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T1-T2 Interplay in the Complex Immune Landscape of Severe Asthma

Marc Gauthier[1] | Sagar L. Kale[1] | Anuradha Ray[1,2]

[1]Pulmonary Allergy Critical Care and Sleep Medicine, Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA | [2]Department of Immunology, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

Correspondence: Marc Gauthier (gauthierm@upmc.edu) | Anuradha Ray (raya@pitt.edu)

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ABSTRACT

Asthma is orchestrated by an aberrant immune response involving a complex interplay between multiple inflammatory cell types. An increase in Th2 cells in the asthmatic airway is a hallmark of asthma, and biologics blocking their effector functions have been life-changing for many severe asthma patients who poorly respond to immunosuppression by corticosteroids. However, studies in the past decade have highlighted not only other cell types that also produce Th2 cytokines boosting the Type 2/T2 phenotype but also a heightened IFN-γ response, primarily from T cells, referred to as a Type 1/T1 immune response. Data derived from studies of immune cells in the airways and mouse models of severe asthma suggest a role of IFN-γ in corticosteroid resistance, airway hyperreactivity, and also airway remodeling via effects on other cell types including mast cells, eosinophils, airway epithelial cells, and airway smooth muscle cells. The simultaneous presence of T1 and T2 immune responses is detectable in the sickest of asthma patients in whom corticosteroids suppress the T2 but not the T1 response. This article has reviewed our current understanding of the complex T1–T2 interplay in severe asthma highlighting mediators that impact both arms which may be targeted alone or in combination for disease alleviation.

1 | Introduction

Asthma is increasingly recognized as a complex disease composed of multiple endotypes with unique natural histories, biomarkers, and responses to therapy [1]. Asthma is highly prevalent, affecting 4%–9% of the population globally [2], with difficult-to-control and severe asthma accounting for 4%–20% of asthma patients depending upon location [2, 3]. Corticosteroids and bronchodilators have been the mainstay of asthma treatment for a half century [4]; however, these medications are often insufficient to control severe disease. An improved understanding of the inflammatory underpinnings of asthma has been necessary to advance asthma care for these patients. In our discussion of interactions between type 1 and type 2 inflammation in the asthmatic airway, we begin with type 2 inflammation, most commonly associated with asthma and responsive to broad immunosuppression by corticosteroids, then also include type 1 inflammation, which can be induced alone or in parallel in a subset of the sickest patients and is poorly responsive to corticosteroids, even when used at high doses. There is clearly an unmet need for the treatment of these patients since most existing biologics developed to treat poorly controlled asthma target various arms of T2 inflammation only.

2 | Type 2 Inflammation in Asthma

The importance of inflammatory pathways in identifying treatment response in asthma has long been recognized. The emergence of corticosteroid therapy in the 1950s brought

about a recognition that patients with sputum eosinophilia were more likely to have a favorable clinical response to this treatment [5]. A study by Woodruff et al. showed that Type 2 (T2) inflammation could be detected in approximately 50% of asthma subjects, and that when randomized to fluticasone therapy, only the T2 high individuals showed improvement in forced expiratory volume at 1 s (FEV1), confirming the importance of T2 inflammation in corticosteroid-responsive asthma [6]. T2-targeted biologic therapies have shown significant efficacy in reducing asthma exacerbations and improving FEV1, but only when targeted to patients with markers of T2 inflammation [7]; no signal was seen in trials with un-differentiated asthma [8]. Furthermore, even in T2-high asthma, the response is not universal. Some patients have near total disease remission on therapy ("super-responders"), while many have partial benefit and some have no improvement [9, 10]. Taken together, these data clearly show that T2 inflammation is not universal in asthma, and that even in those with evidence of increased T2 inflammation, the inability of T2-targeted biologic therapy to universally eliminate disease suggests the presence of additional inflammatory pathways.

3 | Type 1 Inflammation in Asthma

Type 1 (T1) Inflammation is increasingly recognized in asthma. The association of T2 inflammation with asthma led to an early hypothesis that a deficiency in T1 inflammation may be playing a role, but murine models showed mixed results, and a therapeutic trial of recombinant IFN-γ was ineffective [11, 12]. The presence of the T1 chemokine CXCL10 (IP-10) was noted in an ovalbumin (OVA) mouse model of asthma, suggesting a role in the disease, and notably, CXCL10 transgenic mice exposed to the model had worse airway hyperreactivity (AHR) and inflammation [13]. However, in this study, CXCL10 associated with T2 chemokines rather than T1, giving a mixed picture of its role [13]. An analysis of $CD4^+$ and $CD8^+$ T-cells from sputum in asthma and healthy controls showed increased IL-4, IL-5, and IFN-γ with asthma, also suggesting a role for T1 in addition to T2 inflammation [14]. Increasing recognition of the importance of asthma phenotypes suggested that T1 inflammation may not be universal but limited to a subpopulation, raising interest in identifying and characterizing this group [1].

To address this, Raundhal et al. examined bronchoalveolar lavage (BAL) cells recovered from healthy controls, mild–moderate, and severe asthma participants. They observed increased IFN-γ^+ $CD4^+$ cells in severe asthma compared to mild–moderate asthma, and culturing these cells resulted in higher levels of IFN-γ in the supernatant using cells from severe asthma patients, with no difference noted in IL-4, IL-17, or IL-5 [15]. To test this hypothesis, they developed a murine model of severe asthma by combining house dust mite antigen (HDM) with the bacterial second messenger cyclic-di-GMP, which resulted in increased AHR and a significant loss of corticosteroid responsiveness, similar to the clinical phenotypes observed in severe asthma patients [15]. Notably, exposure of *Ifng*$^{-/-}$ mice to the model resulted in loss of the asthma phenotype, while *Il17ra*$^{-/-}$ mice had no difference compared to wild type [15]. This supported a role for IFN-γ in severe asthma and supported T1 inflammation as a corticosteroid-resistant pathway of asthma inflammation.

Gauthier et al. investigated potential mechanisms for corticosteroid resistance. The T1 chemokine CXCL10, previously described in asthma associated with allergy response and T2 inflammation [13, 16], was found to be increased in microarray analysis of BAL cell RNA in severe asthma compared to mild–moderate asthma or healthy controls, correlating with *IFNG* expression. *Cxcl10* was similarly elevated in the murine severe asthma model and associated with *Ifng* expression, supporting similarities between the model and clinical phenotype [17]. Prior work hypothesized that IFN-γ contributed to corticosteroid resistance by inhibiting glucocorticoid receptor (GR) translocation to the nucleus [18]. However, in vitro analysis of THP-1 cell response to dexamethasone in the presence of IFN-γ showed preserved activation of GR response elements by reporter assay and downstream corticosteroid-induced *DUSP1* gene expression [17]. This study showed that the ability of cells to respond to corticosteroids was not impaired by the presence of T1 inflammation, yet the clinical phenotype was rendered steroid resistant.

Confirming these findings, Oriss et al. examined the role of the T1 transcription factor IRF5 and found that *Irf5*$^{-/-}$ mice exposed to the severe asthma model had reductions in lung *Ifng* and *IL17a* expression, with restoration of corticosteroid sensitivity seen by improvements in AHR and lung inflammation [19]. These improvements were not seen in an HDM-only mild–moderate asthma model, suggesting that IRF5 was critical to the T1 inflammatory pathway in severe disease [19]. Regarding clinical relevance, *IRF5* expression was higher in severe asthma compared to mild–moderate disease in BAL cell RNA [19].

While pathways of T1 inflammation were being clarified, the clinical importance of this pathway was becoming more apparent. The presence of T1 inflammation can be identified in approximately 20%–30% of asthma subjects [17], and a pattern has been seen across multiple cohorts including the Immune Mechanisms in Severe Asthma (IMSA) cohort [20], multiple iterations of the NHLBI Severe Asthma Research Program (SARP) in the United States [17, 21–23], the UBIOPRED (Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes) cohort in Europe [24], and in pediatric studies [25]. Analysis of microarray data from the entire SARP I/II cohort showed that ~20% had elevated expression of *IFNG* in the BAL and that this correlated with disease severity, oral corticosteroid use, and exacerbations [21].

Additional work sought to understand what mechanisms were important for Th1 recruitment to the lung. CXCL9 (MIG) and CXCL10 signal through the receptor CXCR3 [26], which is important for the recruitment of Th1 cells to sites of inflammation in multiple autoimmune diseases [27–29]. Th1 cells release IFN-γ, which can then drive additional local production of CXCL9 and CXCL10, creating a feed-forward loop to drive targeted inflammation, which is critical for bacterial and viral response [30], but may be counterproductive in autoimmune conditions. CXCR3 had shown variable effects in mild–moderate asthma models, with *Cxcr3*$^{-/-}$ mice showing improvement in

an OVA model [31] but worsening in an HDM model [32], likely due to limited and variable contributions of T1 inflammation in these models. Notably, in the severe asthma model that used a combination of HDM and cyclic-di-GMP, *Cxcr3*$^{-/-}$ mice showed no change from wild type C57B6/6 mice, with preserved AHR, inflammation and Th1 recruitment to the lung [21], suggesting an additional pathway capable of Th1 recruitment. While Th1 cells in blood are generally CXCR3^{+}, they can also express CCR5 [33], a receptor for the chemokine CCL5 (RANTES), with dual positivity noted primarily at sites of inflammation rather than in the blood [34]. This dual positivity was suspected to play a role in asthma, and while *Ccr5*$^{-/-}$ mice also had no change in phenotype in the severe asthma model, use of the CCR5 inhibitor maraviroc in *Cxcr3*$^{-/-}$ mice significantly reduced Th1 and Th17 recruitment to the lungs [21]. Notably, use of maraviroc in wild type mice resulted in significant improvement in lung inflammation and AHR despite an intact Cxcr3-induced pathway in these mice, suggesting a unique role for CCL5 in asthma [21]. Maraviroc was also effective when dosed post-sensitization but pre-challenge, suggesting CCR5 may be important in eliciting asthmatic responses after sensitization has been established [21].

The cellular sources of T1 inflammation were investigated further in the IMSA cohort. This cohort utilized high-dimensional flow cytometry with 40 combined surface and intracellular markers by cytometry time-of-flight (CyTOF) to analyze cells obtained by BAL from healthy control, mild–moderate, and severe asthma participants [20]. Camiolo et al. then used K-means clustering to identify immunophenotyped clusters based on BAL cellular profiles, with identification of a healthy control/mild asthma group, a T2^{High} innate-cell dominant group, and a T1^{High}/T2^{variable}/Lymphocyte dominant group [20]. Networking of correlated cell types identified CD4^{+} and CD8^{+} Tissue Resident Memory T-cells (TRMs) along with CD161^{+}CCR5^{+} T-cells as key contributors to the cellular definition of the T1^{High}/Lymphocyte dominant group, with these cells strongly positive for IFN-γ [20]. Notably, the CD161^{+}CCR5^{+} cells showed the strongest negative association in the cohort with FEV1, suggesting a particular role in driving AHR [20]. Another analysis of participants in the Wessex Asthma Cohort (WATCH) in the UK similarly identified CD4^{+} cytotoxic TRMs producing non-Th2 chemokines including CCL5 from bronchoscopy airway brushings, with significantly increased presence in severe asthma [35]. Additionally, a recent study of children with refractory wheeze has identified similar T1 TRM and CD161^{+}CCR5^{+} cellular profiles [36].

Notably, the tissue resident memory T-cells identified by Camiolo et al. were strongly CXCR3^{+} and CCR5^{+} [22] again suggesting a role for CCL5 in TRM maintenance and activation. Consistent with this, utilizing the severe asthma mouse model, researchers assessed the ability of maraviroc to block TRM reactivation by inhibiting CCR5 and showed that mice exposed to maraviroc only during a 4-week rest phase (following sensitization and challenge but prior to reactivation challenge) had a significant decrease in IFN-γ^{+} and IL-17^{+} TRMs despite no change in overall CXCR3^{+}CCR5^{+} TRMs in the lung, consistent with impaired reactivation [22]. These data all support a unique role for T1 inflammation in asthma, likely mediated through T1 CD4^{+} and CD8^{+} TRM cells and possibly also CD4^{+}CD161^{+}CCR5^{+} cells in the airways that may lead to refractory corticosteroid-resistant disease.

4 | Evidence for Combined Inflammation

While many studies have examined specific inflammatory pathways in isolation, there is increasing recognition that these pathways are frequently present together, and how they interact with each other may be as important in determining disease severity and treatment response as the individual pathways themselves. As first described, T1 and T2 inflammation are classically counter-regulated [37]; IFN-γ opposes the development of Th2 cells while IL-4 and IL-10 inhibit Th1 development [38, 39]. This counterbalance led to a trial of subcutaneous recombinant IFN-γ for steroid-dependent asthma which showed no change in FEV1 despite a reduction in blood eosinophils by 31% [12]. Trials such as these raised doubts as to the ability of T1 cytokines to effectively blunt T2-mediated asthma through these counter-regulatory mechanisms.

Airway targeted studies in asthma are showing a role for combined inflammation. CD4^{+} and CD8^{+} cells from induced sputum in mild to moderate asthma showed increased numbers of IL-4^{+}/IL-5^{+} as well as IFN-γ^{+} cells compared to healthy controls, with the association of CD4^{+} (for T2) and CD8^{+} (T1 and T2) with AHR [14]. Analysis of airway brushings by RNAseq from healthy control and mild asthma showed elevated T2 and T1 signals in asthma; although elevations were not associated with each other, both signals associated with airway obstruction [40]. Data from the SARP I/II cohort similarly showed orthogonal elevations in T1 and T2 inflammation in severe asthma, but importantly subjects with combined T1 and T2 inflammation showed a high fraction of exhaled nitric oxide (FeNO) and had the most severe disease [23]. Consistent with this, analysis of sputum RNAseq data from the SARP III cohort identified elevated T1 and T2 signaling, again in an orthogonal pattern, with combined T1 and T2 subjects having the most severe disease [41]. Notably, when this cohort was treated with systemic corticosteroids, while the T2 signal was responsive to corticosteroid treatment, clinical response in T2 individuals was limited to those with isolated T2 high disease, with mixed T1/T2 individuals showing no clinical response despite steroid mediated T2 reduction [41]. Further clinical analysis of the SARP III cohort by sputum identified participants with increased *CCL5* expression in sputum which correlated with *CXCL9* and *CXCL10* expression consistent with CCL5 as a T1 chemokine [22]. Notably, CCL5^{High} participants despite being T1^{High} had increased T2 biomarkers (absolute blood eosinophil counts and FeNO), suggesting combined disease [22].

