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Review

The basic immunology of asthma

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SUMMARY

In many asthmatics, chronic airway inflammation is driven by IL-4-, IL-5-, and IL-13-producing Th2 cells or ILC2s. Type 2 cytokines promote hallmark features of the disease such as eosinophilia, mucus hypersecretion, bronchial hyperresponsiveness (BHR), IgE production, and susceptibility to exacerbations. However, only half the asthmatics have this "type 2-high" signature, and "type 2-low" asthma is more associated with obesity, presence of neutrophils, and unresponsiveness to corticosteroids, the mainstay asthma therapy. Here, we review the underlying immunological basis of various asthma endotypes by discussing results obtained from animal studies as well as results generated in clinical studies targeting specific immune pathways.

Asthma is a chronic inflammatory disease of the airways leading to cough, wheeze, shortness of breath, and chest tightness. Asthma symptoms are driven by the inflammation of the airways, which triggers processes such as mucus production, remodeling of the airway wall, and bronchial hyperresponsiveness (BHR, which is the tendency of smooth muscle cells to react to nonspecific stimuli such as cold air. Asthma often starts at a young age (childhood-onset asthma), but some patients can develop asthma later in life (late-onset asthma). Childhood-onset and late-onset asthma differ in many ways. Late-onset asthma is more severe and less associated with allergy than childhood-onset asthma. In children, atopy, lower-lung function, and respiratory-tract infections especially with rhinovirus, represent major risk factors for the persistence of asthma. Whether the underlying inflammation present in asthmatic children drives the pathogenicity of respiratory viruses or whether frequent viral infections at young age set the stage for asthma to develop is still not understood. Because inflammation is so central in asthma pathogenesis, it is not surprising that the primary goal of asthma treatment has been to achieve the control of symptoms and underlying inflammation to avoid future disease exacerbations.

Unsupervised clustering methodology to look at the disease course and clinical features of asthma has revealed that this is a heterogeneous disease, reflected by major patient-specific differences in age of onset, associated risk factors, and degrees of severity, comorbidity, and response to treatment (Wu et al., 2019). We also realize now that not all asthma patients have reversible airway obstruction, and persistent airway obstruction caused by airway-wall remodeling and mucus plugging of the airways is now seen as one of the biggest unmet medical needs of clinical asthma care (Dunican et al., 2018). Historically, only two main forms of asthma have been identified: allergic and nonallergic asthma, but this has turned out to be an oversimplification.

Allergic asthma tends to begin in childhood and is associated with T helper 2 (Th2) cell responses, which are also seen in other allergic conditions such as atopic dermatitis or allergic rhinitis. This form of asthma is induced by early life encounters with environmental allergens such as house dust mite (HDM), pollen, cockroach, or animal dander but can also be induced later in life when a new, e.g., occupational allergen, is encountered. Upon recognizing allergens, allergen-specific Th2 cells produce type 2 cytokines (interleukin [IL]-4, IL-5, IL-9, and IL-13), that lead to the accumulation of high numbers of eosinophils in the airway wall, mucus overproduction, and synthesis of immunoglobulin E (IgE) by allergen-specific B cells, which can be detected in the serum or through a positive skin-prick test. Although the mechanisms and environmental risk and protective factors behind allergic sensitization in childhood are well understood and extensively modeled in mice (reviewed in Lambrecht and Hammad, 2017), why the disease localizes to the airways, and persists into adulthood, is less clear. Disease onset coincides, however, with a very crucial period of immune system and lung structural development in early childhood. Lifelong homeostasis and susceptibility to immune-mediated diseases such as asthma are shaped during the neonatal period. Therefore, alterations in the lung environment during this "window of opportunity" could lead to changes in immune cell and organ behavior, that persist long after the initial trigger is gone (de Kleer et al., 2016a; Saglani et al., 2018). In contrast to allergic asthma, non-allergic asthma is usually late onset, is more common in females and in obese patients (Pakkasela et al., 2020), and can sometimes be very difficult to treat. Late-onset asthma phenotypes were classified into Th2 and non-Th2 late-onset asthma. The non-Th2 form is often associated with obesity, aging, and smoking. The Th2-associated form is often accompanied by recurrent and chronic rhinosinusitis with nasal polyps (CRSwNP) and with sensitivity to aspirin and can be associated with high eosinophil numbers in the airways (Bachert et al., 2020).

As with many chronic inflammatory diseases, clinicians now realize that the division of asthma into just two clinical forms

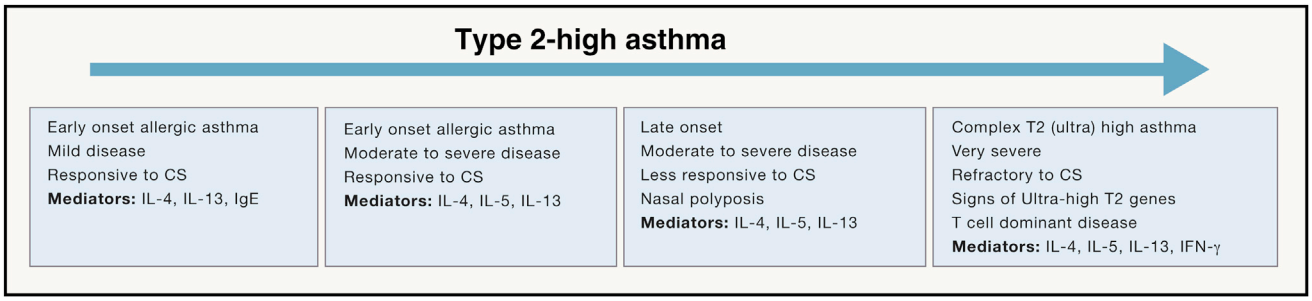

Figure 1. Schematic of our current understanding of type 2-high asthma
Type 2 asthma is characterized by high production of IL-4, IL-5, and IL-13, which leads to asthma features with the accumulation of type 2-associated cells such as eosinophils and mast cells in lung tissue and to mucus production. Four asthma phenotypes have been identified, ranging from mild allergic early-onset to severe late-onset Th2 high to ultra-high asthma.

has been an oversimplification. In the last years, the classification of asthma phenotypes has evolved into asthma endotypes such as the type 2-high or -ultra-high (essentially eosinophilic; see Figure 1) and type 2-low (non-eosinophilic, sometimes neutrophilic, and metabolic; see Figure 2; Peters et al., 2019). Endotypes are defined by underlying pathophysiological mechanisms, which might lead to direct differences in responsiveness to common therapies such as inhaled corticosteroids or specific biologicals (Fahy, 2015; Lötvall et al., 2011; Lambrecht et al., 2019). As such, the type 2-high endotype is orchestrated by Th2-associated cytokines such as IL-4, IL-5, and IL-13, with ultra-type 2-high asthma reflecting a more severe form of the disease (Figure 1). The type 2-low endotype is more complex and no biomarkers have been identified so far. Therefore, type 2-low asthma generally includes all asthmatic patients with no type 2-high inflammation (Figure 2).

In this review, we will focus on the underlying immunological basis of the various asthma endotypes, highlighting articles where authors imply causality through rigorous experimentation. We will discuss results obtained from animal studies in which molecular pathways have been unraveled in great molecular detail and results generated in clinical studies targeting particular pathways using molecule-specific biologics.

PATHOPHYSIOLOGY OF ASTHMA

The main feature of asthma is airway obstruction, which is caused by a reduction in the diameter of the airways. The narrowing of the airways is mediated by chronic inflammation of the airway wall, characterized by the infiltration and activation of immune cells such as dendritic cells (DCs), eosinophils, neutrophils, lymphocytes, innate lymphoid cells (ILCs), and mast cells. A complex interplay between these immune cell types and with neighboring structural cells such as epithelial cells leads to the development of asthma features such as BHR which, in most cases, is reversible by the use of bronchodilators. However, in more severe forms of asthma, airway obstruction does not always normalize following therapy. In such patients, the development of long-lasting mucus plugs in smaller airways might explain the fixed airway obstruction (Dunican et al., 2018). In addition, in patients with severe asthma, other mechanisms, including airway remodeling (which consists of airway smooth muscle hyperplasia, goblet cell metaplasia, and exaggerated subepithelial collagen deposition), might also contribute greatly to the pathogenesis of asthma.

TYPE 2-HIGH ASTHMA IS CONTROLLED BY AIRWAY EPITHELIAL CELLS

Our understanding on the role of epithelial cells (ECs) in asthma has advanced greatly in the last years. Nowadays, ECs are considered as more than just a physical barrier between the body and the outside world and are recognized as immunologically active cells that can orchestrate inflammatory responses to external triggers. In asthma, the epithelial cell barrier is altered by the loss of tight junction proteins that keep them together, leading to increased permeability. Allergens such as HDM or cockroach, endowed with enzymatic activities, are able to cleave intercellular junctions leading to the loss of cell-cell contacts (Wan et al., 1999). Epithelial cell damage is actually a feature present in all phenotypes of asthma and correlates with disease severity (Papi et al., 2018). Moreover, changes in EC functions are seen at very young age (Carsin et al., 2016), leading to the idea that ECs may contribute in some way to the initiation of asthma early in life.

