

1 **Is There a Role for CD8+ Tc2 Cells in Steroid Resistant Asthma?**

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Different types of inflammation and effector cells contribute to asthma pathogenesis, with genetics and varied environmental triggers dictating the effector pathways (reviewed in ¹). As a consequence, patients may differ from each other as well at different stages of their disease. Predominant effector molecules in asthmatic airway inflammation include type 2 cytokines, IL-4, IL-5, and IL-13. IL-17 and airway neutrophilia may play a role in response to certain triggers. For several decades, type 2 cytokine release has been associated with CD4⁺ Th2 cells and corticosteroid responsiveness. Other cell types are now shown to be sources of these cytokines including mast cells and other T cell subsets, including type 2 innate lymphoid cells and Th9 cells, activated in response to epithelial cytokines IL-25 and IL-33 and TSLP. Despite inhaled and often oral corticosteroids, many asthmatic patients exhibit persistent symptoms, type 2 inflammation, airway eosinophilia, and airway obstruction. Different molecular signatures of the airway inflammatory cells have been demonstrated as a means to classify asthma, but for the most part have not assigned these signatures to specific cellular subsets.

In experimental models of asthma, predominantly in mice, contributing subsets have been defined and characterized¹. In addition to type 2-secreting CD4⁺ Th2 cells and type 2 innate lymphoid cells (ILCs), CD8⁺ cells have been shown capable of releasing significant amounts of IL-5 and IL-13. IFN- γ -producing CD8⁺ Tc1 cells exposed to IL-4 undergo transdifferentiation to IL-5- and IL-13-producing Tc2 cells responsible for the full array of lung allergic responses¹ (Figure). IL-4 was required for *Gata3* expression in these cells and IL-4-dependent recruitment of GATA3 to the IL-13 promoter. Of note, these CD8⁺ Tc2 cells were corticosteroid-insensitive compared to CD4⁺ Th2 cells¹. Terminal transcriptional differentiation to IL-13 production was regulated by

40 activation of the steroidogenic enzyme, cytochrome P₄₅₀ family 11 subfamily A member 1 (*Cyp11A1*)¹.
41 Pulmonary Tc2 cell recruitment was facilitated by upregulation of the leukotriene B₄ receptor,
42 BLT1¹. A unique feature of these converted CD8⁺ Tc2 cells was their sensitivity to hypoxia². Via
43 activation of hypoxia-inducible factor 1 α , exposure to a hypoxic environment resulted in
44 expansion of these cells, increasing IL-13-secreting cells capable of enhancing the full array of
45 allergen-driven responses.

46 The extent to which murine studies can be extrapolated to human asthma is controversial.
47 Studies focusing on the role of CD8⁺ T cells in asthma are infrequent. Nonetheless, a role for
48 CD8⁺ T cells has been suggested in human asthma with an overlap of the features described in
49 mouse CD8⁺ effector Tc2 cells. Human CD8⁺ T cells are similarly more resistant to
50 corticosteroids than CD4⁺ T cells¹. Therapeutic corticosteroids in asthma result in significant
51 decreases in numbers of CD4⁺ but not CD8⁺ T cells in peripheral blood³. Expression of *CYP11A1*
52 in CD8⁺ T cells is sensitive to vitamin D, and an epistatic effect between genetic variants in
53 *CYP11A1* and the vitamin D receptor (VDR) was protective against the development of
54 childhood asthma⁴.

55 A role for CD8⁺ T cells has been suggested in fatal asthma, in smoking asthmatics, and virus-
56 induced asthma (reviewed in ¹). Where directly examined, numbers were similar or even
57 greater than CD4⁺ T cells in the airways. Similar to mouse CD8⁺ Tc2 cells, human Tc2 cells
58 express the prostaglandin D₂ (PGD₂) receptor CRTH2, the cysteinyl leukotriene receptor 1
59 (CysLT1) and BLT1⁵. When activated, these elicit Tc2 cell chemotaxis and production of
60 chemokines and type 2 and other cytokines, resulting in eosinophil recruitment and survival⁵.