All these data support an important interaction between T1 and T2 inflammation. While these inflammatory pathways are noted as orthogonal in cohort studies (Figure 1), the presence of T1 inflammation appears to impact the response of T2 inflammation to corticosteroids. Although classically counterbalanced, there is clear data that in some asthma patients, this regulatory process is impaired or may be reversed, with T1 inflammation possibly promoting T2 inflammation [22]. The mechanisms underlying these potential interactions involving multiple cell types and their mediators (Figure 2) and the resulting clinical impact are the focus of the remainder of this review.

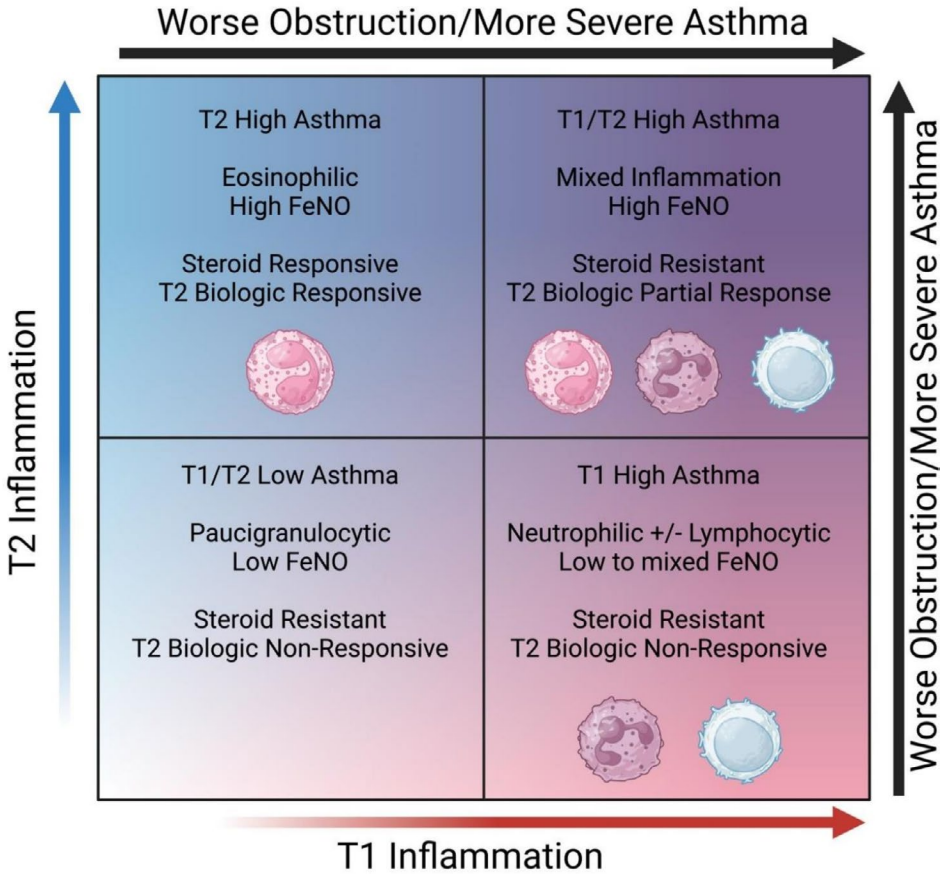

FIGURE 1 | Conceptual diagram for the effect of overlapping T1 and T2 inflammation in asthma. T1 and T2 inflammation are orthogonal, but as they coexist, they exert combined effects that result in a combined worsening of disease secondary to both inflammatory pathways, leading to a poor response to currently available T2 targeted therapies. Created in BioRender.com.

4.1 | T1 Inflammation and Mast Cells

Mast cells are not only integral to allergic reactions; it is clear they also mediate asthma pathophysiology [42, 43]. In the airways of healthy individuals, mast cells are present in the submucosa, but in asthmatic airways, their numbers increase in the airway epithelium [44, 45] and smooth muscle [46]. Mast cells harbor a variety of pre-formed mediators such as histamine and proteases, and upon activation, can also synthesize other pro-inflammatory molecules that include leukotrienes, prostaglandin D2, and also T2 and other cytokines [42, 43]. Upon release, these mediators cause immediate local reactions such as bronchoconstriction and edema, followed by late phase reactions that promote the infiltration of other cell types such as T cells and eosinophils into the airways. While various stimuli can cause mast cell activation triggering the release of these mediators, cross-linking of surface IgE upon binding of the cognate antigen/allergen is the best known. The monoclonal anti-IgE antibody omalizumab blocks the binding of IgE to its high-affinity receptor FcεRI and was the first biologic approved for the treatment of severe asthma [47, 48]. The ability of omalizumab to alleviate immediate and late phase reactions in asthma patients in response to inhaled allergens to which the subjects were sensitized confirmed the pathogenic role of mast cells in allergic asthma. However, not all asthma patients respond to anti-IgE therapy, and as discussed below, more recent studies provide evidence of non-T2 and non-IgE-associated mast cell gene/activation signatures in human asthma.

Mast cells are strictly tissue resident, limited in abundance, and not present in circulation, which is an impediment to functional studies of the cells. Although T2 cytokines are most commonly associated with mast cell function in asthma, including immunoglobulin isotype switching to IgE, T1/IFN-γ has also been implicated. Human mast cells derived by growth factor-induced differentiation of CD34+ progenitors were shown to express a functional, high-affinity IgG receptor, FcγRI, whose expression increased several-fold upon IFN-γ exposure [49]. FcγRI aggregation induced by anti-IgG binding subsequent to IFN-γ exposure promoted mast cell degranulation with the release of histamine and multiple cytokines, including TNF-α and IL-13 [49]. Responses induced via FcγRI stimulation were found to be indistinguishable from those stimulated via FcεRI [50]. In a mouse model of chronic asthma induced by repeated challenge by OVA without inclusion of an adjuvant for sensitization, IFN-γ/IFNγRI-mediated mast cell activation was associated with mixed granulocytic airway inflammation, AHR, airway remodeling, and production of several pro-inflammatory cytokines in the airways [51]. In a study of CD4+ T cells present in bronchoalveolar lavage (BAL) of a subset of mild–moderate and severe asthma patients enrolled in the SARP I/II cohort, a higher Th1/IFN-γ immune profile was identified in severe asthma compared to that in mild–moderate asthma [15].

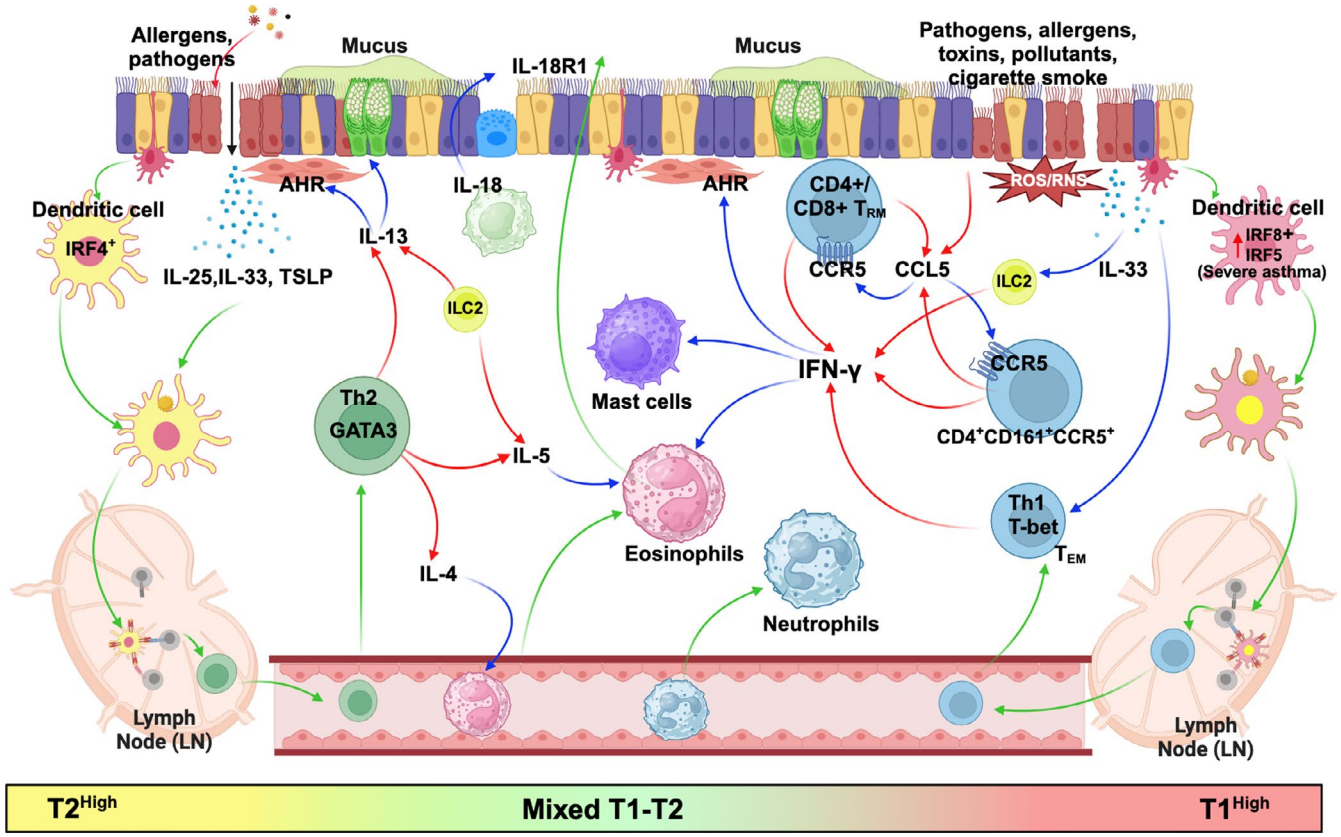

FIGURE 2 | T1-T2 interactions in the asthmatic airway. Exposure of the airway mucosa to allergens, pathogens, and pollutants activates the immune system via the release of a variety of mediators to induce T cell differentiation. IFN-γ, the key effector cytokine of the T1 immune response, acts on multiple cell types, including eosinophils, mast cells, and epithelial cells, and also induces AHR. Multiple T cell populations identified by cell surface markers such as effector/memory (T_{EM}), tissue-resident memory (T_{RM}) and pathogenic $CD4^+$ $CD161^+$ $CCR5^+$ are generated with the potential for short- or long-term residence in the subepithelium and airspaces that can be reactivated by reexposure to the cognate antigens or by bystander mechanisms, resulting in chronic secretion of IFN-γ and chemokines such as CCL5, a chemoattractant of T1 cells. T2 cells and ILC2s are components of the T2 arm of the immune response generated or activated upon exposure to allergens or alarmins, respectively, and can also undergo repeated reactivation. While T1 and T2 immune responses antagonize each other, their coexistence is evident in a subset of severe asthma patients. T1 and T2 cytokines, as well as the myriad mediators released by the different cell types, promote oxidative and nitrosative stress in the airway epithelium. Created in BioRender.com.

A follow-up study of BAL cells of the same cohort and a mouse model of $T1^{high}$ severe asthma revealed that while increased expression of the IFN-γ-induced chemokine CXCL10 in the airways of both mice and humans marked the corticosteroid-resistant T1 phenotype, CXCL10 was also associated with a mast cell gene signature [17]. In a subsequent study of BAL cells of patients enrolled in the IMSA program that employed high-dimensional multiomics and machine learning approaches, a severe asthma-enriched patient group with a dominant T cell response displayed a high T1 profile and showed association with a mast cell gene signature [20]. Figure 3 depicts interactions between mast cells and a T1 immune response. Another study from the UBIOPRED program studied mast cell activation signatures in the sputum of asthma patients [24]. The investigators interrogated mast cell activation signatures by comparing the sputum transcriptome with published mast cell signatures generated from cultured human blood progenitor-derived mast cells that were either unstimulated or stimulated by FcεRI activation, LPS, IL-33, or IFN-γ. The relationship of the mast cell activation signatures with asthma severity and sputum granulocyte status was examined and also mapped to three sputum molecular clusters of severe asthma, labeled as transcriptome-associated clusters (TACs) 1–3, that the investigators had previously described [52]. The study revealed differential enrichment of gene signatures depending on clinical severity, granulocytic profile, and molecular phenotype, showing that mast cell activation via pathways other than FcεRI is evident in asthma across clinical severity and associates with differential granulocytic inflammation. While the study did not detect differential IFN-γ-activated mast cell gene signatures across clinical severity, the IFN-γ-activated signature associated with the molecular cluster TAC2, these patients showing the highest level of sputum neutrophilia, serum C-reactive protein levels, and incidence of eczema [52]. The relationship between IFN-γ and mast cells may also be bidirectional given that mediators produced by a human mast cell line were shown to promote IFN-γ production by both $CD4^+$ and $CD8^+$ T cells [53]. While mast cell activation is typically associated with degranulation and release of mediators, it is possible that the impact of IFN-γ on mast cells does not cause overt degranulation but instead results in a slower process of release of mediators called piecemeal degranulation, also known as transgranulation [54]. During this process, the mast cells release their granules but retain the granule membranes in the cytoplasm

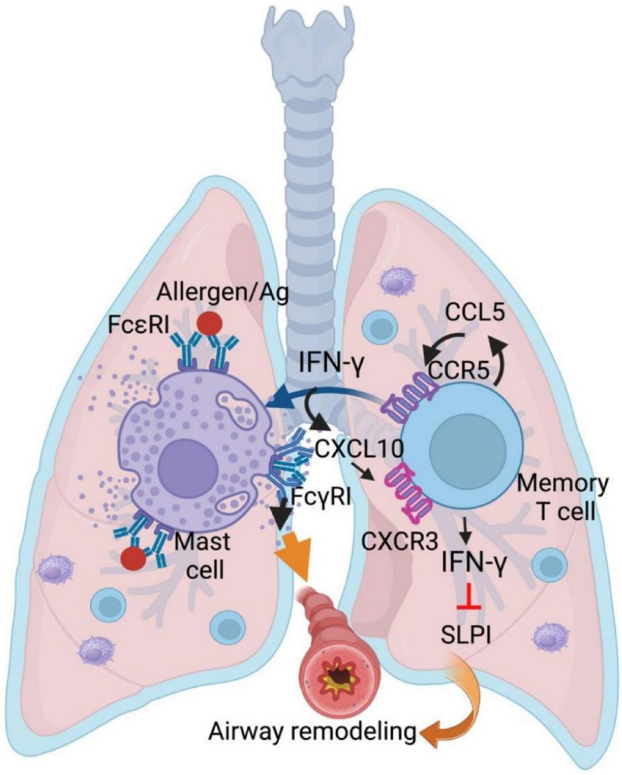

FIGURE 3 | Interaction between mast cells and T1 cells in the asthmatic airway. Mast cells express both the high-affinity receptor for IgE, FcεRI, and FcγRI, the expression of the latter being augmented severalfold by IFN-γ secreted by memory T cells in the tissue (T_{RM}, T_{EM} and $CD4^+CD161^+CCR5^+$ T cells). Both FcεRI and FcγRI activation can induce the release of mast cell mediators. Increased levels of the T1-associated chemokines CXCL10 and CCL5 track closely in the severe asthmatic airway, together contributing to enhancing the T1 response. IFN-γ/IFNγRI-mediated mast cell activation has been implicated in promoting all features of asthma, including airway remodeling. Suppression of IFN-γ-mediated SLPI expression from airway epithelial cells also has implications in airway remodeling. Created in BioRender.com.

such that the cells remain chronically activated with the release of one or more mediators but do not undergo complete degranulation as induced during an acute hypersensitivity reaction.