Epithelial-derived cytokines contribute to type 2-high asthma

Lung ECs express a myriad of pattern recognition receptors such as toll-like receptors (TLRs), nucleotide-binding oligomerization do-main (NOD)-like receptors (NLRs), C-type lectin receptors (CLRs), retinoic acid-inducible gene (RIG)-I-like receptors (RLRs), protease-activated receptors, and purinergic receptors. These receptors allow ECs to respond to a variety of external triggers by producing chemokines and cytokines (Figure 3). In mice, allergen exposure is able to trigger the production of IL-1α, granulocyte-macrophage colony-stimulating factor (GM-CSF) (Willart et al., 2012), macrophage colony stimulating factor (M-CSF) (Moon et al., 2018), and transforming growth factor β (TGF-β) (Denney et al., 2015) by ECs, but the best-studied epithelial-derived cytokines in the context of asthma are IL-33, thymic stromal lymphopoietin (TSLP), and IL-25. These "alarmins" are produced by ECs in response to the allergens HDM, *Aspergillus fumigatus*, and the cat dander protein Feld1, mainly

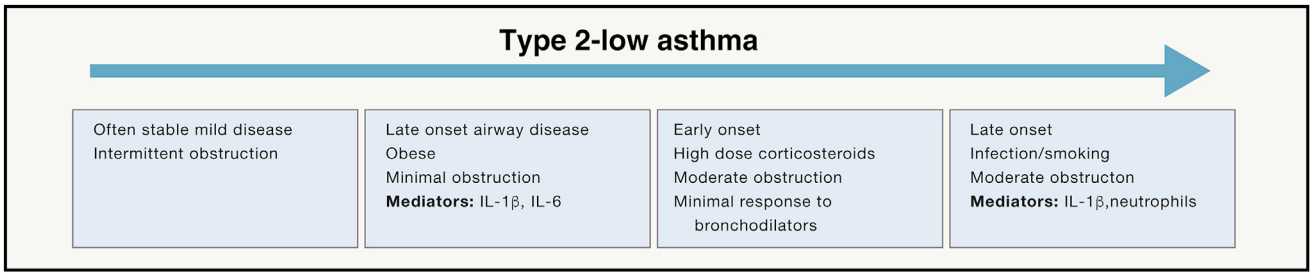

Figure 2. Schematic of our current understanding of type 2-low asthma
Type 2-low asthma is characterized by lack of type 2 biomarkers, presence of neutrophils, obesity, and/or unresponsiveness to corticosteroids. Different phenotypes with a broad range of severity have been identified.

in a TLR4/Myd88-dependent manner (Cayrol et al., 2018; Hammad et al., 2009; Willart et al., 2012). All these epithelial-derived cytokines can contribute to the establishment of Th2 responses in murine models of eosinophilic asthma (Lambrecht et al., 2019).

In humans, the exact contribution of these epithelial-derived alarmins to asthma is difficult to assess, but certainly they are also present in patients with different phenotypes of asthma. IL-33 and IL1RL1, encoding the IL-33 receptor ST2, are among the most highly replicated susceptibility loci for asthma (Moffatt et al., 2010). IL-33 is increased in epithelial cells and bronchoalveolar lavages from asthmatic patients and correlates with disease severity (Li et al., 2018). Patients with allergic eosinophilic asthma have increased serum IL-33 levels and higher ST2 expression on blood and suptum eosinophils compared to non-allergic and non-eosinophilic phenotypes (Gasiuniene et al., 2019).

IL-25 transcripts and proteins are elevated in type 2-high asthmatic patients, and patients with higher levels of IL-25 had greater airway hyperresponsiveness, more airway and blood eosinophils, higher serum IgE, more subepithelial thickening, and higher expression of Th2 signature genes (Cheng et al., 2014).

TSLP mRNA expression is also increased in the airway mucosa of asthmatic patients and correlates with disease severity (Ying et al., 2005). It was shown that TSLP could contribute to ILC2 longevity and be responsible for their resistance to corticoid treatment (Kabata et al., 2013). Although classically considered as a Th2-associated cytokine, TSLP expression in bronchoalveolar lavages has been shown to also correlate with neutrophilic inflammation (Mitchell et al., 2018).

Epithelial cell-dendritic cell interactions are required in asthma

Our understanding of the mode of action of these epithelial-derived cytokines mainly comes from murine models. Indeed, the absence of epithelial-derived cytokines or cytokine signaling in these models failed to lead to Th2 immunity to allergens. It was shown that the way epithelial-derived cytokines participate in Th2 immunity in the lung comes from their ability to activate a number of immune cells involved in asthma, such as eosinophils, Th2 cells, mast cells, basophils (Chan et al., 2019), but also ILC2s and DCs (Roan et al., 2019; Figure 3).

The initiation of Th2 responses in the lungs and in other organs has been attributed to a subset of conventional DCs (conventional type 2 or cDC2s) that require IRF4 for their development (Deckers et al., 2017). Several epithelial-derived cytokines, such as IL-33, TSLP, IL-1α, or GM-CSF, have the ability to target SIRPα$^+$CD11b$^+$ cDC2s involved in T helper 2 cell differentiation (Moon et al., 2020; Willart et al., 2012). On the contrary, bona fide lung XCR1$^+$CD103$^+$ cDC1s and monocyte-derived DCs are not able to induce Th2 responses and were even shown to protect from asthma development through the production of the Th1-associated cytokine IL-12 that can inhibit Th2 responses (Conejero et al., 2017).

To be able to induce proper Th2 immunity, cDC2s that have been exposed to epithelial-derived cytokines need to migrate from the lung tissue to the draining lymph nodes, a process potentially controlled by ILC2-derived IL-13 and by type I interferon (IFN) (Halim et al., 2014; Webb et al., 2017). The question of why type I IFN, normally associated with anti-microbial responses, has evolved to Th2 immune responses remains unclear. Results from our lab have shown that, following exposure to Th2-inducing triggers such as allergen or helminths, type I IFN induces a very peculiar subset of inflammatory cDC2s that has hybrid characteristics of cDC1s (IRF8 expression) and monocyte-derived cells (CD64 expression) (Bosteels et al., 2020). The exact contribution of this subset to the initiation of Th2 immunity needs to be clarified, since experimental data clearly identify CD64$^-$ cDC2s and not inflammatory CD64$^+$ cDC2s as the driver of Th2 cell differentiation (Bosteels et al., 2020).

DCs have an important role not only in sensitization to allergens but also in ongoing asthma both in humans and in mice. The number of DCs is increased in the airways of patients with asthma. In the lung, most conventional CD1c-expressing DCs found in type 2-high patients express the high-affinity receptor FcεRI, suggesting a role for IgE and these DCs in Th2 airway inflammation, and suggesting they are highly related to cDC2s (Dutertre et al., 2019; Naessens et al., 2020). The difficulty of obtaining lung tissue samples from patients with ongoing asthma has made functional studies on DCs difficult. However, in a recent study, sputum was used as a proxy for the lung compartment, and the Siebold group has identified inflammatory CD11b$^+$IRF4$^+$CD103$^-$ DCs expressing thymic stromal lymphopoietin (TSLPR) and type 2-associated chemokines in patients with severe type 2-high asthma (Peters et al., 2019). This finding therefore provides a rationale for targeting CD11b$^+$ cDC2s in patients with type 2-high asthma. However, whether these cells require type I IFN to maintain type 2