Numerous clinical studies observed associations between airway CD8⁺ T cell frequencies and asthma, with increased numbers in bronchoalveolar lavage (BAL) fluid over CD4⁺ T cells, and correlations with airway hyperresponsiveness (reviewed in ⁵). Increased numbers of CD8⁺ cells in BAL fluid were associated with eosinophilic asthma⁵. In bronchial biopsies of asthmatics, CD8⁺ T cells were enriched in the lamina propria despite regular inhaled corticosteroids (ICS)⁶. In the large U-BIOPRED study, increased CD3⁺ and CD8⁺ (but not CD4⁺) T cells characterized asthmatics with the highest submucosal eosinophilia, high fractional exhaled nitric oxide (FeNO), frequent exacerbations, and high oral corticosteroid use⁷. In a 7.5-year prospective follow-up of adults with atopic asthma⁶, declines in post-bronchodilator forced expiratory volume in 1s (FEV₁) correlated not with bronchial biopsy eosinophils or airway remodeling but with bronchial biopsy CD8⁺ (and not CD4⁺) T cells. These findings were confirmed at a 14-year follow-up⁸.

Beyond these associations, what is known about type 2 cytokine-secreting human CD8⁺ T cells specifically? Several studies reported increased peripheral blood Tc2 cell frequencies in asthma; an association stronger than with Th2 cells (reviewed in ⁵). BAL fluid CD8⁺ T cell lines produced more IL-5 in asthmatics than in healthy controls⁵. 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 cells⁵. Resting blood CD8⁺ cells produced more IL-4 in asthmatics than in healthy individuals⁵. Peripheral blood CD8⁺IL-4⁺ cells were increased in allergic asthma and CD8⁺IL-5⁺ cells were significantly increased in eosinophilic asthma; this was not true for CD4⁺IL-5⁺ cells⁹. A subset of CD8⁺IL-6Rα⁺ T effector memory cells expressing the type 2-associated nuclear transcription factor GATA3, produced high levels of IL-5 and IL-13,

and were increased in peripheral blood in asthma¹⁰. Similarly, peripheral blood CD8⁺IL-13⁺ Tc2 cells were increased in asthma and correlated with severity and with an eosinophilic phenotype, while increases in CD4⁺IL-13⁺ Th2 cells were observed only in mild disease and not in eosinophilic disease⁵. Tc2 frequencies were associated with nasal polyposis and smoking. Moreover, frequencies of peripheral blood CD8⁺IL13⁺ Tc2 cells were positively correlated with type 2 lung inflammation, measured by sputum T cell IL-4 expression, despite no association with blood CD4⁺IL13⁺ Th2 cells⁵. In a separate cohort, using CRTH2 as a surface marker for type 2 cells, CD4⁺CRTH2⁺ Th2 cells were not associated with eosinophilic disease; in contrast, peripheral blood CD8⁺CRTH2⁺ cells were strongly associated with a severe eosinophilic phenotype⁵. The findings of stronger disease associations for Tc2 than Th2 cells emerge from several studies where specifically examined. Although CD4⁺IL-13⁺ Th2 cells were enriched in bronchial biopsies, but only in mild, steroid-naive, atopic asthma; in the same study, increases in CD8⁺IL-13⁺ Tc2 cells were detected in bronchial biopsies and BAL in eosinophilic asthma⁵.

The combination of molecular reprogramming, corticosteroid insensitivity, and expansion under hypoxia position CD8⁺ Tc2 cells as important contributors to asthma pathophysiology, especially in corticosteroid-resistant asthma. Increased frequencies and activation of CD8⁺ Tc2 cells in blood and airway tissues, and the associations with eosinophilic phenotypes despite corticosteroids that are often stronger than those observed with Th2 cells, support this notion. Although bulk transcriptomic tissue studies have revealed convergence of asthma disease pathology on common pathways, the cell type-specific molecular pathology is often undefined. Future studies involving large patient cohorts followed longitudinally, whole-exome sequencing and improved single cell technologies (such as single nucleus RNA sequencing), will allow for

105 more precise identification of asthma-driven molecular changes, their association with specific
106 cell types and deleterious genetic variants, and correlations with clinical severity. Only in this
107 way will we be able to more directly implement personalized medicine with the advent of many
108 new biologics.