4.2 | Mixed Granulocytic Inflammation in Asthma

4.2.1 | Eosinophils in Asthma

Eosinophils have long been described in asthma, with increased eosinophil numbers in the airways associated with clinical disease severity, exacerbation risk, and clinical symptoms. Increased sputum eosinophils were associated with greater frequencies of asthma exacerbations in the prior year in the SARP III cohort [55], and a population-based chart review study in the UK showed that asthmatic patients with baseline elevated blood eosinophil counts had a higher risk of exacerbation in the following year, with the level of risk directly correlated to the eosinophil counts [56]. Eosinophils also track with markers of disease symptomatology. Sputum eosinophilia and blood eosinophil counts track with mucus plugging on CT scan, correlating with worse obstruction and increased frequency of bronchitis symptoms [57]. Another analysis from the SARP III cohort utilizing expiratory CT scans to assess segmental air trapping in the lung showed that sputum and blood eosinophilia were associated with higher segmental air trapping scores, suggesting again a role for eosinophils in driving asthma pathology and symptomatology [58].

Significant literature has shown the importance of eosinophils in asthma pathogenesis, and multiple treatment modalities are now utilized targeting eosinophils in asthma. Sputum eosinophils were recognized early on as strong predictors of corticosteroid responsiveness in asthma [59] and can effectively guide inhaled corticosteroid dosing in mild to moderate asthma [60]. Eosinophils are generally corticosteroid responsive, while inhaled β-agonists do not have any significant effect on these cells; inhaled corticosteroids can significantly reduce eosinophil populations in the lamina propria and epithelium in the lung [61]. However, despite these data suggesting strong corticosteroid responsiveness, eosinophils can persist in the presence of corticosteroid therapy. A study by Wenzel et al. performed bronchoscopies with endobronchial biopsies on healthy controls, mild, moderate, and severe asthma, with severe asthma defined by systemic corticosteroid dependence [62]. While eosinophil numbers in the tissue fell from mild to moderate disease, in severe asthma a split was noticed, with 20 of 34 subjects (59%) having tissue eosinophilia that was at or above the levels seen in mild to moderate asthma despite systemic corticosteroid therapy [62]. A similar finding was reported by Jatakanon et al. in induced sputum, with marked eosinophilia in some severe asthma subjects that was at or above the level seen in mild to moderate asthma participants [63]. Another bronchoscopy study in children identified a similar pattern, with 32% of children having steroid-refractory eosinophilic inflammation [64]. These data support the idea that not all eosinophils are fully steroid sensitive, and other pathways may contribute to eosinophil persistence in the presence of corticosteroid therapy.

Eosinophils rely on interleukin-5 (IL-5) signaling via the surface receptor IL-5R for chemotaxis as well as pro-persistence signaling once these cells are established in the tissues [65, 66]. In the last decade, three therapeutic antibodies have been developed to target this pathway, either by binding IL-5 directly (mepolizumab, reslizumab) or by binding the receptor via the IL-5Rα subunit (benralizumab), with significant reductions in exacerbations and improvements in lung function [7]. However, many patients have persistent disease despite complete suppression of the IL-5/IL-5R pathway, with only 50% achieving remission with regard to exacerbations and 20%–33% obtaining remission with regard to lung function and symptoms [67]. These data support the concept that alternative non-eosinophilic mechanisms of asthma contribute significantly to disease in eosinophilic patients or that mechanisms of eosinophil persistence in some patients are not IL-5 dependent.

4.2.2 | Eosinophils and Type 1 Inflammation

While classically associated with T2 inflammation through the IL-5 and eotaxin pathways, eosinophils, like mast cells, have been shown to interact with T1 inflammatory cytokines.

Eosinophils isolated from whole blood could be maintained in vitro with recombinant IFN-γ exposure to a degree similar to that seen with IL-5 treatment [68]. IFN-γ treatment of human eosinophils also promoted the release of CCL5 from these cells [69] and exposure of IL-5 transgenic mouse eosinophils ex vivo to IFN-γ resulted in a T1 dominant transcription profile including CXCL9, CXCL10, and CCL5 [70], suggesting that IFN-γ was also able to affect the transcriptional programming of these cells. In addition to IFN-γ responsiveness, eosinophils have also been shown to express the T1-chemokine receptor CXCR3 [71], the cognate receptor for the T1 chemokines CXCL9, CXCL10, and CXCL11. As mentioned above, elevations in CXCL9 and CXCL10 have been observed in asthma [17, 72–74], suggesting a pathogenic role that could contribute to eosinophil activation. Stimulation of eosinophils isolated from healthy donors with CXCL10 increased eosinophil activation with increases in ICAM expression and superoxide production, in addition to increased production of T1 chemokines and IFN-γ production [75]. These data support a role for T1 signaling in eosinophil persistence and activation.

While T1 inflammatory signals can clearly interact with eosinophils in vitro and ex vivo, there is increasing evidence that these interactions are clinically relevant. In a murine study, adoptively transferred eosinophils from sensitized IL-5 transgenic mice into naïve mice were able to produce lung inflammation and AHR, as well as promote the recruitment of eosinophils, neutrophils, and macrophages [76]. Eosinophils in this model resulted in a significant induction of IFN-γ, and importantly, the transfer of activated eosinophils from *Ifng*$^{-/-}$ mice resulted in significantly reduced inflammation and AHR, suggesting that T1 chemokines were important for eosinophil induction of AHR and inflammation [76]. In a study examining exercise-induced bronchospasm (EIB) as a marker of indirect AHR, EIB^{+} asthma subjects had greater epithelial but not subepithelial eosinophil numbers. RNAseq with pathway analysis showed that T2 inflammation was increased in EIB+ subjects, as expected, but the study also identified *IFNG* as a key node correlating with intraepithelial eosinophil location [77].

There is increasing evidence that eosinophils are not a monolithic cell type, but that subpopulations exist that are relevant to disease. The interaction of eosinophils with their environment can yield distinct transcriptional programs. Treatment of peritoneal eosinophils isolated from IL-5 transgenic mice with either IFN-γ or IL-4 resulted in distinct transcriptional profiles, with IFN-γ producing a T1 profile, as noted above, and IL-4 producing a more classic T2 profile, suggesting that the inflammatory milieu is critical for determining eosinophilic transcriptional responses. Beyond this T1 vs. T2 paradigm, there is also recognition of differential eosinophil function within tissue determined by tissue residency (Figure 4). Work by Mesnil et al. identified Siglec-F^{int}CD125^{int}CCR3^{+} cells in the lung as resident eosinophils, populating mouse lungs by 7 days of age and then persisting throughout the life span of the mouse [78]. These cells were different from inflammatory eosinophils (Siglec-F^{high}CD125^{+}CCR3^{+}) which were rapidly recruited to the lung with exposure to an HDM asthma model, with inflammatory eosinophils favoring a T2 response and resident eosinophils functioning as immunomodulatory cells in this model. Importantly, despite both expressing IL-5R, resident eosinophils were not dependent on IL-5 for survival and persisted in mice treated with anti-IL5 therapy, suggesting the potential for anti-IL5 resistant eosinophils in humans [78]. These cells could also be identified in human blood samples [78] and a study in COPD and asthma showed the presence of resident eosinophils in peripheral blood in both conditions, although with significantly elevated inflammatory eosinophils seen exclusively in asthma [79]. These data support the role of unique eosinophil subsets in human disease, some of which are T1 responsive. Our understanding of the spatial positioning of these cells in asthma and how they relate to disease severity is an ongoing area of inquiry.

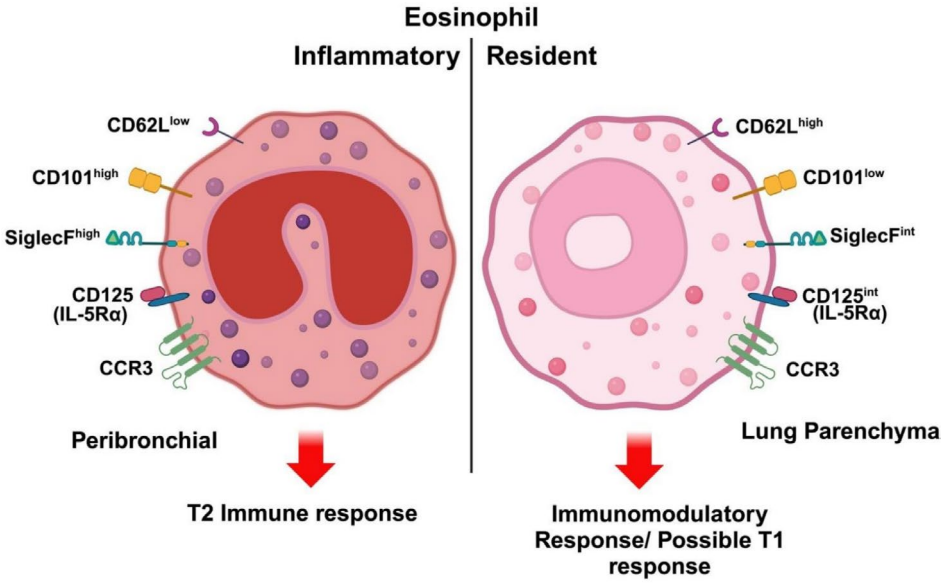

FIGURE 4 | Resident and inflammatory eosinophils can be distinguished by differential expression of surface markers. Created in BioRender.com.

4.2.3 | Combined Neutrophilic and Eosinophilic Inflammation in Asthma

The rise of anti-IL5 and anti-IL5Ra therapies has led to the division of asthma into eosinophilic and non-eosinophilic groups based on perceived likelihood of response to these therapeutics, with non-eosinophilic asthma consisting of neutrophilic and pauci-granulocytic types based on sputum cellular content. However, this ignores substantial overlap in neutrophilic and eosinophilic disease, a subject of significant importance in asthma.

Airway and sputum neutrophilia are associated with increased asthma severity. Neutrophilia is associated with increased T1 inflammation [41] and notably, IFN-γ can direct neutrophil chemotaxis to sites of inflammation by upregulation of CCR1 and CCR3, allowing chemotaxis via a CCL5 gradient [80]. In the study by Wenzel et al., the number of neutrophils in airway biopsies steadily increased with disease severity, with severe asthma showing the highest levels of neutrophil infiltration of the airways [62]. A similar pattern was also noted in induced sputum, with sputum neutrophil content rising with disease severity [63]. While this association had been postulated to be secondary to corticosteroid exposure associated with disease severity [81], there is increasing evidence that neutrophils are not bystander cells, but actively contribute to disease [82]. Neutrophils were identified upon histological examination of the airways in sudden onset fatal asthma [83]. In a murine model, sensitization of mice to allergen combined with endotoxin led to neutrophilic inflammation and the development of neutrophilic extracellular traps (NETs) through a process of netosis [84]. NETosis is critical for neutrophil response to pathogens, as NETs successfully trap pathogens such as bacteria, preventing spread and allowing phagocytosis [85]. Moreover, the residual cytoplasts trigger additional inflammation to drive recruitment of further neutrophils and enhance inflammation as part of the inflammatory response [84, 85]. There is evidence for this process in human asthma as well, with detectable NETs and cytoplasts in BAL [84] along with extracellular DNA which is associated with lower Asthma Control Test scores and higher rates of chronic bronchitis symptoms [86]. Notably, neutrophils produce nitric oxide as a key part of the NETosis pathway, which may contribute to FeNO elevation in some patients with neutrophilia and severe asthma independent of T2 inflammation [87]. This process may also not be exclusive to neutrophils, as eosinophils have also been described to produce extracellular NETs through an autoimmune mediated process, which notably was also refractory to corticosteroid therapy [88]. In a follow-up study of severe asthmatic subjects on anti-IL5 therapy, failure of therapy response was associated with the presence of anti-eosinophil peroxidase antibodies, suggesting these autoimmune eosinophil features, if present, may also be refractory to eosinophil-targeted biologic therapy [89].

Neutrophilic and eosinophilic inflammation are not mutually exclusive. In the Wenzel et al. study, lung tissue neutrophils were highest in the eosinophilic severe asthma group [62]. Work in the SARP I/II cohort by Hastie et al. showed that cohort participants with sputum eosinophilia (≥2%) and neutrophilia (≥40%) had the lowest lung function, increased symptoms, and higher health care use [90]. A follow-up study in the SARP III cohort looked at this pattern longitudinally and observed that combined neutrophilic-eosinophilic asthma had the lowest FEV1 at baseline and through the 3 years of follow-up compared to all other subjects [91]. This suggests that combined inflammatory disease is particularly severe and challenging to treat. Recent work seeking to understand this link has explored the role of CCL5 in combined disease. In the SARP III cohort, CCL5 expression in sputum was elevated compared to the healthy control range, and CCL5 expression tracked closely with the T1 chemokines CXCL9 and CXLC10, suggesting its role as a predominantly T1 chemokine, but also $CCL5^{High}$ participants had increased T2 biomarkers [22]. $CCL5^{High}$ subjects had both increased sputum lymphocytes and neutrophils consistent with T1 inflammation, and also increased eosinophils, with an overall significant increase in mixed eosinophilic-neutrophilic disease compared to $CCL5^{Low}$ subjects [22]. Logistic regression analysis confirmed that this association was unique to CCL5, rather than CXCL9 or CXCL10, suggesting a unique role as a bridging molecule between T1 and T2 inflammation and resulting in combined neutrophilic and eosinophilic inflammation in asthma [22].