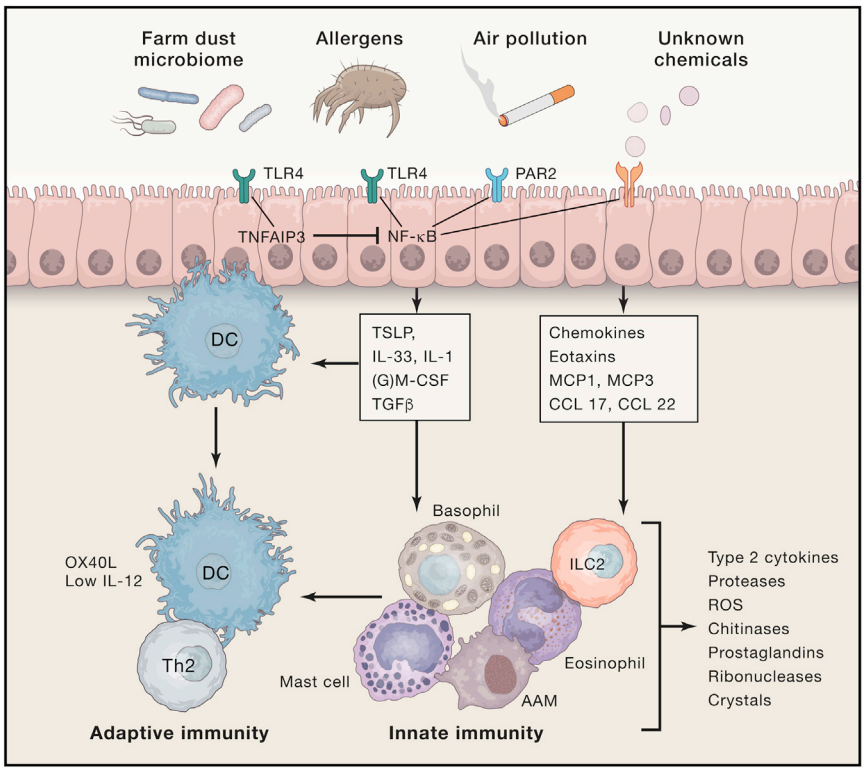

Figure 3. Early innate immune response driving asthma

Most allergens and many air pollutants trigger production by epithelial cell of cytokines and chemokines, through activation of toll-like receptors (TLRs) or protease-activated receptors (PARs). This then leads to activation of dendritic cells (DCs) that migrate to the draining nodes to promote Th2 development, and to activation of innate immune cells that get recruited to the airways, and subsequently produce many mediators that contribute to airway inflammation. Cytokines such as thymic stromal lymphopoietin (TSLP), interleukin-33 (IL-33) and granulocyte-macrophage colony stimulating factor (GM-CSF), and chemokines such as monocyte chemokine proteins (MCPs), eotaxins, and C-C chemokines (CCL17 and CCL22) are all potential drug targets for new biologicals. Some environmental exposures such as chronic exposure to farm dust can suppress the airway epithelial response to allergens because these induce expression of a negative regulator of nuclear factor kB (NF-kB) activation called TNF-a induced protein-3 (TNFAIP3). In this way, chronic farm dust exposure can suppress manifestations of asthma.

immunity and are related to an inflammatory cDC2 subset identified in murine models remains to be addressed. In mice, early experiments have shown that the depletion of CD11c$^+$ DCs during ongoing allergen challenge suppressed all features of asthma (van Rijt et al., 2005). Despite yielding important results, one caveat with this strategy of CD11c$^+$ cell depletion is that it affects all subsets of lung DCs. Therefore, the exact contribution of DC subsets to inflammation in ongoing asthma still needs to be addressed using more cell-specific depletion strategies, or by purifying DC subsets to look at their gene-expression profiles. Using the latter strategy, our group has found that monocyte-derived DCs, which accumulate after an allergen challenge, were producing chemokines involved in the attraction of Th2 cells and eosinophils to the lungs but were not good at promoting Th2 cell differentiation (Bosteels et al., 2020; Plantinga et al., 2013). However, a recent paper proposed that cDC1s were the drivers of eosinophil accumulation in the lung following repeated exposure to allergens (Yi et al., 2018). How each subset exactly contributes to the maintenance of asthma features still needs to be addressed both in patients and in murine models of the disease.

ADAPTIVE TYPE 2 IMMUNITY IN ASTHMA

All asthma patients with airway eosinophilia, reflected by high sputum and/or blood eosinophilia, are now said to be type 2-high patients, and some endotypes of asthma even have an ultra-type 2-high immune response (Peters et al., 2019). Within the type 2-high subtype, the major pathways involved in airway inflammation (i.e., IL-4, IL-13, and IL-5 signaling) are driven by T helper type 2 (Th2) cells, which are induced or restimulated in the lung by DCs under the influence of epithelial-derived cytokines. Such effector Th2 cells have been shown to arise from IL-4-committed T follicular helper cells upon allergen challenge in mice (Ballesteros-Tato et al., 2016). Whether such a differentiation path of Th2 cells also exist in humans is not known.

Th2 cells and their cytokines control type 2-high asthma

The idea that eosinophilic asthma is a Th2-driven disease comes from several observations. First, allergen-specific Th2 cells and their associated cytokines are clearly present in the bronchoalveolar lavage of asthmatic patients and mice with allergic eosinophilic asthma (Coquet et al., 2015; Robinson et al., 1992; Tibbitt et al., 2019). Then, circulating allergen-specific Th2 cells producing IL-4, IL-5, IL-13, and even IL-9 can be found in the blood of allergic asthmatic patients (Seumois et al., 2020). In addition, early studies performed in murine models of type 2-high asthma induced by the inhaled antigen ovalbumin (OVA) have demonstrated that the depletion of CD4$^+$ T cells prevented asthma development and that, on the opposite side, the adoptive transfer of *in vitro*-polarized Th2 cells from mice with transgenic expression of an OVA peptide-specific T cell antigen receptor (TCR) lead to the induction of asthma features (Cohn et al., 1997). Interestingly, Th2 cells obtained from humans with type 2-high asthma and from murine models of allergic eosinophilic asthma show similar patterns of gene expression. These cells produce high levels of Th2 cytokines, express specific receptors recognizing epithelial-derived signals, and, unexpectedly, are characterized by the expression of the nuclear receptor PPAR-γ (Tibbitt et al., 2019). PPAR-γ in Th2 cells seems to be crucial to drive the pathogenicity of these cells since it contributes to Th2 cytokine production, by upregulating the expression of the IL-33 receptor (Chen et al., 2017).

Although ovalbumin-induced models are not very physiological (sensitization is achieved by intraperitoneal administration of the protein emulsified in alum), they are characterized by a pure Th2 response (IL-4, IL-5, and IL-13 production and presence of antigen-specific IgE), and they have allowed us to obtain a detailed picture of Th2 cell effector functions. Studies in these models have led to the finding that, by acting on the IL-4Rα, IL-4 was shown to induce IgE immunoglobulin class switching by B cell, to promote bronchial hyperresponsiveness, and induce the expression of adhesion molecules such as ICAM-1 and VCAM1 that allow the extravasation of inflammatory cells such as eosinophils into inflamed lungs (Godar et al., 2018). Many of these effects are also induced by IL-13 that also uses the IL-4Rα for signaling (Godar et al., 2018). Whereas IL-13 was originally thought to not be involved in stimulating IgE class-switching, recently an IL-13-producing T follicular helper (T_{FH}) cell subset boosting anaphylactic IgE was identified in mice and humans (Gowthaman et al., 2019). Studies in mice also showed that IL-5 drives the recruitment and the activation of eosinophils, but its effect on driving BHR has been controversial (Corry et al., 1996).

IL-9: A real player in type 2-high asthma?

IL-9 is the most enigmatic type 2 effector cytokine in asthma. It is produced by a multitude of cells including highly differentiated Th2 cells in allergic asthmatic patients (Seumois et al., 2020), Th9 cells (Veldhoen et al., 2008), and human eosinophils and neutrophils (Gounni et al., 2000; Sun et al., 2018). In humans, studies have shown increased IL-9 expression in bronchoalveolar lavages from patients with asthma (Erpenbeck et al., 2003) and by PBMCs from patients with allergic eosinophilic asthma (Seumois et al., 2020). However, the function of IL-9 in type 2-high asthma is unknown. In mice, the role of IL-9 has been studied more effectively. In murine models of type 2-high asthma, IL-9 seems to be a critical player in allergic airway inflammation since the administration of blocking antibodies in the airways or the use of IL-9-deficient mice reduced all the features of asthma, including BHR (Du et al., 2020). On the contrary, the administration of Th9 cells to mice induced asthma features (bronchial hyperresponsiveness, eosinophilia, and mucus production) similar to a transfer of Th2 cells (Staudt et al., 2010). Experiments in mice have shown a clear role for IL-9 in driving several pathological features of asthma. More evidence on its role in patients with type 2-high asthma is still needed.

Resident memory T cells: The new players in asthma

In mice and in humans, memory lymphocytes consist of circulating (central memory and effector memory) and non-circulating cells (resident memory located in peripheral tissues). Mainly $CD4^+$ resident memory T (Trm) cells have been studied in the context of eosinophilic asthma. These cells reside in the lung very long after cessation of allergen exposure and can be found in the lungs of mice subjected to experimental asthma and in patients with type 2-high asthma (Hondowicz et al., 2016; Vieira Braga et al., 2019). Trm cells produce more Th2 cytokines that circulating Th2 cells and get reactivated rapidly *in situ* after re-exposure to allergens, although the mechanism of allergen-specific Trm cell reactivation in tissues is still not very clear. A possible hypothesis is that this could be mediated by $CD11b^+$ dendritic cells (DCs) presenting the antigen locally without migration to the lymph nodes. Given the heterogeneity of lung DCs (Plantinga et al., 2013), it is not clear whether these $CD11b^+$ DCs are conventional DCs or monocyte-derived cells.

