***: $p<0.001$. Reproduced and modified from [88] with permission.

transcription factor GATA3, produce high levels of IL-5 and IL-13 and are increased in the peripheral blood of asthmatics [98].

Tc2 cells in human airway tissue

As with peripheral blood, BAL fluid $CD8^+$ T-cell lines produced more IL-5 in asthma than in health [113]. Likewise, unstimulated sputum $CD8^+$ T-cells produced more IL-4 and IL-5 in asthma than in health, and this increase was related more closely to the severity of AHR for $CD8^+$ than $CD4^+$ T-cells, both in terms of the magnitude and the statistical strength of the association [102]. Similarly, both $CD8^+$ and $CD4^+$ IL-4 producing T-cells were increased in BAL fluid in asthma and eosinophilic bronchitis, but the strength of this association was stronger for Tc2 than Th2 cells [107].

BLT1 is the high-affinity receptor for leukotriene B4 expressed on a variety of cell types [114]. In allergen-sensitised and -challenged mice, it was the $CD8^+BLT1^+$ and not the $CD8^+BLT1^-$ T-cell subset that was associated with IL-13-mediated asthma and dependent on LTB4 release from mast cells [115–118]. Using BLT1 as a surface marker of type 2 cells, we found $CD8^+BLT1^+$ and $CD8^+BLT1^+IL\text{-}13^+$ cells to be more increased in BAL fluid in asthma than in health, with the highest expression of IL-13 found in $CD8^+$ rather than $CD4^+$ cells [107]. Furthermore, this $CD8^+BLT1^+IL\text{-}13^+$ subset in BAL correlated with the presence of asthma and with markers of atopy (serum IgE), airway remodelling (reticular basement membrane thickness) and the degree of airflow obstruction (FEV_1 and forced expiratory flow at 25–75% of forced vital capacity) [119].

This finding of a stronger disease association for Tc2 than Th2 cells emerges consistently from multiple studies, including data from cohorts studied in Southampton and Oxford (UK). While some increases in $CD4^+IL\text{-}13^+$ Th2 cells were demonstrated in bronchial biopsies in asthma overall, Th2 cells were only significantly enriched in mild, steroid-naïve, atopic asthma, but were not associated with airway eosinophilia [88]. Th2-cells were not enriched in steroid-treated moderate asthma, or subjects with severe

asthma on high dose inhaled or oral steroids [4]. In contrast, in the same cohort we observed increases in $CD8^+IL\text{-}13^+$ Tc2 cells in eosinophilic asthma, in bronchial biopsies and in BAL fluid. Moreover, these high bronchial biopsy $CD8^+IL\text{-}13^+$ Tc2 frequencies were associated with high bronchodilator reversibility.

How might Tc2 cells contribute to inflammation in eosinophilic asthma? The onset of severe eosinophilic asthma commonly follows a viral respiratory tract infection, which could promote $CD8^+$ T-cell activation, and in the presence of IL-4, this would favour transcriptional reprogramming and conversion to Tc2 cells. These cells might then be further activated either in an antigen-specific manner by aeroallergens or viruses, or in a TCR-independent manner by inflammatory mediators. Since Tc2 cells express CRTH2 and CysLT1 (receptors for prostaglandin D2 (PGD_2) and leukotriene E_4 (LTE_4), respectively), we investigated their ligands in the airways in asthma. PGD_2 was increased in the airways of all severe asthma patients, but elevated LTE_4 was specific to the severe eosinophilic phenotype [88]. *In vitro*, PGD_2 and LTE_4 were chemattractants for Tc2 cells. This attraction was synergistic and could be inhibited by a CRTH2 antagonist, and to a lesser extent by the CysLT1 antagonist montelukast. PGD_2 and LTE_4 synergistically enhanced Tc2 production of IL-5 and IL-13, and this effect was more potent in Tc2 than Th2 cells. A specific source of these inflammatory mediators could be mast cells. IgE cross-linking of mast cells triggered release of PGD_2 and LTE_4 [88], which could induce both migration and production of IL-5 and IL-13 by Tc2 cells. In turn, supernatants from stimulated Tc2 cells contained IL-5 and granulocyte–macrophage colony-stimulating factor and could directly activate eosinophils and upregulate eotaxin release from bronchial epithelial cells [88].

Conclusion

In summary, in addition to the many descriptions of involvement of $CD4^+$ Th2 cells in human asthma, increasingly, studies have shown increased frequencies and activation of Tc2 cells both in peripheral blood and in airway tissues. In a number of these studies, the strength of Tc2 cell associations with eosinophilic phenotypes and with markers of airways inflammation and bronchial hyperreactivity were consistently stronger than those observed with Th2 cells, and were consistent with experimental findings in allergen-sensitised mice. Recent mechanistic human studies have begun to elucidate how these cells could participate in driving the type 2 pro-inflammatory responses in severe eosinophilic asthma. Phenotypic differences between T-cell subsets suggest that Tc2 cells may be less steroid-responsive, and are implicated in a variety of triggers known to elicit asthma exacerbations. Given the advances in technology and approaches to limited cell number analyses in lung tissue and BAL fluid, it is important to combine determinations of both $CD4^+$ and $CD8^+$ T-cell numbers and function in the various phenotypes of asthma, not only in steady state, but over the longitudinal course of asthma in the same patient and in cohorts.

Many questions remain unresolved, including what are the original environmental or genetic triggers for Tc2 development? What are the critical epigenetic modifications which maintain these cells as a persistent population expressing common transcriptional programmes? And ultimately, of greatest interest, how might these underappreciated contributors to severe asthma be targeted therapeutically?

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