4.3 | T1 Immunity, Tissue Resident Memory (T_{RM}) Cells and Asthma

$CD4^+$ and $CD8^+$ T_{RM} cells are non-emigrating memory T cells in different tissues that persist for prolonged periods of time [92–94]. They upregulate molecules that allow them to remain within the tissue and downregulate CCR7, a chemokine receptor required for entry into lymphoid tissues. In lung transplant patients, donor T_{RM} cells were detected in allograft biopsy for up to a year after transplant, coupled with concurrent establishment of new recipient T_{RM} cells during this time [95]. In the lung, the establishment of Th2-type T_{RM} cells was associated with neuronal signaling in early life [96]. While it has been challenging to attach unique cell surface molecules to T_{RM} cells, $CD4^+$ T_{RM} cells frequently express the activation marker CD69, also expressed by effector/memory T cells in tissues (but not in blood) and these cells may also express CD103, the α_e integrin. $CD69^+$ T_{RM} type cells have also been detected in lymph nodes [97, 98]. $CD8^+$ T_{RM} cells in all tissues express CD103 [93, 99]. The level of expression of both CD69 and CD103 by T_{RM} cells varies in different tissues, with $CD8^+$ TRM cells in the lung and the intestines found to express higher levels of CD103 than those in other tissues [93, 99]. T_{RM} cells, identified in the lung, intestines, skin, liver, and brain, provide protective immunity against invading pathogens through rapid, local production of effector cytokines such as IFN-γ and TNF-α and this protective function of T_{RM} cells in different tissues is well documented. In the lung, T_{RM} cells have been shown to protect against infections by influenza [100], respiratory syncytial virus (RSV) [101] and SARS-CoV2 [102]. However, it appears that T_{RM} cells decline in the lung over time, as evident in both mice after influenza infection [103, 104] and in humans after RSV infection [105]. The reason for this decline is unclear, although various alternative mechanisms such as cell death and egress brought about by tissue damage or lack of tonic (non-canonical) TGF-β signaling via CD103 have been proposed [93]. While a protective role of antigen-specific resident memory T cells that can rapidly mount defense against a repeat infection by the same pathogen can be easily envisioned,

their persistence over long periods of time in large numbers may also prove pathogenic, especially given that cytokine-mediated bystander activation of T_{RM} cells has been described [106–108]. This is of particular concern in individuals with chronic diseases such as asthma, especially corticosteroid-refractory severe asthma, in whom $CD8^+$ and $CD4^+$ T_{RM} cells expressing IFN-γ have been identified in bronchoalveolar lavage cells [20, 35]. These T_{RM} cells are also strongly associated with gene modules expressing CCL5 and its receptor CCR5 [20]. Interrogation of a mouse model of steroid-refractory severe asthma [15] revealed the presence of IFN-γ+ T_{RM} cells in the mouse lungs as well [22]. Notably, targeting CCR5 using the CCR5 antagonist maraviroc in the mouse model reduced the frequency of IFN-γ+ and IL-17+ T_{RM} cells upon allergen challenge, suggesting that CCR5 antagonism may reduce reactivation of T_{RM} cells in asthmatic airways [22]. Although the antigen specificity of IFN-γ+ T_{RM} cells in asthmatic airways has yet to be described, viral antigen carriage [109], auto-antigens (when autoimmunity is a comorbidity) [110] or chronic stimulation by inflammatory cytokines such as IL-18 [111] or others that enable memory T cell maintenance, such as IL-15 [112, 113] may independently or in combination contribute to the long-term maintenance and activation of T_{RM} cells. Uncontrolled IFN-γ production by T_{RM} cells in the lungs may not only worsen lung function [15] but may also induce premature tissue senescence [114] and epithelial barrier disruption [115].

4.4 | T1-T2 Interaction and the Airway Epithelium

Airway epithelial injury and sloughing, barrier dysfunction coupled with impaired repair, goblet cell hyperplasia, mucus plug formation, persistent airway inflammation, and airway remodeling are common features of asthma irrespective of the underlying immune heterogeneity and/or disease severity [116]. Airway epithelial cells (AECs) form a physical barrier that acts as a first line of defense between the environment and the lung. However, constant provocation by multiple environmental factors, including allergens, toxins, pathogens, and particulate matter, has the potential to disrupt the AEC barrier [117] resulting in barrier dysfunction [118]. Allergens with proteolytic activity, such as Derp 1 present in the fecal pellets of the common allergen house dust mite or in cockroach allergen, cleave tight junction proteins in the epithelium and compromise epithelial barrier integrity [119, 120]. Interaction of allergens with the epithelium can induce the production of multiple pro-inflammatory cytokines and chemokines in susceptible individuals, and proteolytic allergens also augment the release of alarmins from the epithelial cells. The allergens interact with dendritic cells (DCs) both in the epithelium and in the subepithelium when the barrier is breached, and the processing of these allergens by DCs and their activation and migration to lung-draining lymph nodes results in Th2 differentiation [121]. In addition, the alarmins also activate ILC2s with secretion of Th2 cytokines [121]. Although T1-mediated AEC barrier disruption has not been adequately studied, studies of intestinal epithelial cells have revealed that IFN-γ stimulation leads to endocytosis of TJ proteins, occludin, JAM-A, and claudin [122]. In studies of autoimmune polyendocrinopathy candidiasis–ectodermal dystrophy (APECED), an inherited autoimmune disease caused by loss-of-function mutations in the autoimmune regulator (*AIRE*) gene, increased IFN-γ production was observed in $Aire^{-/-}$ mice, resulting in barrier disruption in the oral mucosa with promotion of fungal infection [115]. It is possible that a $T1^{high}$ immune response in severe asthma also promotes barrier dysfunction in the airways.

Like the T2 cytokines IL-4 and IL-13, IFN-γ also induces NOS2 in AECs with the production of nitric oxide (NO). Given that FeNO is a key biomarker of human asthma, FeNO levels in the asthmatic airway may reflect the combined action of T1 and T2 on the airway epithelium. Indeed, IL-13 and IFN-γ were shown to synergistically promote NOS2 in normal human bronchial epithelial cells (NHBE) and induce nitrosative stress [123]. In a UBIOPRED study, analysis of transcriptome data of bronchial biopsies also revealed an increase in oxidative stress signature correlating with IFN-γ and corticosteroid insensitivity [124] that may be further augmented by T2 inflammation [123]. However, as in lymphocytes, antagonism between T1 and T2 cytokines has also been noted in AECs. In a study of genes induced by either IFN-γ or IL-4 alone or their combination, the cytokines were found to oppose each other's ability to induce gene expression, although a subset of genes was spared from this effect [125]. Interestingly, CCR5 was among IFN-γ-induced genes not inhibited by IL-4 [125].

As discussed above, increased IFN-γ level in a mouse model of severe asthma was associated with enhanced central airway resistance (Rn), which was only partially responsive to the corticosteroid dexamethasone [15]. IFN-γ levels in both the mouse model of severe asthma and in human severe asthma inversely correlated with the expression of serine leukocyte protease inhibitor (SLPI) in the AECs. Forced expression of SLPI in the mice subjected to the severe asthma model reduced Rn, which was further reduced when SLPI was combined with dexamethasone [19]. This inverse relationship between IFN-γ and SLPI was also documented in prior studies [126, 127]. Emphysema caused by IFN-γ overexpression in mice led to upregulation of multiple chemokines, including CCL5, and neutralization of CCR5 reduced inflammatory cytokine levels in the lung and airway remodeling with an increase in SLPI levels [128]. In a study of wound healing in the skin, increased generation of TGF-β was observed in $Slpi^{-/-}$ mice, which promoted wound healing [129]. Given that TGF-β is a central mediator of airway remodeling, it is possible that SLPI deficiency caused by a $T1^{high}$ immune status in severe asthma contributes to airway remodeling that also results in persistent AHR [130]. In a more recent study, IFN-γ was shown to increase the expression of IL-31RA in primary airway smooth muscle (ASM) cells from mice [110]. Treatment of mice with IFN-γ increased total airway resistance (Rrs) which was in attenuated $Il31ra^{-/-}$ mice. It will be interesting to determine whether IL-31RA also plays a role in IFN-γ-induced increase in Rn. Similar to the effects of IL-13, IFN-γ exposure also caused upregulation of *MUC5AC* and *GOB5* expression in the AECs and induced goblet cell hyperplasia [110]. Collectively, whether an IFN-γ-SLPI-TGF-β axis plays a role in airway remodeling in severe asthma in humans remains to be determined in future studies.

Whether genetic predisposition and epigenetic mechanisms influence the effect of T1 and T2 cytokines on AECs and ASM cells to promote cellular stress and airway remodeling awaits future investigation.

5 | Airway Dysbiosis

The airway is not a sterile location but is home to its own unique flora of bacteria, fungi, and potentially viruses [131] and changes in this balance may play an important role in airway/lung disease [132]. While there has been much interest in the role of the microbiome in disease onset in asthma [133–135], research has increasingly supported a potential role for the microbiome to contribute to asthma severity [136]. Disruptions in microbiome diversity, termed dysbiosis [137], may contribute to severity and drive molecular pathways important to certain asthma endotypes.

The composition, nature, and diversity of the respiratory microbiome not only play a major role in tolerance versus disease prevalence but also influence disease severity [136, 138, 139]. Overall, less diversity in the lung microbiota in comparison to healthy individuals is associated with asthma and general atopy [140]. In a recent study of participants with poorly controlled severe asthma, reduced diversity with increased abundance of Haemophilus and Moraxella taxa was correlated with higher sputum neutrophil percentages, whereas individuals characterized by higher eosinophil percentages had a greater frequency of Streptococcus, Neisseria, and Gemella [141]. Along with its strong association with the risk of development of asthma and its severity, the variance in microbiome also influences the responsiveness to inhaled corticosteroids (ICS). The prevalence of Haemophilus, Moraxella, and Streptococcus has also been implicated in steroid refractory severe asthma. A study by Goleva et al. demonstrated specific expansion of Gram-negative bacteria rich in endotoxin levels in corticosteroid resistant asthmatics as compared to corticosteroid sensitive asthmatics [142]. While the specific mechanisms behind microbiome-induced corticosteroid resistance remain unclear, the microbiome is known to modulate the immune response and may impart corticosteroid unresponsiveness by interacting with components of the innate immune system. Culturing macrophages from BAL fluid in the presence of *Haemophilus parainfluenzae* did in fact render these cells less responsive to corticosteroids [142]. Corticosteroids can also be metabolized by components of the microbiome, as demonstrated using human colonic bacteria [143] and bacterial families associated with corticosteroid biodegradation pathways were enriched in ICS non-responders as compared to the responders [144]. These data support a direct involvement of the microbiome in metabolizing steroids, thus blunting their effectiveness. Another issue that has been raised is the possibility of steroid-induced changes in lung microbiota [145] which might also be detrimental, although the confounding factor of increased steroid need in severe asthma has made sorting out causality and association challenging. Potentially supporting a causal effect is the efficacy of macrolide therapy in asthma, with post hoc analysis of the AMAZES trial for chronic azithromycin therapy in severe asthma showing significant reductions in Haemophilus burden without overall changes in total bacterial load [146]. Overall, however, the data support the hypothesis that disruptions in the bacterial airway microbiome can contribute to asthma severity.

The microbiome has the potential to affect the inflammatory profile of the lung. This is already well described in T2 inflammation with allergic bronchopulmonary aspergillosis, where non-invasive airway colonization is able to elicit a robust immune response to impact asthma management [147]. Chronic airway inflammation from bacterial dysbiosis or viral carriage may lead to persistent T1 elevation in response, contributing to mixed inflammation in asthma. Notably, mouse models of T1-dependent severe asthma have been able to generate this phenotype by pairing traditional allergen (HDM) with a bacterial second messenger (cyclic-di-GMP) to obtain the resulting T1 dominant inflammatory signal with asthma features (AHR, mucus production, peribronchovascular inflammation) [15]. Clinical support for this hypothesis exists as well, with sputum T1 inflammatory signal in the SARP III cohort showing increased prevalence of viral transcripts (despite no viral symptoms) [109]. A pediatric study of severe asthma utilizing bronchoscopy for lower airway sampling showed a high prevalence of T1 inflammation associated with recoverable respiratory viruses and bacteria [25]. Notably, lower airway bacterial and viral signatures were not returned in all participants in either study, suggesting that these were not the sole cause of T1 inflammation in asthma. However, these data support the idea that disruption in the microbiome may lead to persistent T1 inflammation in some patients, resulting in mixed inflammatory disease and impacting asthma control.

5.1 | T1 Inflammation and Alarmins

The epithelial-derived cytokines IL-25, TSLP, and IL-33, called alarmins, have been implicated in asthma pathogenesis, an important effect of these molecules being stimulation of T2 cytokine secretion by ILC2s, which express the receptors for these molecules. Allergens with protease activity that include those present in fungi and viruses have been shown to cause ILC2 activation via the release of IL-33, IL-25, or TSLP from airway epithelial cells in mice and humans [148–150]. Interestingly, allergen-experienced ILC2s persist after initial activation and respond more vigorously to a subsequent stimulation similar to memory T cells [151]. However, the effector function of these alarmins, the most studied being IL-33, is not just limited to the promotion of T2 responses. In a mouse model of airway provocation induced by virus, bacteria, or cigarette smoke, lung resident ILC2s showed decreased GATA-3 but increased T-bet and IFN-γ expression in a process related to IL-12 and IL-18 signaling [152]. A similar observation was made in humans [153]. A heightened IL-33 response has been associated with multiple respiratory viral infections [154]. However, while an exaggerated IL-33 response induced by a viral infection is undoubtedly deleterious in the setting of asthma, IL-33 was shown to exert a protective role during viral infections by promoting a cytotoxic antiviral $CD8^+$ T cell response and preserving the stemness of $Tcf1^+$ $CD8^+$ T cells during chronic viral infection [155]. Collectively, this brings into focus a complex circuitry among cell types and mediators when discussing T1–T2 relationships in severe asthma. An increase in IL-18R1 expression and downstream signaling was documented in the airways of severe asthma patients [111]. More recently, the density of intraepithelial eosinophils in asthmatic airways was shown to positively associate with IL-18R1 and IFN-γ [77]. In coculture experiments, IL-18 and IL-33 both increased the expression of *IFNG*, *IL13*, and *IL18* in eosinophils, suggesting that when epithelial cells, eosinophils, and immune cells such as ILC2s and TRM cells are in close apposition, both T1 and T2

immune responses can exist simultaneously in the peripheral tissue, and as discussed above, they do not necessarily oppose each other as evident during T cell priming and differentiation in lymphoid tissues [39]. That being said, because of the inherent tendency of one arm of the immune response to cross-regulate the other, blocking one completely may upregulate the other, as discussed in the following section.