One outstanding question is whether effector Th2 cells and $CD4^+$ Trm cells perform overlapping functions or whether each cell population can contribute differently to asthma pathogenesis. An interesting feature of allergen-specific lung Trm cells is that, at least in murine models, they are sufficient to induce features of asthma. Indeed, experiments performed in mice showed that reducing the number of circulating memory cells while keeping the pool of lung Trm cells intact was sufficient to induce several features of the disease (Hondowicz et al., 2016; Vieira Braga et al., 2019). This was mainly explained by the difference in localization of circulating and resident-memory T cells in the lung. Circulating memory Th2 cells were found to preferentially localize in the parenchyma and were responsible for eosinophil and T cell recruitment to the lung. Trm cells were mainly found near the airways and induced mucus production, eosinophil activation, and bronchial hyperresponsiveness (Rahimi et al., 2020). These data are in line with the fact that expression of the IL-33 receptor, ST2, was found on lung Trm cells in mice (Bošnjak et al., 2019) and suggest that Trm cells could be directly affected by epithelial-derived IL-33 to perform at least some of their functions. Moreover, $ST2^+$ memory Th2 have also been shown to persist in lymphoid structures under the influence of IL-33-producing lymphatic endothelial cell and immunofibroblasts, which could also produce a favorable niche for Trm cell survival (Shinoda et al., 2016). The field of Trm cell biology is still in its infancy and the use of specific mouse models allowing to target Trm cells would help to pinpoint their exact contribution to asthma pathogenesis. Moreover, more data on Trm cells in human asthma are needed to confirm whether or not they share, at least, some similarities with murine Trm cells regarding their localization and functions.

CONTRIBUTION OF B CELLS AND IgE TO ASTHMA

After Th2 cells have been generated in lung-draining lymph nodes, part of them will interact locally with B cells, which will mature into plasma cells and antibody-producing cells. Under the influence of the Th2 cytokines IL-4 and IL-13, B cells will preferentially produce IgE. Although IgE production mainly takes place in secondary lymphoid organs, there is evidence that this event can also happen in the lung mucosa of patients with asthma (so-called local IgE production) (Manise et al., 2013).

The role of IgE is very complex and is linked to its ability to affect several immune and structural cells involved in allergic asthma. IgE can bind to the high-affinity FcεRI and the low-affinity CD23 receptors. FcεRI is expressed on basophils, mast cells, eosinophils, and DCs but also on airway smooth muscle cells, endothelial cells, and epithelial cells (Redhu and Gounni, 2013). In asthma, IgE is mainly produced from a pool of memory IgG-positive B cells that class switch to IgE and become long-lived plasma cells in response to IL-4 and/or IL-13 derived from T_{FH} cells (Hoof et al., 2020).

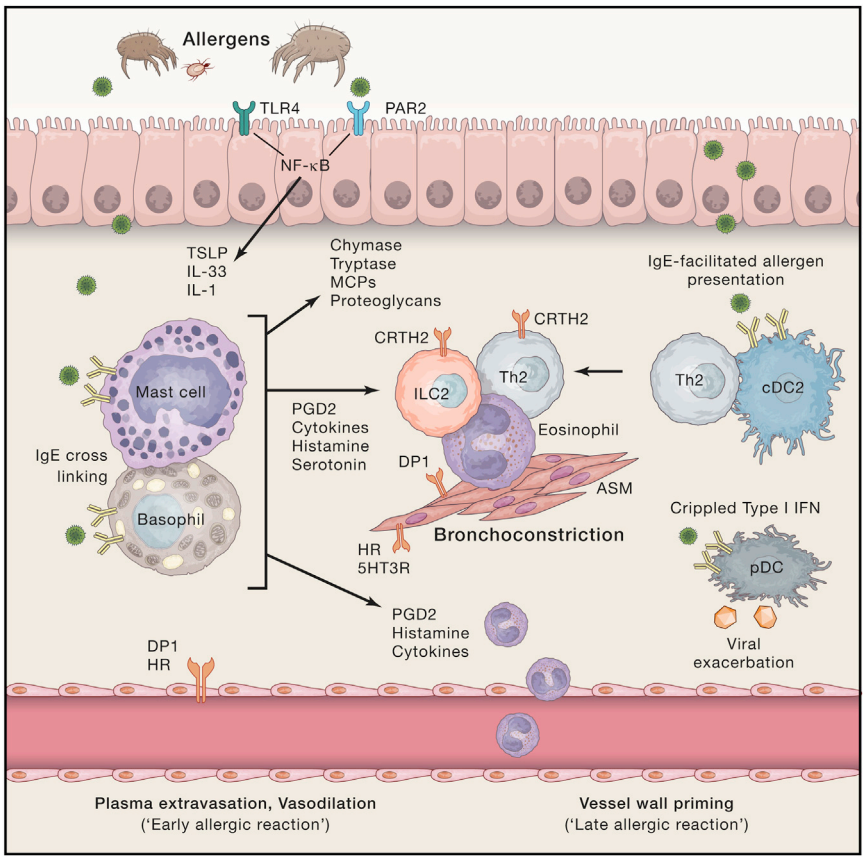

Figure 4. IgE effector functions in asthma
When allergens crosslink allergen-specific IgE on mast cells and basophils, this leads to immediate degranulation and release of many mediators such as prostaglandin D2 (PGD2) that can act on the type 1 prostaglandin D2 receptor (DP1) receptor or the CRTH2 (chemokine receptor expressed by Th2 cells, a.k.a.DP2) receptor or serotonine acting on the HT5R to cause inflammation and immediate bronchoconstriction, in the so-called early asthmatic response. Histamine will also cause bronchoconstriction and will cause plasma leakage from nearby vessels. Cytokines released with PGD2 will prepare the vessel wall for later extravasation of leukocytes, and in this way mast cells and basophils can contribute to the late-phase response as well. Crosslinkining of IgE on antigen-presenting type 2 conventional DCs (cDC2s) leads to improved antigen uptake and better presentation of Th2 cells that further contribute to the late allergic response by producing cytokines and activating the recruited cell types. When allergens crosslink IgE on plasmacytoid DCs (pDCs), however, this leads to suppressed capacity of these cells to produce type I interferons (IFN), thus contributing to the increased susceptibility of asthmatics to respiratory viral infections that cause exacerbations of disease.

Effects of IgE on mast cells and basophils

The interaction between IgE and FcεRI on mast cells and basophils is an important part of the allergic cascade. The crosslinking of two adjacent IgE molecules by the allergen activates mast cells and basophils to release biologically active preformed mediators such as histamine and neutral proteases such as tryptase and chymase. They also produce large amounts of lipid mediators (cysteinyl leukotriens [CysLT] or prostaglandin D2 [PGD2]) as well as Th2-associated cytokines (IL-4, IL-5, IL-13, IL-9), all reinforcing the pro-inflammatory Th2 environment present (Figure 4). The interaction of IgE with mast cells and basophils is responsible for the rapid phase of the allergic response, which is characterized by an increased vascular permeability and increased cellular recruitment in the lung. In addition, in the airways, mast cells localize near the submucosal mucous glands, and the release of PGD2, LTC4, IL-4, and IL-13 can trigger mucus hyperproduction by goblet cells (Carter and Bradding, 2011). Finally, the localization of mast cells in airway smooth muscles is a key feature in the pathogenesis of asthma and contributes to smooth muscle hypertrophy and hyperplasia, and to the establishment of BHR (Elieh Ali Komi and Bjermer, 2019).

IgE: More than basophil and mast cell activation

Over the years, it has become obvious that IgE mediates more than just the early phase of the allergic response. The two receptors for IgE are expressed by airway smooth muscle cells. As such, airway smooth muscle cells can also respond directly to IgE by producing cytokines and chemokines but also by proliferating and contracting, therefore contributing to airway hyperreactivity (Ferreira et al., 2018; Figure 4).

In addition, IgE can also bind to FcεRI on DCs and facilitate allergen presentation to memory Th2 lymphocytes. IgE-mediated allergen presentation actually decreases the threshold to mount allergen-specific Th2 cell responses (Khan and Grayson, 2010; Figure 4). This, in turn, leads to an increased production of allergen-specific IgE by B cells, and this vicious cycle contributes to pathogenic mechanisms in asthma. Another way IgE contributes to asthma pathogenesis through DCs is through its ability to hamper plasmacytoid DC functions when binding FcεRI. Plasmacytoid DCs are cells specialized in type I IFN production and play an important role in anti-viral responses. By binding on plasmacytoid DCs, IgE reduces intracellular type 1 IFN signaling (Schroeder et al., 2005), which could have detrimental effects upon encounter with respiratory viruses during which type I IFNs are crucial for viral clearance (Figure 4).

EOSINOPHILS: MORE THAN A MARKER OF TYPE 2-HIGH ASTHMA

Development and recruitment of eosinophils

Eosinophils develop in the bone marrow from $CD34^+$ stem cells. The commitment of these stem cells to the eosinophil lineage requires expression of specific transcription factors, namely, GATA-1, PU.1, and CCAAT/enhancing binding protein (a.k.a C/EBP). Eosinophil progenitors mature in the bone marrow and acquire IL-5R expression before being released into blood vessels. After entering the circulation, and in the absence of inflammation,

eosinophils either stay in the bone marrow or enter various peripheral tissues where they will exert homeostatic functions including maintenance of plasma cells or metabolic homeostasis (Wu et al., 2011).