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References:

1. Gelfand EW, Joetham A, Wang M, Takeda K, Schedel M. Spectrum of T-lymphocyte activities regulating allergic lung inflammation. *Immunol Rev* 2017;278:63-86.
2. Ning F, Takeda K, Schedel M, Domenico J, Joetham A, Gelfand EW. Hypoxia enhances CD8(+) TC2 cell-dependent airway hyperresponsiveness and inflammation through hypoxia-inducible factor 1alpha. *J Allergy Clin Immunol*, in press, 2019.
3. Corrigan CJ, Haczku A, Gemou-Engesaeth V, Doi S, Kikuchi Y, Takatsu K, et al. CD4 T-lymphocyte activation in asthma is accompanied by increased serum concentrations of interleukin-5. Effect of glucocorticoid therapy. *Am Rev Respir Dis* 1993;147:540-7.
4. Schedel M, Jia Y, Michel S, Takeda K, Domenico J, Joetham A, et al. 1,25D3 prevents CD8(+)Tc2 skewing and asthma development through VDR binding changes to the Cyp11a1 promoter. *Nat Commun* 2016;7:10213.
5. Hilvering B, Hinks TSC, Stoger L, Marchi E, Salimi M, Shrimanker R, et al. Synergistic activation of pro-inflammatory type-2 CD8(+) T lymphocytes by lipid mediators in severe eosinophilic asthma. *Mucosal Immunol* 2018;11:1408-19.
6. van Rensen EL, Sont JK, Evertse CE, Willems LN, Mauad T, Hiemstra PS, et al. Bronchial CD8 cell infiltrate and lung function decline in asthma. *Am J Respir Crit Care Med* 2005;172:837-41.
7. Kuo CS, Pavlidis S, Loza M, Baribaud F, Rowe A, Pandis I, et al. A transcriptome-driven analysis of epithelial brushings and bronchial biopsies to define asthma phenotypes in U-BIOPRED. *Am J Respir Crit Care Med* 2017;195:443-55.

- 142 8. den Otter I, Willems LN, van Schadewijk A, van Wijngaarden S, Janssen K, de Jeu RC, et
143 al. Lung function decline in asthma patients with elevated bronchial CD8, CD4 and CD3
144 cells. Eur Respir J 2016;48:393-402.
- 145 9. Stoeckle C, Simon HU. CD8(+) T cells producing IL-3 and IL-5 in non-IgE-mediated
146 eosinophilic diseases. Allergy 2013;68:1622-5.
- 147 10. Lee N, You S, Shin MS, Lee WW, Kang KS, Kim SH, et al. IL-6 receptor alpha defines
148 effector memory CD8+ T cells producing Th2 cytokines and expanding in asthma. Am J
149 Respir Crit Care Med 2014;190:1383-94.

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Figure Legend

Interleukin (IL)-4 produced by cells including CD4 Th2 cells leads to differentiation of type 2 CD8⁺ (Tc2) cells by transcriptional reprogramming of Tc1 cells. IL-4 acts on Tc2 cells via JAK1/3 and pSTAT6 to induce expression of the type 2 master transcription factor GATA3. GATA3 expression enhances expression of HIF1 α and type 2 cytokines, including IL-5 and IL-13 which drive a number of key features of asthma pathology. These include eosinophil recruitment and survival, bronchial hyperreactivity, and goblet cell metaplasia. Tc2 cells express the prostaglandin D2 (PGD₂) receptor chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2), the cysteinyl leukotriene receptor 1 (CysLT₁) and leukotriene B4 receptor (BLT1) which respond to eicosanoids induced by inflammatory stimuli, such as cross-linking of immunoglobulin E (IgE) on mast cells. HIF1 α , hypoxia-inducible factor 1-alpha; JAK, Janus kinase; LT, leukotriene; pSTAT6, phosphor signal transducer and activator or transcription 6.