5.2 | Implications for Therapy in Asthma

The presence of multiple pathways has implications for disease management as this can affect the response of disease to therapeutics. When two pathways are present contributing to disease, therapy targeted towards a single pathway may be ineffective for the other pathway, leading to only partial response. While T2 inflammation is classically corticosteroid responsive [6, 41], T1 inflammation is resistant to corticosteroid therapy [41, 156], decreasing the responsiveness of asthma to corticosteroid therapy. As already discussed above, the ChIP assay demonstrated GR-STAT1 cooperation at interferon response elements in the CXCL10 promoter, suggesting that corticosteroid exposure may augment T1 inflammation over time [17].

Biologic therapies in asthma primarily target components of the T2 pathway with no observed effect on T1 inflammation [7]. While tezepelumab is approved in all asthma, including T2-low, data from the NAVIGATOR study showed efficacy was tied to the degree of T2 inflammation [157]. While partial response in participants who meet biomarker criteria has been recognized for some time, a recent report examining biomarkers of clinical response to mepolizumab and omalizumab showed that negative correlations between T1 and T2 chemokines predicted response better than absolute levels, suggesting patients with inflammation in both pathways are less likely to respond to these therapies [158]. Dual pathway elevation is increasingly recognized as a potential issue with dupilumab, which significantly suppresses T2 inflammation through blockade of the IL-4Rα subunit. As T2 inflammation is able to provide a brake to T1 inflammation [38], in patients where both pathways are increased, the loss of T2 inflammation has the potential to lead to unrestrained T1 inflammation. This potential risk is observed in reports of increased incidence of T1 and IL-17 mediated diseases, including psoriasis [159] and seronegative arthritis [160, 161].

Other mechanisms may also contribute to disease management challenges when multiple pathways are present. In addition to one pathway augmenting the other pathway, as we have noted above, the potential for T1 inflammation augmenting aspects of T2 inflammation in asthma, one pathway may also impact the ability of the other pathway to respond to treatment. This possibility is illustrated in the persistence of eosinophils in bronchial biopsies and induced sputum in severe asthma despite corticosteroid therapy [62, 63]. Notably, analysis of RNAseq data from bronchial brushings in the IMSA and SARP III cohorts showed that the expression of corticosteroid response genes (upregulated in subjects on inhaled corticosteroids) positively correlated with asthma clinical outcomes, but that the presence of T1 inflammatory gene signature identified asthma subjects with poor corticosteroid gene response to ICS, suggesting an inhibitory effect [162]. Full analysis of the SARP III sputum RNAseq dataset confirmed this, showing that while T2 inflammation responded to systemic triamcinolone administration regardless of T1 status, clinical benefit from systemic corticosteroid therapy in terms of FEV1 improvement was seen only in participants who were $T1^{Low}/T2^{High}$, with no significant response in the $T1^{High}/T2^{High}$ group, again supporting an inhibitory effect of T1 inflammation on clinical response to corticosteroid therapy [109].

6 | Concluding Remarks

It is increasingly clear that despite having broad immunosuppressive functions, corticosteroids fail to temper the T1 arm in the context of a mixed T1–T2 immune response observed in the sickest of asthma patients. Thus, suppression of the T2 immune response alone is inadequate to achieve clinical benefit in patients who harbor a mixed immune response in their airways, requiring a novel approach for their treatment. One such approach is to break the critical nodes in the circuitry among multiple cell types that feed the mixed inflammatory response. As discussed in this article, increased expression of molecules such as IL18R1, CCL5, and IL-33 has the potential to incite both the T1 and T2 arms by promoting interplay between multiple cell types to fuel the asthma phenotype. It is important to note that among these molecules, IL-33 was shown to defend against viral infections by promoting $CD8^+$ T cell function. Therefore, while blocking the deleterious Th2-promoting effects of IL-33 using itepekimab would be desirable, it would also have the unintended consequence of blocking its beneficial anti-viral effect in individuals particularly prone to viral infections and ensuing asthma exacerbations. As discussed, interactions between T1 and T2 immune responses in asthma not only involve the T1 and T2 cells but also multiple other cell types and their mediators, and thus treatment of mixed T1–T2 disease needs to take into consideration both the complex effector functions of some of the key players as well as the concept of immune cross-regulation for optimal clinical response.

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Conflicts of Interest

Anuradha Ray completed a speaker agreement with Regeneron Pharmaceuticals. Marc Gauthier is a Principal Investigator of a study sponsored by Regeneron Pharmaceuticals.

Data Availability Statement

The authors have nothing to report.

References

1. M. Gauthier, A. Ray, and S. E. Wenzel, "Evolving Concepts of Asthma," *American Journal of Respiratory and Critical Care Medicine* 192, no. 6 (2015): 660–668, https://doi.org/10.1164/rccm.201504-0763PP.

2. A. P. Rabe, W. J. Loke, K. Gurjar, A. Brackley, and D. E. Lucero-Prisno Iii, "Global Burden of Asthma, and Its Impact on Specific Subgroups:

Nasal Polyps, Allergic Rhinitis, Severe Asthma, Eosinophilic Asthma," *Journal of Asthma and Allergy* 16 (2023): 1097–1113, https://doi.org/10.2147/jaa.s418145.

3. P. P. Hekking, R. R. Wener, M. Amelink, A. H. Zwinderman, M. L. Bouvy, and E. H. Bel, "The Prevalence of Severe Refractory Asthma," *Journal of Allergy and Clinical Immunology* 135, no. 4 (2015): 896–902, https://doi.org/10.1016/j.jaci.2014.08.042.

4. Global Initiative for Asthma, "2023 GINA Report, Global Strategy for Asthma Management and Prevention," updated July 2023, www.ginasthma.org.

5. H. M. Brown, "Treatment of Chronic Asthma With Prednisolone; Significance of Eosinophils in the Sputum," *Lancet* 2, no. 7059 (1958): 1245–1247.

6. P. G. Woodruff, B. Modrek, D. F. Choy, et al., "T-Helper Type 2-Driven Inflammation Defines Major Subphenotypes of Asthma," *American Journal of Respiratory and Critical Care Medicine* 180, no. 5 (2009): 388–395, https://doi.org/10.1164/rccm.200903-0392OC.

7. G. G. Brusselle and G. H. Koppelman, "Biologic Therapies for Severe Asthma," *New England Journal of Medicine* 386, no. 2 (2022): 157–171, https://doi.org/10.1056/nejmra2032506.

8. P. Flood-Page, C. Swenson, I. Faiferman, et al., "A Study to Evaluate Safety and Efficacy of Mepolizumab in Patients With Moderate Persistent Asthma," *American Journal of Respiratory and Critical Care Medicine* 176, no. 11 (2007): 1062–1071, https://doi.org/10.1164/rccm.200701-085OC.

9. S. Gerday, S. Graff, C. Moermans, et al., "Super-Responders to Anti-IL-5/Anti-IL-5R Are Characterised by High Sputum Eosinophil Counts at Baseline," *Thorax* 78, no. 11 (2023): 1138–1141, https://doi.org/10.1136/thorax-2022-219781.

10. E. S. Harvey, D. Langton, C. Katelaris, et al., "Mepolizumab Effectiveness and Identification of Super-Responders in Severe Asthma," *European Respiratory Journal* 55, no. 5 (2020): 1902420, https://doi.org/10.1183/13993003.02420-2019.

11. G. Lack, H. Renz, J. Saloga, et al., "Nebulized but Not Parenteral IFN-Gamma Decreases IgE Production and Normalizes Airways Function in a Murine Model of Allergen Sensitization," *Journal of Immunology* 152, no. 5 (1994): 2546–2554.

12. M. Boguniewicz, L. C. Schneider, H. Milgrom, et al., "Treatment of Steroid-Dependent Asthma With Recombinant Interferon-Gamma," *Clinical and Experimental Allergy* 23, no. 9 (1993): 785–790.

13. B. D. Medoff, A. Sauty, A. M. Tager, et al., "IFN-Gamma-Inducible Protein 10 (CXCL10) Contributes to Airway Hyperreactivity and Airway Inflammation in a Mouse Model of Asthma," *Journal of Immunology* 168, no. 10 (2002): 5278–5286.

14. S. H. Cho, L. A. Stanciu, S. T. Holgate, and S. L. Johnston, "Increased Interleukin-4, Interleukin-5, and Interferon-Gamma in Airway $CD4^+$ and $CD8^+$ T Cells in Atopic Asthma," *American Journal of Respiratory and Critical Care Medicine* 171, no. 3 (2005): 224–230, https://doi.org/10.1164/rccm.200310-1416OC.

15. M. Raundhal, C. Morse, A. Khare, et al., "High IFN-Gamma and Low SLPI Mark Severe Asthma in Mice and Humans," *Journal of Clinical Investigation* 125, no. 8 (2015): 3037–3050, https://doi.org/10.1172/JCI80911.

16. D. Tworek, P. Kuna, W. Mlynarski, P. Gorski, T. Pietras, and A. Antczak, "MIG (CXCL9), IP-10 (CXCL10) and I-TAC (CXCL11) Concentrations After Nasal Allergen Challenge in Patients With Allergic Rhinitis," *Archives of Medical Science: AMS* 9, no. 5 (2013): 849–853, https://doi.org/10.5114/aoms.2013.37198.

17. M. Gauthier, K. Chakraborty, T. B. Oriss, et al., "Severe Asthma in Humans and Mouse Model Suggests a CXCL10 Signature Underlies Corticosteroid-Resistant Th1 Bias. JCI," *Insight* 2, no. 13 (2017): 94580, https://doi.org/10.1172/jci.insight.94580.

18. J. J. Li, W. Wang, K. J. Baines, et al., "IL-27/IFN-Gamma Induce MyD88-Dependent Steroid-Resistant Airway Hyperresponsiveness by Inhibiting Glucocorticoid Signaling in Macrophages," *Journal of Immunology* 185, no. 7 (2010): 4401–4409, https://doi.org/10.4049/jimmunol.1001039.

19. T. B. Oriss, M. Raundhal, C. Morse, et al., "IRF5 Distinguishes Severe Asthma in Humans and Drives Th1 Phenotype and Airway Hyperreactivity in Mice. JCI," *Insight* 2, no. 10 (2017): e91019, https://doi.org/10.1172/jci.insight.91019.

20. M. J. Camiolo, X. Zhou, T. B. Oriss, et al., "High-Dimensional Profiling Clusters Asthma Severity by Lymphoid and Non-Lymphoid Status," *Cell Reports* 35, no. 2 (2021): 108974, https://doi.org/10.1016/j.celrep.2021.108974.

21. M. Gauthier, S. L. Kale, T. B. Oriss, et al., "Dual Role for CXCR3 and CCR5 in Asthmatic Type 1 Inflammation," *Journal of Allergy and Clinical Immunology* 149, no. 1 (2022): 113–124, https://doi.org/10.1016/j.jaci.2021.05.044.

22. M. Gauthier, S. L. Kale, T. B. Oriss, et al., "CCL5 Is a Potential Bridge Between Type 1 and Type 2 Inflammation in Asthma," *Journal of Allergy and Clinical Immunology* 152 (2023): 94–106, https://doi.org/10.1016/j.jaci.2023.02.028.

23. B. D. Modena, E. R. Bleecker, W. W. Busse, et al., "Gene Expression Correlated With Severe Asthma Characteristics Reveals Heterogeneous Mechanisms of Severe Disease," *American Journal of Respiratory and Critical Care Medicine* 195, no. 11 (2017): 1449–1463, https://doi.org/10.1164/rccm.201607-1407OC.

24. A. Tiotiu, Y. Badi, N. Z. Kermani, et al., "Association of Differential Mast Cell Activation With Granulocytic Inflammation in Severe Asthma," *American Journal of Respiratory and Critical Care Medicine* 205, no. 4 (2022): 397–411, https://doi.org/10.1164/rccm.202102-0355oc.

25. J. A. Wisniewski, L. M. Muehling, J. D. Eccles, et al., "T(H)1 Signatures are Present in the Lower Airways of Children With Severe Asthma, Regardless of Allergic Status," *Journal of Allergy and Clinical Immunology* 141, no. 6 (2018): 2048–2060, https://doi.org/10.1016/j.jaci.2017.08.020.

26. A. Antonelli, S. M. Ferrari, D. Giuggioli, E. Ferrannini, C. Ferri, and P. Fallahi, "Chemokine (C-X-C Motif) Ligand (CXCL)10 in Autoimmune Diseases," *Autoimmunity Reviews* 13, no. 3 (2014): 272–280, https://doi.org/10.1016/j.autrev.2013.10.010.

27. A. Antonelli, M. Rotondi, P. Fallahi, et al., "Increase of Interferon-Gamma Inducible Alpha Chemokine CXCL10 but Not Beta Chemokine CCL2 Serum Levels in Chronic Autoimmune Thyroiditis," *European Journal of Endocrinology* 152, no. 2 (2005): 171–177, https://doi.org/10.1530/eje.1.01847.

28. A. Antonelli, C. Ferri, P. Fallahi, et al., "CXCL10 (Alpha) and CCL2 (Beta) Chemokines in Systemic Sclerosis–A Longitudinal Study," *Rheumatology (Oxford, England)* 47, no. 1 (2008): 45–49, https://doi.org/10.1093/rheumatology/kem313.