IL-5 represents the key cytokine for mediating maturation of eosinophils in the bone marrow. IL-5 is not a chemoattractant but mobilizes eosinophils from the bone marrow during allergic inflammation. IL-5 is also driving activation, proliferation, and survival of eosinophils in peripheral tissues. In general, CCR3, the receptor for eotaxin, along with expression of several adhesion molecules such as VCAM1, allow the recruitment of eosinophils from the bloodstream into inflamed tissues.

Different eosinophil populations in tissues

In humans, two distinct population of eosinophils, normodense and hypodense, have been identified based on their respective density. The hypodense population is more activated and is increased in the blood and BAL of asthmatic patients. Moreover, hypodense eosinophils are sensitive to corticosteroid treatments (Kuo et al., 1994).

In mice, similar as in humans, two subsets of eosinophils have been described based on the inflammatory status of the organ. In the absence of inflammation, IL-5-independent resident eosinophils (Siglec F^{int} $CD101^{low}$) are present in the parenchyma. Following HDM exposure, IL-5-dependent inflammatory eosinophils (Siglec F^{int} $CD101^{low}$) are found around the airways. Resident eosinophils are related to human normodense eosinophils, whereas inflammatory eosinophils are related to human hyposense eosinophils (Mesnil et al., 2016).

Role of eosinophils in asthma pathogenesis

It is now clear from the literature that eosinophils exert both immunomodulatory and pro-inflammatory functions. In type 2-high asthma, eosinophils are recruited from the bloodstream to the lung where they get activated by IL-5 released by Th2 cells. Activated eosinophils might exert their biological effects in the lung through a myriad of factors including cytotoxic proteins (e.g., major binding protein [MBP], eosinophil peroxidase [EPO], eosinophil cationic protein [ECP], and eosinophil-derived neurotoxin [EDN]), Th2 cytokines (IL-4, IL-5, IL-9, IL-13, and IL-25), acute proinflammatory cytokines (e.g., tumor necrosis factor [TNF]-α, IL-1β, IL-6, and IL-8), chemokines, and lipid mediators (e.g., leukotriene C4). All these factors contribute to several features of asthma including BHR and goblet cell metaplasia. Moreover, persistent airway inflammation associated with eosinophils leads to continued damage to lung structural cells induced by the release of cytotoxic granule proteins (MBP, EPO, EDN) (Cañas et al., 2018). Eosinophil-associated fibrogenic factors (such as TGF-β) will, in turn, lead to airway remodeling characterized by smooth muscle thickening, goblet cell metaplasia, and extracellular matrix protein deposition (Kanda et al., 2020). Moreover, the localization of activated eosinophils and the release of granule content near airway nerves can also change the tone of parasympathetic and sensory nerves and promote BHR (Drake et al., 2018).

Role of extracellular traps and eosinophil-derived crystals in asthma

Upon appropriate stimulation, eosinophils can form extracellular traps (EETs), which are extracellular DNA fibers (Ueki et al., 2016). Although they have an important function in immunity against extracellular pathogens, EETs and extracellular histone-rich DNA are also contributing to the pathogenesis of asthma. Indeed, peripheral EET-forming eosinophils are elevated in severe asthmatics and can activate lung epithelial cells to produce IL-33 and TSLP (Choi et al., 2020). In eosinophils, EET formation is also strongly linked with the production of Charcot-Leyden crystals (CLCs) (Persson et al., 2019). These are bipyramidal crystals made of the protein Galectin-10, one of the most abundant cytoplasmic proteins of eosinophils (Su, 2018). CLC crystals and soluble galectin-10 are found in increased amounts in asthmatic patients (Nyenhuis et al., 2019) and CLCs have been mostly described inside mucus plugs (Persson et al., 2019). It is believed that the presence of EET together with CLCs could profoundly alter the rheology of the mucus, turning it into a more elastic gel that is harder to cough up. In mouse models, CLCs were found to be immunogenic and promoted the development of Th2 immunity and were shown to represent an interesting therapeutic target since their dissolution using a specific antibody prevented Th2 immunity, IgE synthesis and mucus production in a humanized mouse model (Persson et al., 2019). In CRSwNP, CLCs can also be abundantly found. Here, crystals seem to promote neutrophil recruitment and induction of NETosis in neutrophils (Gevaert et al., 2020). The crystals therefore seem to lodge in a mucus hydrogel that is stuck in the airways, which could be a very localized trigger for creating a favorable niche for type 2 immune cells to persist for long times.

Despite the increased attention these eosinophil-derived crystals have received these last years, many questions still remain as to their precise function and evolutionary development. Do these crystals contribute to asthma or are they just a marker for the presence of eosinophils? Do these crystals elicit any type of response from immune or neighboring structural cells in the lung? Are they present in all patients with type 2-high asthma or only in more severe cases who are refractory to treatment? More research on the contribution of these CLCs to asthma is warranted to address these aspects.

ILC2s: DOES THEIR FUNCTION OVERLAP WITH TH2 CELLS IN TYPE 2-HIGH ASTHMA?

Characterization and activation of ILC2s

Natural ILCs mainly reside in mucosal tissues such as the gastrointestinal tract, the reproductive tract, and the respiratory tract, where they contribute to homeostasis, immunosurveillance, immunoregulation, and tissue repair (Ricardo-Gonzalez et al., 2018). In contrast to these resident ILCs, inflammatory ILCs can respond to microbes, helminths, and allergens by cytokine production within a few hours and migrate from one site of exposure to another. ILCs have a phenotype close to the one of lymphocytes but lack the expression of TCR, BCR, and any myeloid lineage

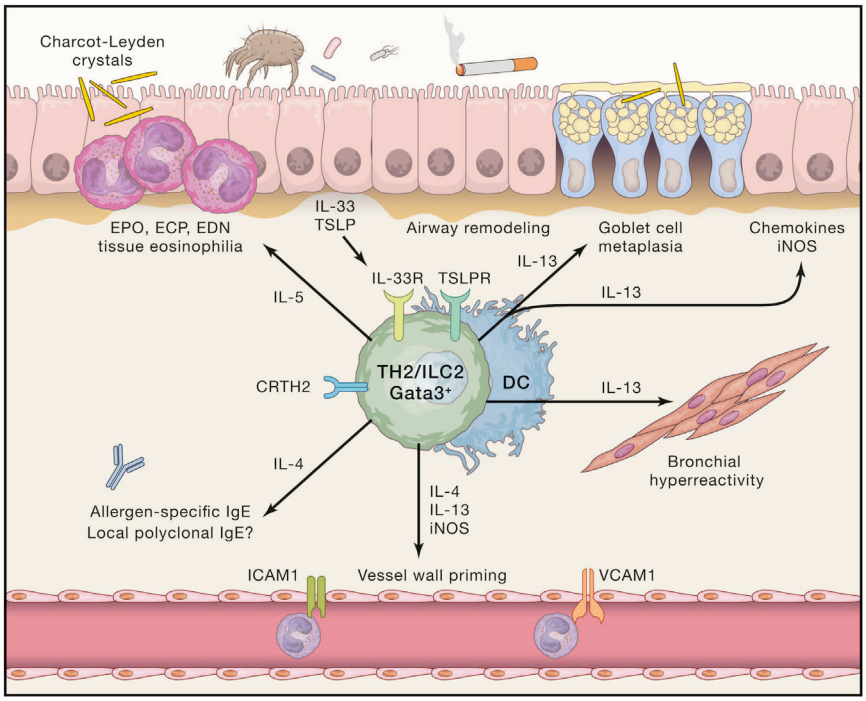

Figure 5. Central role of Th2 and innate lymphocytes in controlling key features of type 2-high asthma

Type 2-high asthma is defined by presence of airway eosinophils and by the biomarker iNOS in exhaled air. It is often accompanied by goblet cell metaplasia of the airway epithelium and high-level production of the airway mucin MUC5AC. Th2 memory lymphocytes get restimulated by dendritic cells (DCs) upon allergen recognition, and subsequently produce IL-4, IL-5, and IL-13. This scenario likely occurs in allergic asthmatics. In some patients with type 2-high asthma, innate lymphocytes type 2 (ILC2) get activated directly by epithelial cytokines such as TSLP and IL-33 to produce the cytokines IL-5 and IL-13. Th2 cells and ILC2s share many features such as expression of cytokine receptors, transcription factor GATA3, and CRTH2 (chemokine receptor expressed by Th2 cells). IL-5 drives the development and activation of airway eosinophils. These eosinophils are the source of galectin-10 which forms Charcot-Leyden proteins in the airways. Eosinophil activation also leads to their degranulation and release of toxic substances such as eosinophil peroxidase (EPO) that can crosslink airway mucus, and eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (EDN) that are very damaging to lung structural cells and also activate innate immune cells. IL-13 drives iNOS production in airway epithelial cells, goblet cell metaplasia and bronchial hyperreactivity. IL-4 promotes IgE synthesis (allergen-specific in the case of allergic asthma) and primes the vessel wall for extravasation of eosinophils through induction of vascular cell adhesion molecule (VCAM-1) and intercellular adhesion molecule (ICAM-1), by acting on IL-4R. When IL-4R is blocked, this can explain increased blood eosinophilia, with reduced tissue eosinophilia.

markers. Different groups of ILCs (ILC1, ILC2, and ILC3) with unique phenotypes and different functions have been identified (Meininger et al., 2020). Recent studies have shown that the specific gene signature of ILCs depends on the tissue microenvironment in humans (Yudanin et al., 2019) and in mice (Zeis et al., 2020). As such, ILCs perform tissue-specific functions that depend on the signals these cells receive. ILC2s resemble Th2 cells since they also express the prototypical Th2-associated transcription factor GATA-3 and produce IL-5, IL-9, and IL-13 (Figure 5). Like effector Th2 cells, ILC2s also heavily rely on PPAR-γ regulation (Xiao et al., 2020). ILCs store fatty acid nutrients in lipid droplets and use these droplets as an emergency energy source to proliferate when stimulated by cytokines (Karagiannis et al., 2020).

ILC2s mainly respond to the epithelial-derived cytokines IL-33, IL-25, and TSLP, which induce not only their proliferation but also activation (Guo et al., 2015; Huang et al., 2015). ILC2s in the lung can also get activated by the neuropeptide neuromedin U (NMU) in the presence of high levels of epithelial-derived cytokines, showing that neuronal activation following allergen exposure can enhance the effect of epithelial-derived cytokines on ILC2s (Wallrapp et al., 2017).

Role of ILC2s in asthma

In humans, ILC2s were found to be an important player in asthma. The number of ILC2s is increased in the blood and in the bronchoalveolar lavage of patients with asthma (Winkler et al., 2019). In human asthmatics, allergen challenge also led to expression of TL1A, a known ligand for DR3 expressed by ILC2s. Allergen-induced TL1A thus might contribute to the expansion and activation of human ILC2s (Machida et al., 2020). The *ex vivo* co-culture of ILC2s with human bronchial epithelial cells showed that IL-13 derived from ILC2s could disturb lung epithelial cell barrier function (Sugita et al., 2018). ILC2s also seem to be more active in females and in patients with CRSwNP, potentially explaining the gender bias in non-allergic type 2-high asthmatics (Wang et al., 2020).

The mechanism by which ILC2s contribute to type 2 responses in the lung have started to be slowly unraveled with the use of murine models (Figure 5). However, the results obtained in these models are difficult to translate to patients with asthma because immunologists have mainly used models of allergic eosinophilic asthma, which do not reflect the human non-allergic eosinophilic asthma. In mice, the accumulation of ILC2s in allergic lung can be explained by the fact that HDM exposure is sufficient to induce the production of IL-33 by lung epithelial cells, a process that is greatly facilitated in early life (de Kleer et al., 2016a; Hammad et al., 2009). Under the influence of IL-33, ILC2s produce IL-13 and IL-5, thus promoting key asthma features such as tissue eosinophilia and BHR (Klein Wolterink et al., 2012; Figure 5). However, ILC2s can also act very early on in the process of Th2 sensitization, by promoting DC migration to the draining nodes via release of IL-13 (Halim et al., 2014), thus facilitating induction of Th2 immunity to allergens. Moreover, in parasitic infections, ILC2s have been shown to have antigen-presenting capacities through their expression of major histocompatibility complex class II (MHCII) and

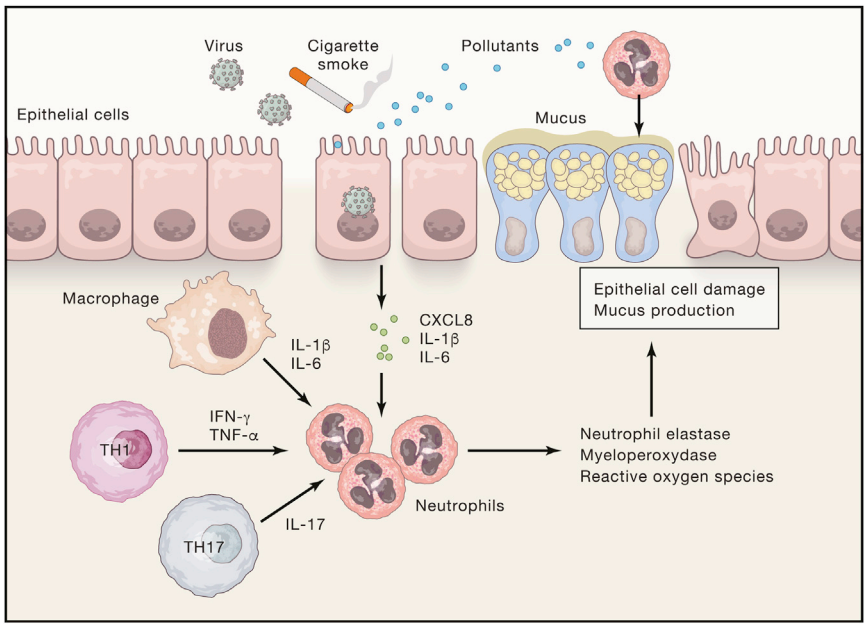

Figure 6. Proposed role of neutrophils in non-type 2 asthma

The airway epithelium and alveolar macrophages can get activated by environmental triggers such as microbes or pollutants. In response to these stimuli, they produce pro-inflammatory cytokines (IL-1β, IL-6) and the epithelium also produces CXCL8 (also known as IL-8), a potent neutrophil attractant. Under the influence of the cytokine environment, Th1 and Th17 cells are induced and further contribute to neutrophil recruitment and activation. Activated neutrophils release factors such as neutrophil elastase, myeloperoxidase, or ROS that will induce epithelial cell damage and contribute to increased mucus production.

costimulatory molecules (Angkasekwinai et al., 2017). Whether they perform similar functions following allergen exposure in the lung remains to be addressed.

The main issue that remains is whether ILC2 and Th2 cells overlap in their functions in asthma, given that they produce similar cytokine patterns. One way to address this question would be to have models where ILC2s can be specifically deleted. Moreover, it would be interesting to assess their real contribution to disease features in allergic versus non allergic asthma. Although murine models are available for the former, they are still missing for the latter.

NON- (OR LOW-) TYPE 2 ASTHMA: ALL DOWN TO NEUTROPHILS?

Non- or low-type 2 phenotypes of asthma are defined by the absence of Th2 cytokine signatures (such as blood and sputum eosinophilia, increased FeNO) rather than by the existence of alternative immune signatures. Non-type 2 asthma has been associated with later age at onset of the disease, use of high dose of corticosteroids, and obesity (Tliba and Panettieri, 2019; Figure 2). What these patients have in common is that they respond poorly to corticosteroids. Despite this, these patients are often treated with these drugs for long periods of time, which contributes to the worsening of their obesity. Obese patients with a type 2-low signature are often female gender with late-onset asthma. In these patients, studies have identified increases in the IL-6 pathway in epithelial brushings and sputum cells (Li et al., 2020).

The most consistent pathways identified in type 2-low asthma patients are those related to the inflammasome, especially those related to the IL-1β pathway. By discarding patients with sputum eosinophils, the U-BIOPRED consortium has identified a cluster of patients with inflammasome expression, neutrophilic inflammation, and high corticosteroids usage (Rossios et al., 2018). Interestingly, a study from the Severe Asthma Research Program has identified patients with similar characteristics, but, in addition, these patients had extracellular DNA in their sputum, which was identified as neutrophil-derived extracellular traps (Lachowicz-Scroggins et al., 2019). This group of patients also had sputum neutrophils, caspase-1 activation, and high IL-1β levels, consistent with the activation of the inflammasome pathway (Figure 6). Neutrophilic asthma has been proposed as a phenotype of asthma. However, clinical trials, performed in patients with neutrophilic asthma using CXCR2 antagonists to block the recruitment of neutrophils by CXCL8, failed to change any of the asthma outcomes (O'Byrne et al., 2016). Therefore, whether neutrophils have a function in asthma or are simply bystander cells is still an open question (Figure 6). A recent study using children and adolescents with neutrophilic asthma has shown that children with high neutrophil counts had increased concentrations of granule proteins (myeloperoxidase and elastase) suggesting that these cells might contribute to lung tissue damage. In addition, exposure of primary neutrophils to BAL fluids from children with high neutrophil counts increased their phagocytic capacities and formation of NETs, which are cytotoxic for epithelial cells (Grunwell et al., 2019). These data indicate that neutrophils can be real effector cells in severe non-type 2 asthma and that the environment in the lung of these patients drive neutrophil pathogenic functions.