29. A. Antonelli, P. Fallahi, S. M. Ferrari, et al., "Serum Th1 (CXCL10) and Th2 (CCL2) Chemokine Levels in Children With Newly Diagnosed Type 1 Diabetes: A Longitudinal Study," *Diabetic Medicine: A Journal of the British Diabetic Association* 25, no. 11 (2008): 1349–1353, https://doi.org/10.1111/j.1464-5491.2008.02577.x.

30. A. Antonelli, C. Ferri, S. M. Ferrari, M. Colaci, and P. Fallahi, "Immunopathogenesis of HCV-Related Endocrine Manifestations in Chronic Hepatitis and Mixed Cryoglobulinemia," *Autoimmunity Reviews* 8, no. 1 (2008): 18–23, https://doi.org/10.1016/j.autrev.2008.07.017.

31. Y. Lin, H. Yan, Y. Xiao, et al., "Attenuation of Antigen-Induced Airway Hyperresponsiveness and Inflammation in CXCR3 Knockout Mice," *Respiratory Research* 12 (2011): 123, https://doi.org/10.1186/1465-9921-12-123.

32. Z. Liu, H. Chen, X. Chen, J. Gao, and Z. Guo, "Characteristics of Allergic Pulmonary Inflammation in CXCR3Knockout Mice

Sensitized and Challenged With House Dust Mite Protein," *PLoS One* 11, no. 10 (2016): e0162905, https://doi.org/10.1371/journal.pone.0162905.

33. S. Qin, J. B. Rottman, P. Myers, et al., "The Chemokine Receptors CXCR3 and CCR5 Mark Subsets of T Cells Associated With Certain Inflammatory Reactions," *Journal of Clinical Investigation* 101, no. 4 (1998): 746–754, https://doi.org/10.1172/JCI1422.

34. J. J. Campbell, C. E. Brightling, F. A. Symon, et al., "Expression of Chemokine Receptors by Lung T Cells From Normal and Asthmatic Subjects," *Journal of Immunology* 166, no. 4 (2001): 2842–2848, https://doi.org/10.4049/jimmunol.166.4.2842.

35. S. Herrera-De La Mata, C. Ramírez-Suástegui, H. Mistry, et al., "Cytotoxic CD4(+) Tissue-Resident Memory T Cells Are Associated With Asthma Severity," *Med* 4, no. 12 (2023): 875–897, https://doi.org/10.1016/j.medj.2023.09.003.

36. N. Bryant, L. M. Muehling, K. Wavell, W. G. Teague, and J. A. Woodfolk, "Rhinovirus as a Driver of Airway T-Cell Dynamics in Children with Severe Asthma," 2024. *bioRxiv: The Preprint Server for Biology*, 2024.11.15.623877, https://doi.org/10.1101/2024.11.15.623877.

37. T. R. Mosmann, H. Cherwinski, M. W. Bond, M. A. Giedlin, and R. L. Coffman, "Two Types of Murine Helper T Cell Clone. I. Definition According to Profiles of Lymphokine Activities and Secreted Proteins," *Journal of Immunology* 136, no. 7 (1986): 2348–2357.

38. A. O'Garra, "Cytokines Induce the Development of Functionally Heterogeneous T Helper Cell Subsets," *Immunity* 8, no. 3 (1998): 275–283.

39. R. J. Creusot and N. A. Mitchison, "How DCs Control Cross-Regulation Between Lymphocytes," *Trends in Immunology* 25, no. 3 (2004): 126–131, https://doi.org/10.1016/j.it.2004.01.005.

40. N. R. Bhakta, S. A. Christenson, S. Nerella, et al., "Interferon-Stimulated Gene Expression, Type-2 Inflammation and Endoplasmic Reticulum Stress in Asthma," *American Journal of Respiratory and Critical Care Medicine* 197 (2017): 313–324, https://doi.org/10.1164/rccm.201706-1070OC.

41. J. V. Fahy, N. D. Jackson, S. P. Sajuthi, et al., "Asthma Severity and Corticosteroid Response Depend on Variable Type 1 and Type 2 Inflammation in the Airway," 2023. medRxiv, 2023.10. 05.23296609, https://doi.org/10.1101/2023.10.05.23296609.

42. M. L. Fajt and S. E. Wenzel, "Mast Cells, Their Subtypes, and Relation to Asthma Phenotypes," *Annals of the American Thoracic Society* 10, no. Suppl (2013): S158–S164, https://doi.org/10.1513/AnnalsATS.201303-064AW.

43. S. J. Galli, N. Gaudenzio, and M. Tsai, "Mast Cells in Inflammation and Disease: Recent Progress and Ongoing Concerns," *Annual Review of Immunology* 38 (2020): 49–77, https://doi.org/10.1146/annurev-immunol-071719-094903.

44. S. Balzar, H. W. Chu, M. Strand, and S. Wenzel, "Relationship of Small Airway Chymase-Positive Mast Cells and Lung Function in Severe Asthma," *American Journal of Respiratory and Critical Care Medicine* 171, no. 5 (2005): 431–439, https://doi.org/10.1164/rccm.200407-949OC.

45. M. C. Altman, Y. Lai, J. D. Nolin, et al., "Airway Epithelium-Shifted Mast Cell Infiltration Regulates Asthmatic Inflammation via IL-33 Signaling," *Journal of Clinical Investigation* 129, no. 11 (2019): 4979–4991, https://doi.org/10.1172/jci126402.

46. C. E. Brightling, P. Bradding, F. A. Symon, S. T. Holgate, A. J. Wardlaw, and I. D. Pavord, "Mast-Cell Infiltration of Airway Smooth Muscle in Asthma," *New England Journal of Medicine* 346, no. 22 (2002): 1699–1705, https://doi.org/10.1056/NEJMoa012705.

47. H. Milgrom, R. B. Fick, Jr., J. Q. Su, et al., "Treatment of Allergic Asthma With Monoclonal Anti-IgE Antibody. rhuMAb-E25 Study Group," *New England Journal of Medicine* 341, no. 26 (1999): 1966–1973, https://doi.org/10.1056/NEJM199912233412603.

48. W. Busse, J. Corren, B. Q. Lanier, et al., "Omalizumab, Anti-IgE Recombinant Humanized Monoclonal Antibody, for the Treatment of Severe Allergic Asthma," *Journal of Allergy and Clinical Immunology* 108, no. 2 (2001): 184–190, https://doi.org/10.1067/mai.2001.117880.

49. Y. Okayama, A. S. Kirshenbaum, and D. D. Metcalfe, "Expression of a Functional High-Affinity IgG Receptor, Fc Gamma RI, on Human Mast Cells: Up-Regulation by IFN-Gamma," *Journal of Immunology* 164, no. 8 (2000): 4332–4339, https://doi.org/10.4049/jimmunol.164.8.4332.

50. Y. Okayama, D. D. Hagaman, and D. D. Metcalfe, "A Comparison of Mediators Released or Generated by IFN-Gamma-Treated Human Mast Cells Following Aggregation of Fc Gamma RI or Fc Epsilon RI," *Journal of Immunology* 166, no. 7 (2001): 4705–4712, https://doi.org/10.4049/jimmunol.166.7.4705.

51. M. Yu, M. R. Eckart, A. A. Morgan, et al., "Identification of an IFN-Gamma/Mast Cell Axis in a Mouse Model of Chronic Asthma," *Journal of Clinical Investigation* 121, no. 8 (2011): 3133–3143, https://doi.org/10.1172/JCI43598.

52. C. S. Kuo, S. Pavlidis, M. Loza, et al., "T-Helper Cell Type 2 (Th2) and Non-Th2 Molecular Phenotypes of Asthma Using Sputum Transcriptomics in U-BIOPRED," *European Respiratory Journal* 49, no. 2 (2017): 602135, https://doi.org/10.1183/13993003.02135-2016.

53. F. L. de Pater-Huijsen, M. J. de Riemer, R. M. Reijneke, et al., "Products From Human Mast Cell Line Cells Enhance the Production of Interferon-Gamma by $CD8^+$ and $CD4^+$ T Cells," *Immunology* 106, no. 1 (2002): 11–19, https://doi.org/10.1046/j.1365-2567.2002.01411.x.

54. A. M. Dvorak, "Basophils and Mast Cells: Piecemeal Degranulation In Situ and Ex Vivo: A Possible Mechanism for Cytokine-Induced Function in Disease," *Immunology Series* 57 (1992): 169–271.

55. L. C. Denlinger, B. R. Phillips, S. Ramratnam, et al., "Inflammatory and Comorbid Features of Patients With Severe Asthma and Frequent Exacerbations," *American Journal of Respiratory and Critical Care Medicine* 195, no. 3 (2017): 302–313, https://doi.org/10.1164/rccm.201602-0419OC.

56. D. B. Price, A. Rigazio, J. D. Campbell, et al., "Blood Eosinophil Count and Prospective Annual Asthma Disease Burden: A UK Cohort Study," *Lancet Respiratory Medicine* 3, no. 11 (2015): 849–858, https://doi.org/10.1016/S2213-2600(15)00367-7.

57. E. M. Dunican, B. M. Elicker, D. S. Gierada, et al., "Mucus Plugs in Patients With Asthma Linked to Eosinophilia and Airflow Obstruction," *Journal of Clinical Investigation* 128, no. 3 (2018): 997–1009, https://doi.org/10.1172/jci95693.

58. C. Leung, M. Tang, B. K. Huang, et al., "A Novel Air Trapping Segment Score Identifies Opposing Effects of Obesity and Eosinophilia on Air Trapping in Asthma," *American Journal of Respiratory and Critical Care Medicine* 209, no. 10 (2024): 1196–1207, https://doi.org/10.1164/rccm.202305-0802oc.

59. H. M. Brown, "Asthma, Allergy and Steroids," *British Journal of Clinical Practice* 15 (1961): 1001–1017.

60. R. H. Green, C. E. Brightling, S. McKenna, et al., "Asthma Exacerbations and Sputum Eosinophil Counts: A Randomised Controlled Trial," *Lancet* 360, no. 9347 (2002): 1715–1721, https://doi.org/10.1016/S0140-6736(02)11679-5.

61. L. A. Laitinen, A. Laitinen, and T. Haahtela, "A Comparative Study of the Effects of an Inhaled Corticosteroid, Budesonide, and a β2-Agonist, Terbutaline, on Airway Inflammation in Newly Diagnosed Asthma: A Randomized, Double-Blind, Parallel-Group Controlled Trial," *Journal of Allergy and Clinical Immunology* 90, no. 1 (1992): 32–42, https://doi.org/10.1016/S0091-6749(06)80008-4.

62. S. E. Wenzel, L. B. Schwartz, E. L. Langmack, et al., "Evidence That Severe Asthma Can Be Divided Pathologically Into Two Inflammatory Subtypes With Distinct Physiologic and Clinical Characteristics," *American Journal of Respiratory and Critical Care Medicine* 160, no. 3 (1999): 1001–1008, https://doi.org/10.1164/ajrccm.160.3.9812110.

63. A. Jatakanon, C. Uasuf, W. Maziak, S. Lim, K. F. Chung, and P. J. Barnes, "Neutrophilic Inflammation in Severe Persistent Asthma," *American Journal of Respiratory and Critical Care Medicine* 160, no. 5 (1999): 1532–1539, https://doi.org/10.1164/ajrccm.160.5.9806170.

64. W. G. Teague, M. G. Lawrence, D.-A. T. Shirley, et al., "Lung Lavage Granulocyte Patterns and Clinical Phenotypes in Children With Severe, Therapy-Resistant Asthma," *Journal of Allergy and Clinical Immunology: In Practice* 7, no. 6 (2019): 1803–1812, https://doi.org/10.1016/j.jaip.2018.12.027.

65. R. Warringa, R. Schweizer, T. Maikoe, P. Kuijper, P. Bruijnzeel, and L. Koendermann, "Modulation of Eosinophil Chemotaxis by Interleukin-5," *American Journal of Respiratory Cell and Molecular Biology* 7 (1992): 631–636.

66. Y. Yamaguchi, Y. Hayashi, Y. Sugama, et al., "Highly Purified Murine Interleukin 5 (IL-5) Stimulates Eosinophil Function and Prolongs In Vitro Survival. IL-5 as an Eosinophil Chemotactic Factor," *Journal of Experimental Medicine* 167, no. 5 (1988): 1737–1742.

67. L. Perez-de-Llano, G. Scelo, T. N. Tran, et al., "Exploring Definitions and Predictors of Severe Asthma Clinical Remission After Biologic Treatment in Adults," *American Journal of Respiratory and Critical Care Medicine* 210, no. 7 (2024): 869–880, https://doi.org/10.1164/rccm.202311-2192OC.

68. K. Ochiai, E. Tanabe, C. Ishihara, et al., "Role of JAK2 Signal Transductional Pathway in Activation and Survival of Human Peripheral Eosinophils by Interferon-Gamma (IFN-γ)," *Clinical and Experimental Immunology* 118, no. 3 (2001): 340–343, https://doi.org/10.1046/j.1365-2249.1999.01068.x.

69. P. Lacy, S. Mahmudi-Azer, B. Bablitz, et al., "Rapid Mobilization of Intracellularly Stored RANTES in Response to Interferon-γ in Human Eosinophils," *Blood* 94, no. 1 (1999): 23–32, https://doi.org/10.1182/blood.v94.1.23.413k13_23_32.

70. A. Dolitzky, G. Shapira, S. Grisaru-Tal, et al., "Transcriptional Profiling of Mouse Eosinophils Identifies Distinct Gene Signatures Following Cellular Activation," *Frontiers in Immunology* 12 (2021): 802839, https://doi.org/10.3389/fimmu.2021.802839.

71. T. Jinquan, C. Jing, H. H. Jacobi, et al., "CXCR3 Expression and Activation of Eosinophils: Role of IFN-Gamma-Inducible Protein-10 and Monokine Induced by IFN-Gamma," *Journal of Immunology* 165, no. 3 (2000): 1548–1556.

72. D. Miotto, P. Christodoulopoulos, R. Olivenstein, et al., "Expression of IFN-Gamma-Inducible Protein; Monocyte Chemotactic Proteins 1, 3, and 4; and Eotaxin in TH1- and TH2-Mediated Lung Diseases," *Journal of Allergy and Clinical Immunology* 107, no. 4 (2001): 664–670, https://doi.org/10.1067/mai.2001.113524.

73. P. A. Wark, F. Bucchieri, S. L. Johnston, et al., "IFN-Gamma-Induced Protein 10 Is a Novel Biomarker of Rhinovirus-Induced Asthma Exacerbations," *Journal of Allergy and Clinical Immunology* 120, no. 3 (2007): 586–593, https://doi.org/10.1016/j.jaci.2007.04.046.

74. T. Hasegawa, T. Okazawa, H. Uga, H. Kurata, and A. Mori, "Serum CXCL9 as a Potential Marker of Type 1 Inflammation in the Context of Eosinophilic Asthma," *Allergy* 74, no. 12 (2019): 2515–2518, https://doi.org/10.1111/all.13924.

75. Y. Takaku, K. Nakagome, T. Kobayashi, K. Hagiwara, M. Kanazawa, and M. Nagata, "IFN-Gamma-Inducible Protein of 10 kDa Upregulates the Effector Functions of Eosinophils Through beta2 Integrin and CXCR3," *Respiratory Research* 12 (2011): 138, https://doi.org/10.1186/1465-9921-12-138.

76. A. Kanda, V. Driss, N. Hornez, et al., "Eosinophil-Derived IFN-γ Induces Airway Hyperresponsiveness and Lung Inflammation in the Absence of Lymphocytes," *Journal of Allergy and Clinical Immunology* 124, no. 3 (2009): 573–582, https://doi.org/10.1016/j.jaci.2009.04.031.

77. R. C. Murphy, Y. Lai, M. Liu, et al., "Distinct Epithelial-Innate Immune Cell Transcriptional Circuits Underlie Airway Hyperresponsiveness in Asthma," *American Journal of Respiratory and Critical Care Medicine* 207, no. 12 (2023): 1565–1575, https://doi.org/10.1164/rccm.202209-1707OC.

78. C. Mesnil, S. Raulier, G. Paulissen, et al., "Lung-Resident Eosinophils Represent a Distinct Regulatory Eosinophil Subset," *Journal of Clinical Investigation* 126, no. 9 (2016): 3279–3295, https://doi.org/10.1172/jci85664.

79. C. C. López, A. S. Santos, A. L. Castellano, et al., "Eosinophil Subtypes in Adults With Asthma and Adults With Chronic Obstructive Pulmonary Disease," *American Journal of Respiratory and Critical Care Medicine* 208, no. 2 (2023): 155–162, https://doi.org/10.1164/rccm.202301-0149OC.

80. R. Bonecchi, N. Polentarutti, W. Luini, et al., "Up-Regulation of CCR1 and CCR3 and Induction of Chemotaxis to CC Chemokines by IFN-Gamma in Human Neutrophils," *Journal of Immunology* 162, no. 1 (1999): 474–479.

81. L. Tien Nguyen, S. Lim, T. Oates, and K. F. Chung, "Increase in Airway Neutrophils After Oral but Not Inhaled Corticosteroid Therapy in Mild Asthma," *Respiratory Medicine* 99, no. 2 (2005): 200–207, https://doi.org/10.1016/j.rmed.2004.06.007.

82. A. Ray and J. K. Kolls, "Neutrophilic Inflammation in Asthma and Association With Disease Severity," *Trends in Immunology* 38 (2017): 942–954, https://doi.org/10.1016/j.it.2017.07.003.

83. S. Sur, T. B. Crotty, G. M. Kephart, et al., "Sudden-Onset Fatal Asthma: A Distinct Entity With Few Eosinophils and Relatively More Neutrophils in the Airway Submucosa?," *American Review of Respiratory Disease* 148, no. 3 (1993): 713–719, https://doi.org/10.1164/ajrccm/148.3.713.

84. N. Krishnamoorthy, D. N. Douda, T. R. Brüggemann, et al., "Neutrophil Cytoplasts Induce T(H)17 Differentiation and Skew Inflammation Toward Neutrophilia in Severe Asthma," *Science Immunology* 3, no. 26 (2018): eaao4747, https://doi.org/10.1126/sciimmunol.aao4747.

85. Y. Li, T. Yang, and B. Jiang, "Neutrophil and Neutrophil Extracellular Trap Involvement in Neutrophilic Asthma: A Review," *Medicine* 103, no. 34 (2024): e39342, https://doi.org/10.1097/md.0000000000039342.

86. M. E. Lachowicz-Scroggins, E. M. Dunican, A. R. Charbit, et al., "Extracellular DNA, Neutrophil Extracellular Traps, and Inflammasome Activation in Severe Asthma," *American Journal of Respiratory and Critical Care Medicine* 199, no. 9 (2019): 1076–1085, https://doi.org/10.1164/rccm.201810-1869oc.

87. P. Chacón, A. Vega-Rioja, B. Doukkali, et al., "Human Neutrophils Couple Nitric Oxide Production and Extracellular Trap Formation in Allergic Asthma," *American Journal of Respiratory and Critical Care Medicine* 210, no. 5 (2024): 593–606, https://doi.org/10.1164/rccm.202305-0889oc.

88. M. Mukherjee, D. C. Bulir, K. Radford, et al., "Sputum Autoantibodies in Patients With Severe Eosinophilic Asthma," *Journal of Allergy and Clinical Immunology* 141, no. 4 (2018): 1269–1279, https://doi.org/10.1016/j.jaci.2017.06.033.

89. M. Mukherjee, D. F. Forero, S. Tran, et al., "Suboptimal Treatment Response to Anti-IL-5 Monoclonal Antibodies in Severe Eosinophilic Asthmatics With Airway Autoimmune Phenomena," *European Respiratory Journal* 56, no. 4 (2020): 2000117, https://doi.org/10.1183/13993003.00117-2020.

90. A. T. Hastie, W. C. Moore, D. A. Meyers, et al., "Analyses of Asthma Severity Phenotypes and Inflammatory Proteins in Subjects Stratified by Sputum Granulocytes," *Journal of Allergy and Clinical Immunology* 125, no. 5 (2010): 1028–1036, https://doi.org/10.1016/j.jaci.2010.02.008.

91. A. T. Hastie, D. T. Mauger, L. C. Denlinger, et al., "Baseline Sputum Eosinophil + Neutrophil subgroups' Clinical Characteristics and Longitudinal Trajectories for NHLBI Severe Asthma Research Program (SARP 3) Cohort," *Journal of Allergy and Clinical Immunology* 146, no. 1 (2020): 222–226, https://doi.org/10.1016/j.jaci.2020.01.039.

92. M. Buggert, D. A. Price, L. K. Mackay, and M. R. Betts, "Human Circulating and Tissue-Resident Memory CD8⁺ T Cells," *Nature Immunology* 24, no. 7 (2023): 1076–1086, https://doi.org/10.1038/s41590-023-01538-6.

93. F. R. Carbone, "Unique Properties of Tissue-Resident Memory T Cells in the Lungs: Implications for COVID-19 and Other Respiratory Diseases," *Nature Reviews. Immunology* 23, no. 5 (2023): 329–335, https://doi.org/10.1038/s41577-022-00815-z.

94. D. L. Farber, N. A. Yudanin, and N. P. Restifo, "Human Memory T Cells: Generation, Compartmentalization and Homeostasis," *Nature Reviews. Immunology* 14, no. 1 (2014): 24–35, https://doi.org/10.1038/nri3567.

95. M. E. Snyder, M. O. Finlayson, T. J. Connors, et al., "Generation and Persistence of Human Tissue-Resident Memory T Cells in Lung Transplantation," *Science Immunology* 4, no. 33 (2019): eaav5581, https://doi.org/10.1126/sciimmunol.aav5581.

96. W. Wang, C. Garcia, F. Shao, et al., "Lung Dopaminergic Nerves Facilitate the Establishment of T(H)2 Resident Memory Cells in Early Life," *Journal of Allergy and Clinical Immunology* 152, no. 2 (2023): 386–399, https://doi.org/10.1016/j.jaci.2023.02.011.

97. T. Sathaliyawala, M. Kubota, N. Yudanin, et al., "Distribution and Compartmentalization of Human Circulating and Tissue-Resident Memory T Cell Subsets," *Immunity* 38, no. 1 (2013): 187–197, https://doi.org/10.1016/j.immuni.2012.09.020.

98. J. J. Thome, N. Yudanin, Y. Ohmura, et al., "Spatial Map of Human T Cell Compartmentalization and Maintenance Over Decades of Life," *Cell* 159, no. 4 (2014): 814–828, https://doi.org/10.1016/j.cell.2014.10.026.

99. N. Lam, Y. Lee, and D. L. Farber, "A Guide to Adaptive Immune Memory," *Nature Reviews. Immunology* 24, no. 11 (2024): 810–829, https://doi.org/10.1038/s41577-024-01040-6.

100. J. R. Teijaro, D. Turner, Q. Pham, E. J. Wherry, L. Lefrancois, and D. L. Farber, "Cutting Edge: Tissue-Retentive Lung Memory CD4 T Cells Mediate Optimal Protection to Respiratory Virus Infection," *Journal of Immunology* 187, no. 11 (2011): 5510–5514, https://doi.org/10.4049/jimmunol.1102243.

101. M. A. Luangrath, M. E. Schmidt, S. M. Hartwig, and S. M. Varga, "Tissue-Resident Memory T Cells in the Lungs Protect Against Acute Respiratory Syncytial Virus Infection," *Immunohorizons* 5, no. 2 (2021): 59–69, https://doi.org/10.4049/immunohorizons.2000067.

102. J. Zhao, J. Zhao, A. K. Mangalam, et al., "Airway Memory CD4⁺ T Cells Mediate Protective Immunity Against Emerging Respiratory Coronaviruses," *Immunity* 44, no. 6 (2016): 1379–1391, https://doi.org/10.1016/j.immuni.2016.05.006.

103. S. L. Hayward, C. D. Scharer, E. K. Cartwright, et al., "Environmental Cues Regulate Epigenetic Reprogramming of Airway-Resident Memory CD8⁺ T Cells," *Nature Immunology* 21, no. 3 (2020): 309–320, https://doi.org/10.1038/s41590-019-0584-x.

104. T. Wu, Y. Hu, Y. T. Lee, et al., "Lung-Resident Memory CD8 T Cells (TRM) are Indispensable for Optimal Cross-Protection Against Pulmonary Virus Infection," *Journal of Leukocyte Biology* 95, no. 2 (2014): 215–224, https://doi.org/10.1189/jlb.0313180.

105. A. Jozwik, M. S. Habibi, A. Paras, et al., "RSV-Specific Airway Resident Memory CD8⁺ T Cells and Differential Disease Severity After Experimental Human Infection," *Nature Communications* 6 (2015): 10224, https://doi.org/10.1038/ncomms10224.

106. C. Ge, I. R. Monk, A. Pizzolla, et al., "Bystander Activation of Pulmonary Trm Cells Attenuates the Severity of Bacterial Pneumonia by Enhancing Neutrophil Recruitment," *Cell Reports* 29, no. 13 (2019): 4236–4244, https://doi.org/10.1016/j.celrep.2019.11.103.

107. J. E. Kohlmeier, T. Cookenham, A. D. Roberts, and S. C. Miller, "Woodland DL. Type I Interferons Regulate Cytolytic Activity of Memory CD8⁺ T Cells in the Lung Airways During Respiratory Virus Challenge," *Immunity* 33, no. 1 (2010): 96–105, https://doi.org/10.1016/j.immuni.2010.06.016.

108. J. S. Low, Y. Farsakoglu, M. C. Amezcua Vesely, et al., "Tissue-Resident Memory T Cell Reactivation by Diverse Antigen-Presenting Cells Imparts Distinct Functional Responses," *Journal of Experimental Medicine* 217, no. 8 (2020): e20192291, https://doi.org/10.1084/jem.20192291.

109. J. V. Fahy, N. D. Jackson, S. P. Sajuthi, et al., "Asthma Severity and Corticosteroid Response Depend on Variable Type 1 and Type 2 Inflammation in the Airway," 2023. medRxiv, 2023.10.05.23296609, https://doi.org/10.1101/2023.10.05.23296609.

110. S. V. Akkenepally, D. J. K. Yombo, S. Yerubandi, et al., "Interleukin 31 Receptor α Promotes Smooth Muscle Cell Contraction and Airway Hyperresponsiveness in Asthma," *Nature Communications* 14 (2023): 8207, https://doi.org/10.1038/s41467-023-44040-1.

111. M. J. Camiolo, X. Zhou, Q. Wei, et al., "Machine Learning Implicates the IL-18 Signaling Axis in Severe Asthma," *JCI Insight* 6, no. 21 (2021): e149945, https://doi.org/10.1172/jci.insight.149945.

112. M. K. Kennedy, M. Glaccum, S. N. Brown, et al., "Reversible Defects in Natural Killer and Memory CD8 T Cell Lineages in Interleukin 15-Deficient Mice," *Journal of Experimental Medicine* 191, no. 5 (2000): 771–780, https://doi.org/10.1084/jem.191.5.771.

113. C. C. Ku, M. Murakami, A. Sakamoto, J. Kappler, and P. Marrack, "Control of Homeostasis of CD8⁺ Memory T Cells by Opposing Cytokines," *Science* 288, no. 5466 (2000): 675–678, https://doi.org/10.1126/science.288.5466.675.

114. G. Desdin-Mico, G. Soto-Heredero, J. F. Aranda, et al., "T Cells With Dysfunctional Mitochondria Induce Multimorbidity and Premature Senescence," *Science* 368, no. 6497 (2020): 1371–1376, https://doi.org/10.1126/science.aax0860.

115. T. J. Break, V. Oikonomou, N. Dutzan, et al., "Aberrant Type 1 Immunity Drives Susceptibility to Mucosal Fungal Infections," *Science* 371, no. 6526 (2021): eaay5731, https://doi.org/10.1126/science.aay5731.

116. D. E. Davies, J. Wicks, R. M. Powell, S. M. Puddicombe, and S. T. Holgate, "Airway Remodeling in Asthma: New Insights," *Journal of Allergy and Clinical Immunology* 111, no. 2 (2003): 215–225, https://doi.org/10.1067/mai.2003.128.

117. J. D. Davis and T. P. Wypych, "Cellular and Functional Heterogeneity of the Airway Epithelium," *Mucosal Immunology* 14, no. 5 (2021): 978–990, https://doi.org/10.1038/s41385-020-00370-7.