In patients with moderate to severe forms of asthma, the levels of IL-17A, IL-17F, and IL-22 are increased in their bronchoalveolar lavage and correlates with disease severity (Al-Ramli et al., 2009). The presence of neutrophils in some asthma patients has generated interest in the cytokine IL-17. However, even using murine models of experimental asthma, its role is still unclear mainly because animal models have yielded confusing results showing that IL-17 can sometimes protect and sometimes favor asthma depending on the timing of IL-17 blockade. In these studies, IL-17 was protective only during the challenge phase (Schnyder-Candrian et al., 2006). Despite the promising results of IL-17 blockade in animal models, the main issue here is that the models used did not reflect human type 2-low asthma. It has been very difficult for immunologists to model neutrophilic inflammation associated with asthma features in mice. To do this, groups had to resort to the use of adjuvants (e.g., high dose of LPS) endowed with pro-Th1 and -Th17 properties. Using

one of such adjuvants called c-di-GMP, researchers have managed to develop a model of asthma with high numbers of lung neutrophils, low Th2 cytokines, high IFN-γ and IL-17 levels, and presence of BHR. When analyzing the contribution of cytokines to asthma features, they found out that IFN-γ and not IL-17 could drive asthma features including BHR (Raundhal et al., 2015). Whether such mouse model could represent human type 2-low asthma is not certain, but sheds light on the fact that other cytokines than IL-17 should be considered to explain neutrophilic asthma. In view of these findings in mice, it is not surprising that clinical trials aiming at blocking the IL-17 pathway in patients failed to show efficacy (Busse et al., 2013). One caveat in the latter study is that the number of neutrophils was not an inclusion criterion, and it is likely that the patients selected were heterogeneous regarding airway neutrophil numbers. Moreover, the IL-17 pathway expression has been identified in some studies but is rather inconsistent, and one study has even shown the presence of tissue eosinophils within a group of asthma patients with a Th17-high signature, showing the possible coexistence of type 2-high and Th17-high signatures in a subset of patients (Choy et al., 2015). Similar findings were recently also reported in mouse models of asthma (Tibbitt et al., 2019; Tortola et al., 2020). To make things even more complex, neutrophils have been shown to influence type 2-high asthma. Indeed, studies have reported that neutrophil extracellular traps induced by rhinovirus were involved in type 2-high asthma exacerbations (Toussaint et al., 2017). A few years later, the same group showed that neutrophil extracellular traps induced by low-dose microbial exposure facilitated the development of type 2-high asthma in mice (Radermecker et al., 2019). These data show that the relationship between Th17-associated neutrophilia and type 2-high asthma is very complex. Therefore, it has been proposed that targeting both Th17 and Th2 pathways might be beneficial in some patients. The dual blockade of IL-13 and IL-17 already showed promising results in mice (Choy et al., 2015) but still needs to be studied in humans, especially in view of the negative trials using IL-17 blockade only.

VIRUS-INDUCED ASTHMA EXACERBATIONS

Asthma exacerbations can be induced by different stimuli, including allergens, pollution, cold air, and microbes. Such agents induced an enhanced inflammation in the lung that worsens the symptoms of the existing disease. Among these agents, respiratory viruses, especially respiratory syncytial virus (RSV) and rhinovirus (RV), are the major drivers of asthma exacerbations in children and adults, respectively (Jartti and Gern, 2017). Respiratory viruses primarily infect lung epithelial cells. In response to viral replication, healthy epithelial cells produce antiviral factors that ultimately lead to viral clearance. In the chronically inflamed airways of asthmatic patients, the antiviral response of epithelial cells might be altered, causing sustained inflammation and exacerbation of symptoms (Busse et al., 2010).

Role of type I interferons

Once the link between viral infections and asthma exacerbation was established, many groups have attempted to identify mechanisms of the underlying virus-induced asthma exacerbation. Following infection, the host cells generally induce an inflammatory response as a way to counteract the virus. Generally, in response to viruses, infected cells produce type I/III IFN. Interestingly, exacerbations are mainly induced in patients with high body mass index, with high eosinophil numbers (type 2-high patients), and in patients with decreased production of type I interferons (especially IFN-β) and type III interferons (IFN-λ) (Denlinger et al., 2017; Wark et al., 2005; Zhu et al., 2019). A recent single-cell RNA sequencing analysis, performed on upper-airway cells of patients with viral infection progressing to asthma exacerbation, showed that higher expression of Th2- and lower expression of type I IFN-related genes were associated with a shorter time to exacerbation (Altman et al., 2019). It is also believed that the levels of IgE may render patients more vulnerable to infections (Denlinger et al., 2017). In this case, the binding of IgE to FcεRI on plasmacytoid DCs was shown to block their capacity to produce type I interferons (Schroeder et al., 2005), allowing the virus to spread easier, and induce more damage and inflammation, leading to asthma exacerbation. In addition, murine models of infection with PVM (pneumovirus of mice), the counterpart of RSV, have highlighted that a defect in type I interferon-producing plasmacytoid DCs predisposes to viral bronchiolitis and asthma. However, the authors have attributed this to TLR7 and not to IgE/FcεRI (Kaiko et al., 2013). Therefore, whether other pathways than IgE contribute to the link between viral infections and asthma in humans remains to be fully addressed.

Epithelial-derived cytokines contribute to virus-induced asthma exacerbations

Because asthma exacerbations are more frequent in patients with blood eosinophils and higher IgE levels, suggestive of the type 2-high endotype, researchers have begun looking at different Th2-associated cell populations and pro-Th2 cytokines to understand the possible reasons underlying virus-induced exacerbations. Recent studies have shown that, during virus-induced asthma exacerbation in mice, IL-33 was produced in high amounts by lung epithelial cells, could suppress antiviral responses by dampening type I IFN production, and could favor asthma exacerbations (Lynch et al., 2016). Single-cell RNA sequencing (RNA-seq) analysis, performed in patients with virus-induced asthma exacerbation, identified a gene core associated with IL-33 and epithelial cell repair. This module also contained CDHR3, a susceptibility locus in childhood asthma encoding for a protein involved in the entrance of Rhinovirus-C in epithelial cells, suggesting that IL-33 in the context of viral infection may leave the epithelium more susceptible to repeated infections (Altman et al., 2019). One caveat in this study is that upper-airway cells were used as a proxy for lower-airway cells. Although some studies have shown that nasal cells are transcriptionally similar to lung cells (Poole et al., 2014), other studies have observed differences in immune responses, including interferon responses (Mihaylova et al., 2018). Future studies using lung cells are needed to confirm the involvement of the identified pathways.

A role for innate type 2 cells in virus-induced asthma exacerbations?

Because exposure to respiratory viruses increases the production of IL-33 and TSLP by lung epithelial cells, it is not really

surprising to observe that infants hospitalized with severe respiratory virus infection had increased numbers of ILC2s in their nasal aspirates compared to children with mild infection (Vu et al., 2019). Similarly, in mice, influenza infection increased ILC2 numbers in the lung (Li et al., 2019). However, during influenza-induced asthma exacerbation, lung ILC2-derived Th2 cytokines were found to be initially lower than those derived from Th2 cells, but ILC2-derived Th2 cytokines peaked at the time of virus clearance (Li et al., 2019). Although influenza virus is not frequently involved in asthma exacerbations, these data nevertheless suggest that, during virus-induced asthma exacerbation, there is probably a division of labor between Th2 cells that contribute early on to increased inflammation and ILC2s that contribute at a later time point to the repair of the lung. In line with this, once the virus was cleared from the airways, ILC2s expressed Amphiregulin, a protein involved in tissue repair (Li et al., 2019). Whether the observations made with influenza also apply for other viruses more relevant to asthma exacerbations remains to be fully addressed.

Viral infections are also always accompanied by high levels of type I (IFN-α/β) and type II (IFN-γ), which are known to inhibit ILC2 functions (Duerr et al., 2016; Moro et al., 2016) and to affect the biology of DCs, which have important functions in Th2 responses to allergens (Webb et al., 2017) as well as in antiviral responses (Bosteels et al., 2020). During viral infections, type I IFN induces a specific population of SIRPα^+IFNAR$^+$ conventional DC2 with high levels of Fc receptors and with strong capacities to activate CD4$^+$ and CD8$^+$ T cell-specific antiviral responses (Bosteels et al., 2020). Such DC population also appears in the lung upon exposure to allergens. The link between type I IFN, IFNAR$^+$ cDC2, and virus-induced asthma exacerbation is very complex and still unclear. It is, however, tempting to speculate that, in a Th2-prone environment as in type 2-high asthma, the low levels of type I IFN present in the lung have an impact on these IFNAR$^+$ cDC2s, leading to less virus-induced migration to the draining lymph nodes, and as a consequence to less virus-specific CD4 and CD8 T cell responses.

With less potent antiviral responses, increased levels of IL-33 induced by respiratory viruses would pave the way for a stronger activation of Th2 cells and ILC2s, both contributing to enhanced asthma features, including bronchial hyperresponsiveness and eosinophilia. However, to make things even more complex, eosinophils themselves have been suggested to have antiviral capacities. Indeed, extracellular eosinophil-derived neurotoxin (EDN) displays RNase activity and can enter viral capsids to degrade RNA from RSV (Samarasinghe et al., 2017). Moreover, mice overexpressing both Eotaxin2 and IL-5 were protected against a lethal pneumovirus infection (Percopo et al., 2014). On the other hand, the depletion of eosinophils with anti-IL-5 antibodies increased the viral load in HDM-sensitized mice (Ravanetti et al., 2017). Moreover, eosinophils from mice and healthy donors are able to capture several respiratory viruses and reduce their infectivity. However, this is not the case for eosinophils obtained from patients with asthma (Sabogal Piñeros et al., 2019). In patients with asthma, the inability of eosinophils to control respiratory viruses may lead to increased viral loads, and, with less type I IFN being produced and less DC-mediated antiviral responses, an ideal scenario would be set to allow frequent virus-induced asthma exacerbations.

BIOLOGICALS IN THE TREATMENT OF ASTHMA

The acknowledgment that asthma is a heterogeneous disease has prompted pharmaceutical companies to develop new drugs to specifically target effector cells, cytokines, or molecules involved in different types of asthma.

Inhibition of eosinophils and IL-5

It is now clear that eosinophils represent a proinflammatory granulocyte that plays a major role in type 2-high asthma. Eosinophilic inflammation in the airways is associated with asthma disease severity (Figure 1), and there is a direct correlation between tissue and blood eosinophil count and the frequency of asthma exacerbations, and the occurrence of irreversible airway obstruction (Graff et al., 2020).

The differentiation, activation, and survival of eosinophils is driven by IL-5, which is secreted by Th2 cells, mast cells, and innate lymphoid cells. Therefore, inhibiting IL-5 or IL-5 signaling seemed like a good option in asthma. The first biologicals targeting the IL-5 pathway was Mepolizumab, an IgG1 antibody directed against IL-5. The first trials using all-comers have proved disappointing (Leckie et al., 2000), but later on trials using biomarker-identified patients showed efficacy of Mepolizumab in reducing corticosteroid use and even improving lung function (Nair et al., 2009). The success of Mepolizumab was also seen with Reslizumab, a similar anti-IL-5 IgG4 antibody (Castro et al., 2015). The third biologics targeting the IL-5 pathway to show clinical efficacy was Benralizumab, a cytotoxic antibody that kills cells expressing the IL-5 receptor alpha chain such as eosinophils and basophils. The depletion of eosinophils with Benralizumab in severe forms of asthma lead to reduced exacerbation rate and reduced need for oral corticosteroids, strengthening the importance of eosinophils in asthma pathobiology (Busse et al., 2019).

Inhibition of IL-4RA

IL-4RA binds both IL-4 and IL-13. Given the importance of both cytokines in driving IgE production by B cells, BHR, goblet cell metaplasia, mucus production, basement membrane thickening, and fibrosis, IL-4RA was considered as an attractive target in asthma.

The use of Dupilumab, a humanized monoclonal antibody that blocks IL-4Rα, was first used in patients with eosinophilic asthma. Patients using Dupilumab showed improved lung function and asthma symptoms (Wenzel et al., 2013). Dupilumab was also found to be efficacious in patients with non-eosinophilic asthma (Wenzel et al., 2016), but the effects were larger for patients with eosinophilic asthma with regards to improved lung function and reduced exacerbation frequency. In some 15% of patients, however, this treatment is associated with a temporary rise in blood eosinophils, most likely because of reduced extravasation from the bloodstream (Castro et al., 2018). Given that it also reduced severity and recurrence of CRSwNP, and features of atopic dermatitis, Dupilumab could be a good choice in asthmatics with comorbid allergic disease (Agache et al., 2020).

Inhibiting IL-9

IL-9 is believed to play a role in asthma although the extent of its involvement in asthma features still remains to be fully addressed, especially in humans. A clinical trial using a humanized anti-IL-9 monoclonal antibody in patients with moderate to severe asthma failed to show efficacy (Oh et al., 2013). It was suggested that the phenotypic heterogeneity of the patients used in this study was a reason for failure. Future studies to identify correlations between Th9/IL-9 and asthma phenotypes should provide a better understanding of the role of IL-9 in asthma and allow identification of subgroups of patients that would benefit from blocking IL-9 therapies, certainly given the recent observations that one of the major discriminators between HDM allergic donors without asthma and HDM allergic asthmatics seems to be T cell production of IL-9 in the latter (Seumois et al., 2020).

Inhibiting IgE

Although the biologics directed against the Th2 cytokines IL-4, IL-13, and IL-5 show exciting results, the first-ever approved biologicals in asthma was in fact directed against IgE. Considering that IgE is involved in the onset of the allergic asthma as well as in the chronic phase of the disease, it is not surprising that omalizumab (a monoclonal antibody that reduces the binding of IgE to its high-affinity receptor leading to the downregulation of FcεRI expression and to an impaired function of several FcεRI^{+} cells; MacGlashan et al., 1997) demonstrated efficacy in clinical trials by reducing exacerbation rates, reducing the doses of corticosteroids needed to control the disease, and by improving lung function (Casale et al., 2019; Holgate et al., 2004; Humbert et al., 2005). Interestingly, the levels of IgE in patients seem to be a poor predictor of response to omalizumab (Bousquet et al., 2004). One retrospective study showed that patients with high eosinophil counts had the greatest benefit (Casale et al., 2019), although this has been refuted (Humbert et al., 2018). It also appears that omalizumab improves lung function in adult and pediatric patients with eosinophilic nonallergic asthma (Bourgoin-Heck et al., 2018; Pillai et al., 2016). This is not surprising since, in this phenotype of asthma, patients show a Th2 cell signature with tissue eosinophilia and can have high total serum IgE (Beeh et al., 2000). Therefore, blocking IgE represents a safe and effective treatment of type 2-high but not type 2-low inflammation (Hanania et al., 2013). This being said, anti-IgE treatment is not the panacea for all type 2-high asthma patients. Indeed, only about 70% of children and adults treated with omalizumab show good to excellent responses to the treatment (Humbert et al., 2018). Deciding which patients should be best treated with which therapy remains a challenging task for physicians.

Inhibiting epithelial-derived cytokines

Given the inability of available anti-type 2 biologicals to prevent all asthma exacerbations and given the complexity in mechanisms involved in asthma, there was a need to investigate other means of curing asthma. Because of their central and very upstream role, epithelial-derived alarmins (IL-1, IL-33, IL-25, and TSLP) have been considered as interesting targets. Biologicals targeting TSLP and IL-33 are the most advanced and have entered clinical trials, although results of the clinical trials have only been published for TSLP. Topline results from the phase 3 Navigator clinical trial using the blocking TSLP antibody Tezepelumab were reported. Very strikingly, the drug improved exacerbation frequency not only in type 2-high patients but also in those with type 2-low disease, and low blood eosinophil counts, making it the first biological on the horizon that may benefit a broad category of asthma patients (Corren et al., 2017).

Anti-type 2 biologics are not a panacea for all patients with type 2-high asthma

Although the use of the new anti-type 2 biologics has changed the life of severe asthmatic patients, clinical trials have shown that not all patients with type 2-high asthma respond well to anti-type 2 biologics. This means that, even if eosinophil count in the blood is sometimes a good biomarker, more is needed to ensure that patients respond to one or another biologics. Other type 2-high biomarkers such as FeNO or periostin can be used together with eosinophil counts to identify asthma subgroups. However, it is very likely that the further identification of novel biomarkers might be necessary to further improve the response to specific biologics. The more extensive use of omics techniques might help identify such new biomarkers in patients who failed to respond to certain agents.

Another point is that, even when considering eosinophilic asthma, some patients will present long-lasting, difficult-to-cough-up, mucus impactions in the airways, caused by eosinophil activation (Dunican et al., 2018). Such more-severe patients also show the presence of eosinophil-derived Charcot-Leyden crystals in these mucus plugs (Persson et al., 2019), which would contribute to fixed-airway obstruction. It remains to be seen whether such patients with such crystals in elastic mucus would benefit from an eosinophil-targeted therapy and show improved lung function or whether an add-on therapy that would be more directed to the crystals would be beneficial.

Finally, the fact that not all type 2-high patients respond to anti-type 2 biologics raises the possibility that non-type 2 pathways might also be at play in such refractory patients. Indeed, inducible nitric oxide synthase (iNOS) is induced not only by Th2 cytokines but also by the Th1 cytokine IFN-γ (Guo et al., 1997), suggesting that elevated FeNO might not solely be explained by type 2 inflammation. In addition, one study has found tissue eosinophils in asthma patients with a Th17-high signature, showing the possible coexistence of type 2-high and Th17-high signatures in a subset of patients (Choy et al., 2015). Therefore, strategies targeting multiple pathways might represent an alternative way to treat subsets of asthmatic patients.

CONCLUSIONS

Clinicians now realize that asthma is a very heterogeneous disease with different endotypes. This is best reflected by the results of the different clinical trials using different biologics in patients with asthma. Asthma is the consequence of a complex interaction between structural cells, such as epithelial cells, and immune cells occurring in the context of exposure to specific environmental triggers in very specific periods of life. Despite the fact that we gradually understand more of the immunology of type 2-high asthma, more investigative work is clearly needed

for type 2-low asthma before we can rationally design therapies based on a well-understood endotype.

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