118. N. Sun, I. Ogulur, Y. Mitamura, et al., "The Epithelial Barrier Theory and Its Associated Diseases," *Allergy* 79 (2024): 3192–3237, https://doi.org/10.1111/all.16318.

119. H. Wan, H. L. Winton, C. Soeller, et al., "Der p 1 Facilitates Transepithelial Allergen Delivery by Disruption of Tight Junctions," *Journal of Clinical Investigation* 104, no. 1 (1999): 123–133, https://doi.org/10.1172/jci5844.

120. S. L. Kale, K. Agrawal, S. N. Gaur, and N. Arora, "Cockroach Protease Allergen Induces Allergic Airway Inflammation via Epithelial Cell Activation," *Scientific Reports* 7 (2017): 42341, https://doi.org/10.1038/srep42341.

121. B. N. Lambrecht and H. Hammad, "Taking Our Breath Away: Dendritic Cells in the Pathogenesis of Asthma," *Nature Reviews Immunology* 3, no. 12 (2003): 994–1003, https://doi.org/10.1038/nri1249.

122. M. Utech, A. I. Ivanov, S. N. Samarin, et al., "Mechanism of IFN-Gamma-Induced Endocytosis of Tight Junction Proteins: Myosin II-Dependent Vacuolarization of the Apical Plasma Membrane," *Molecular Biology of the Cell* 16, no. 10 (2005): 5040–5052, https://doi.org/10.1091/mbc.e05-03-0193.

123. N. Voraphani, M. T. Gladwin, A. U. Contreras, et al., "An Airway Epithelial iNOS-DUOX2-Thyroid Peroxidase Metabolome Drives Th1/

Th2 Nitrative Stress in Human Severe Asthma," *Mucosal Immunology* 7, no. 5 (2014): 1175–1185, https://doi.org/10.1038/mi.2014.6.

124. C. S. Kuo, S. Pavlidis, M. Loza, et al., "A Transcriptome-Driven Analysis of Epithelial Brushings and Bronchial Biopsies to Define Asthma Phenotypes in U-BIOPRED," *American Journal of Respiratory and Critical Care Medicine* 195, no. 4 (2017): 443–455, https://doi.org/10.1164/rccm.201512-2452OC.

125. U. M. Zissler, A. M. Chaker, R. Effner, et al., "Interleukin-4 and Interferon-γ Orchestrate an Epithelial Polarization in the Airways," *Mucosal Immunology* 9, no. 4 (2016): 917–926, https://doi.org/10.1038/mi.2015.110.

126. Z. Wang, T. Zheng, Z. Zhu, et al., "Interferon Gamma Induction of Pulmonary Emphysema in the Adult Murine Lung," *Journal of Experimental Medicine* 192, no. 11 (2000): 1587–1600, https://doi.org/10.1084/jem.192.11.1587.

127. F. Y. Jin, C. Nathan, D. Radzioch, and A. Ding, "Secretory Leukocyte Protease Inhibitor: A Macrophage Product Induced by and Antagonistic to Bacterial Lipopolysaccharide," *Cell* 88, no. 3 (1997): 417–426, https://doi.org/10.1016/s0092-8674(00)81880-2.

128. B. Ma, M. J. Kang, C. G. Lee, et al., "Role of CCR5 in IFN-Gamma-Induced and Cigarette Smoke-Induced Emphysema," *Journal of Clinical Investigation* 115, no. 12 (2005): 3460–3472, https://doi.org/10.1172/jci24858.

129. G. S. Ashcroft, K. Lei, W. Jin, et al., "Secretory Leukocyte Protease Inhibitor Mediates Non-Redundant Functions Necessary for Normal Wound Healing," *Nature Medicine* 6, no. 10 (2000): 1147–1153, https://doi.org/10.1038/80489.

130. D. W. Cockcroft and B. E. Davis, "Airway Hyperresponsiveness as a Determinant of the Early Asthmatic Response to Inhaled Allergen," *Journal of Asthma* 43, no. 3 (2006): 175–178, https://doi.org/10.1080/02770900600566348.

131. B. J. Marsland, K. Yadava, and L. P. Nicod, "The Airway Microbiome and Disease," *Chest* 144, no. 2 (2013): 632–637, https://doi.org/10.1378/chest.12-2854.

132. R. P. Dickson, J. R. Erb-Downward, C. M. Freeman, et al., "Bacterial Topography of the Healthy Human Lower Respiratory Tract," *MBio* 8, no. 1 (2017): e02287-16, https://doi.org/10.1128/mBio.02287-16.

133. M. Teo Shu, D. Mok, K. Pham, et al., "The Infant Nasopharyngeal Microbiome Impacts Severity of Lower Respiratory Infection and Risk of Asthma Development," *Cell Host & Microbe* 17, no. 5 (2015): 704–715, https://doi.org/10.1016/j.chom.2015.03.008.

134. S. M. Teo, H. H. F. Tang, D. Mok, et al., "Airway Microbiota Dynamics Uncover a Critical Window for Interplay of Pathogenic Bacteria and Allergy in Childhood Respiratory Disease," *Cell Host & Microbe* 24, no. 3 (2018): 341–352, https://doi.org/10.1016/j.chom.2018.08.005.

135. A. A. T. M. Bosch, W. A. A. de Steenhuijsen Piters, M. A. van Houten, et al., "Maturation of the Infant Respiratory Microbiota, Environmental Drivers, and Health Consequences. A Prospective Cohort Study," *American Journal of Respiratory and Critical Care Medicine* 196, no. 12 (2017): 1582–1590, https://doi.org/10.1164/rccm.201703-0554OC.

136. Y. J. Huang, S. Nariya, J. M. Harris, et al., "The Airway Microbiome in Patients With Severe Asthma: Associations With Disease Features and Severity," *Journal of Allergy and Clinical Immunology* 136, no. 4 (2015): 874–884, https://doi.org/10.1016/j.jaci.2015.05.044.

137. G. D. Kitsios, M. J. Morowitz, R. P. Dickson, G. B. Huffnagle, B. J. McVerry, and A. Morris, "Dysbiosis in the Intensive Care Unit: Microbiome Science Coming to the Bedside," *Journal of Critical Care* 38 (2017): 84–91, https://doi.org/10.1016/j.jcrc.2016.09.029.

138. J. Durack, H. A. Boushey, and S. V. Lynch, "Airway Microbiota and the Implications of Dysbiosis in Asthma," *Current Allergy and Asthma Reports* 16 (2016): 1–13.

139. K. F. Chung, "Airway Microbial Dysbiosis in Asthmatic Patients: A Target for Prevention and Treatment?," *Journal of Allergy and Clinical Immunology* 139, no. 4 (2017): 1071–1081.

140. I. Hanski, L. von Hertzen, N. Fyhrquist, et al., "Environmental Biodiversity, Human Microbiota, and Allergy Are Interrelated," *National Academy of Sciences of the United States of America* 109, no. 21 (2012): 8334–8339.

141. S. L. Taylor, L. E. X. Leong, J. M. Choo, et al., "Inflammatory Phenotypes in Patients With Severe Asthma Are Associated With Distinct Airway Microbiology," *Journal of Allergy and Clinical Immunology* 141, no. 1 (2018): 94–103, https://doi.org/10.1016/j.jaci.2017.03.044.

142. E. Goleva, L. P. Jackson, J. K. Harris, et al., "The Effects of Airway Microbiome on Corticosteroid Responsiveness in Asthma," *American Journal of Respiratory and Critical Care Medicine* 188, no. 10 (2013): 1193–1201.

143. V. Yadav, S. Gaisford, H. A. Merchant, and A. W. Basit, "Colonic Bacterial Metabolism of Corticosteroids," *International Journal of Pharmaceutics* 457, no. 1 (2013): 268–274.

144. J. Durack, S. V. Lynch, S. Nariya, et al., "Features of the Bronchial Bacterial Microbiome Associated With Atopy, Asthma, and Responsiveness to Inhaled Corticosteroid Treatment," *Journal of Allergy and Clinical Immunology* 140, no. 1 (2017): 63–75.

145. D. R. Denner, N. Sangwan, J. B. Becker, et al., "Corticosteroid Therapy and Airflow Obstruction Influence the Bronchial Microbiome, Which is Distinct From That of Bronchoalveolar Lavage in Asthmatic Airways," *Journal of Allergy and Clinical Immunology* 137, no. 5 (2016): 1398–1405.

146. S. L. Taylor, L. E. X. Leong, F. M. Mobegi, et al., "Long-Term Azithromycin Reduces Haemophilus Influenzae and Increases Antibiotic Resistance in Severe Asthma," *American Journal of Respiratory and Critical Care Medicine* 200, no. 3 (2019): 309–317, https://doi.org/10.1164/rccm.201809-1739OC.

147. P. A. Greenberger, R. K. Bush, J. G. Demain, A. Luong, R. G. Slavin, and A. P. Knutsen, "Allergic Bronchopulmonary Aspergillosis," *Journal of Allergy and Clinical Immunology: In Practice* 2, no. 6 (2014): 703–708, https://doi.org/10.1016/j.jaip.2014.08.007.

148. M. T. Stier, M. H. Bloodworth, S. Toki, et al., "Respiratory Syncytial Virus Infection Activates IL-13-Producing Group 2 Innate Lymphoid Cells Through Thymic Stromal Lymphopoietin," *Journal of Allergy and Clinical Immunology* 138, no. 3 (2016): 814–824, https://doi.org/10.1016/j.jaci.2016.01.050.

149. S. J. Van Dyken, A. Mohapatra, J. C. Nussbaum, et al., "Chitin Activates Parallel Immune Modules That Direct Distinct Inflammatory Responses via Innate Lymphoid Type 2 and γδ T Cells," *Immunity* 40, no. 3 (2014): 414–424, https://doi.org/10.1016/j.immuni.2014.02.003.

150. H. C. Lee, M. B. Headley, Y. M. Loo, et al., "Thymic Stromal Lymphopoietin Is Induced by Respiratory Syncytial Virus-Infected Airway Epithelial Cells and Promotes a Type 2 Response to Infection," *Journal of Allergy and Clinical Immunology* 130, no. 5 (2012): 1187–1196, https://doi.org/10.1016/j.jaci.2012.07.031.

151. I. Martinez-Gonzalez, L. Mathä, C. A. Steer, M. Ghaedi, G. F. Poon, and F. Takei, "Allergen-Experienced Group 2 Innate Lymphoid Cells Acquire Memory-Like Properties and Enhance Allergic Lung Inflammation," *Immunity* 45, no. 1 (2016): 198–208, https://doi.org/10.1016/j.immuni.2016.06.017.

152. J. S. Silver, J. Kearley, A. M. Copenhaver, et al., "Inflammatory Triggers Associated With Exacerbations of COPD Orchestrate Plasticity of Group 2 Innate Lymphoid Cells in the Lungs," *Nature Immunology* 17, no. 6 (2016): 626–635, https://doi.org/10.1038/ni.3443.

153. Y. Ohne, J. S. Silver, L. Thompson-Snipes, et al., "IL-1 Is a Critical Regulator of Group 2 Innate Lymphoid Cell Function and Plasticity," *Nature Immunology* 17, no. 6 (2016): 646–655, https://doi.org/10.1038/ni.3447.

154. G. Murdaca, F. Paladin, A. Tonacci, et al., "Involvement of Il-33 in the Pathogenesis and Prognosis of Major Respiratory Viral Infections: Future Perspectives for Personalized Therapy," *Biomedicine* 10, no. 3 (2022): 715, https://doi.org/10.3390/biomedicines10030715.

155. W. V. Bonilla, A. Fröhlich, K. Senn, et al., "The Alarmin Interleukin-33 Drives Protective Antiviral $CD8^+$ T Cell Responses," *Science* 335, no. 6071 (2012): 984–989, https://doi.org/10.1126/science.1215418.

156. D. O'Connell, B. Bouazza, B. Kokalari, et al., "IFN-Gamma-Induced JAK/STAT, but Not NF-kappaB, Signaling Pathway Is Insensitive to Glucocorticoid in Airway Epithelial Cells," *American Journal of Physiology. Lung Cellular and Molecular Physiology* 309, no. 4 (2015): L348–L359, https://doi.org/10.1152/ajplung.00099.2015.

157. A. Menzies-Gow, J. Corren, A. Bourdin, et al., "Tezepelumab in Adults and Adolescents With Severe, Uncontrolled Asthma," *New England Journal of Medicine* 384, no. 19 (2021): 1800–1809, https://doi.org/10.1056/nejmoa2034975.

158. A. Akenroye, T. Nopsopon, J. J. Hacker, and T. M. Laidlaw, "Ratio of Plasma IL-13/TNF-∝and CXCL10/CCL17 Predicts Mepolizumab and Omalizumab Response in Asthma Better Than Eosinophil Count or Immunoglobulin E Level," *Scientific Reports* 14, no. 1 (2024): 10404, https://doi.org/10.1038/s41598-024-60864-3.

159. C. M. Brumfiel, M. H. Patel, and M. J. Zirwas, "Development of Psoriasis During Treatment With Dupilumab: A Systematic Review," *Journal of the American Academy of Dermatology* 86, no. 3 (2022): 708–709, https://doi.org/10.1016/j.jaad.2021.05.013.

160. Z. N. Willsmore, R. T. Woolf, C. Hughes, et al., "Development of Inflammatory Arthritis and Enthesitis in Patients on Dupilumab: A Case Series," *British Journal of Dermatology* 181, no. 5 (2019): 1068–1070, https://doi.org/10.1111/bjd.18031.

161. C. Bridgewood, M. Wittmann, T. Macleod, et al., "T Helper 2 IL-4/IL-13 Dual Blockade With Dupilumab Is Linked to Some Emergent T Helper 17–Type Diseases, Including Seronegative Arthritis and Enthesitis/Enthesopathy, but Not to Humoral Autoimmune Diseases," *Journal of Investigative Dermatology* 142, no. 10 (2022): 2660–2667, https://doi.org/10.1016/j.jid.2022.03.013.

162. S. P. Ginebaugh, M. Hagner, A. Ray, et al., "Bronchial Epithelial Cell Transcriptional Responses to Inhaled Corticosteroids Dictate Severe Asthmatic Outcomes," *Journal of Allergy and Clinical Immunology* 151, no. 6 (2023): 1513–1524, https://doi.org/10.1016/j.jaci.2023.01.028.