

Overlapping Effects of New Monoclonal Antibodies for Severe Asthma

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Abstract Among the monoclonal antibodies (mAbs) developed for severe asthma treatment, three have already been marketed. Omalizumab was the first, more than 10 years ago; today, mepolizumab and reslizumab are also available in the European Union and the US. Omalizumab blocks free immunoglobulin E (IgE), mepolizumab and reslizumab block an interleukin (IL-5). In the near future, dupilumab and benralizumab are expected to emerge as two new alternatives. Benralizumab blocks the receptor for IL-5 (IL5-R α) and has a direct cytotoxic effect on eosinophils, and dupilumab blocks the α -unit of the heterodimeric receptor for IL-4 and IL-13 (IL-4R α); as a result, dupilumab can block both IL-4 and IL-13. The purpose of this manuscript is to present the pathophysiology of some immunological aspects of severe asthma, describe the adaptive and innate immunity arms as well as their interrelations (stressing the subordination of the adaptive arm to the innate arm), outline the pharmacologic effects of these mAbs, clarify the overlapping effects of the different mAbs, and discuss the differences between mAbs based on their target molecules. Based on the data presented, I propose omalizumab for patients with an allergic phenotype regardless of their peripheral eosinophilic count, and

anti-IL-5 as an alternative in allergic patients with blood eosinophilia in which omalizumab has failed; anti-IL5 for patients with an eosinophilic phenotype and omalizumab as an alternative in patients in whom anti-IL5 fails and IgE ≥ 30 IU/mL (compassionate use). Omalizumab is also proposed for patients with severe chronic asthma allergic to seasonal allergens.

Key Points

Adaptive and innate immunity are interrelated; the former is subordinated to the latter.

The monoclonal antibodies developed for severe asthma treatment block different mediators (immunoglobulin E and interleukins).

The effects of these antibodies may overlap. The treatment response may vary according to the predominance of one or other immunity arm, which may explain why there may be alternative treatments for a given physiopathological mechanism.

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1 A Brief History of Recent Developments in Severe Asthma Treatment

During the last decade, several important changes have been introduced in the treatment of moderate and severe asthma. These changes were designed to act on two pathophysiological aspects of the disease: the modification

of the airway caliber, and its characteristic immunobiological processes.

Historically, the first drugs used to treat asthma were the bronchodilators: initially, short-acting beta-agonists (SABA) and later, in the 1990s, long-acting beta-agonists (LABA). As asthma was considered a reversible airway disease, SABA were considered to be relievers and LABA (with inhaled corticosteroids [ICS]) were considered controller medications. In more chronic severe asthma, a second mechanism influencing the caliber of the airways was then widely identified: sustained bronchoconstriction due to increased cholinergic tone. Next, anticholinergic drugs widely used in chronic obstructive pulmonary disease (COPD) mainly caused by smoking (or more generally, by etiologies other than asthma) were progressively implemented for the treatment of moderate and severe asthma, independently of the cause triggering or causing the condition. First of all, Bateman et al. [1] established the non-inferiority effect of tiotropium compared with salmeterol in maintaining improved lung function in asthma patients. Shortly afterwards, Kerstjens et al. [2] showed that tiotropium was helpful for improving asthma control in poorly controlled asthma patients treated with standard combination therapy (LABA + ICS). Time to first severe exacerbation and time to first asthma worsening were analyzed in T helper type 2 (T_H2)—low and T_H2 —high subgroups defined as total immunoglobulin E (IgE) $\leq$ or $>430 \mu\text{g/L}$ (or 179.2 IU/L) and blood eosinophils $\leq$ or $>0.6 \times 10^9/\text{L}$ (or $600 \text{ cells}/\mu\text{L}$). Time to first severe exacerbation was longer with tiotropium than with placebo in patients with moderate and severe asthma, independently of IgE and blood eosinophils. As a result, tiotropium via mist inhaler was included as an add-on therapy for Global Initiative for Asthma (GINA) step IV patients aged ≥ 12 years with a history of exacerbations [3].

The second revolutionary new treatments introduced were biological treatments, designed to interfere in the different immunopathological steps occurring in asthma [4, 5]. Monoclonal antibodies (mAbs) are preformed antibodies against a target antigen with high specificity against the target extracellular molecule injected into the body [6].

This paper reviews the production, release, and biological effects of the leading mediators and cells involved in bronchial asthma as well as the clinical effects of the mAbs developed (either marketed or soon to be marketed) in order to treat them. The focus is primarily on IgE, the most important interleukins (IL) involved in the different arms of the immunopathology of asthma (IL-5, IL-4 and IL-13), and eosinophils (i.e., their physiological and toxic effects and the benefits of their reduction). This article reviews some aspects of innate and adaptive (or allergic) immunity, tries to interrelate the two arms, and assesses the mode of

action (electronic supplementary material [ESM] 1 and 2) and the effects (or lack of) that mAbs might have on asthma. The intricate pathways described in this review are illustrated in detail in the accompanying ESM.

2 Pathophysiological Pathways of Asthma Susceptible to Blocking

2.1 The Allergic Cascade or Adaptive Immunity

Until very recently, the inflammation associated with asthma was assumed to be primarily driven by T_H2 cells, which release pro-inflammatory cytokines (IL-4, IL-5, IL-13) contributing to eosinophil inflammation and IgE production. Thus, elements in this pathway became natural targets for the development of molecularly targeted biologic therapies. In other words, only the adaptive arm of the asthma inflammatory cascade (currently named the allergic cascade) was considered susceptible for treatment.

The focus was mainly placed on IgE, a young immunoglobulin that originated between 150 and 300 million years ago [7] from a gene duplication of IgY, in which the anaphylactic and opsonic activities of IgY were separated, giving rise to IgE and IgG, respectively [8]. The segregation of anaphylactic and opsonic activities into separate genes allowed a tighter and more specific control of both immune responses [9].

The exposure to allergens exerts a trigger effect on the allergic cascade. At that moment, the IgE produced during the sensitization phase have already reached their receptors on the effector cells (mast cells and basophils) (ESM 3). Working in pairs, the IgE located in their receptors will capture the allergen; then, a crosslinking effect occurs that starts degranulation and the release of mediators (ESM 4 and 5). In addition to the initial pathway through the antigen-presenting cell already described [4] and the crosslinking effect occurring on the effector cells, the allergens can also stimulate the cascade by directly acting on B cells expressing IgE on their surface (ESM 4). During the late phase of the allergic cascade, T_H2 lymphocytes start to release IL-5, which will recruit and stimulate eosinophils (ESM 6), which in turn start to release toxic mediators. Then, the loop may evolve towards chronicity and even in the absence of allergens (or in the presence of a low concentration of allergens) the free IgE (i.e., the IgE not linked to the high and low affinity receptors [Fc ϵ RI and Fc ϵ R2, respectively]) (ESM 7) can perpetuate the process.

This cascade is upgraded not only by the presence of allergens but also by the increased release of IL-4 by T_H2 lymphocytes and through an autocrine reaction, which stimulates the conversion of T_H0 into T_H2 . This IL-4 also has more immunologic effects, described below. The ILs

released in this adaptive immunity cascade are mainly IL-4, IL-13, and IL-5.

The finding that fewer than half of all atopic individuals develop asthma symptoms [10, 11] indicates that factors other than sensitization to the allergen are necessary for the development of the disease. Dendritic cells (DCs) are generally held responsible for initiating and maintaining allergic T_H2 -cell responses to inhaled allergens in asthma. Although the bronchial epithelium was initially believed to function solely as a physical barrier, it is now seen as a central player in the T_H2 -cell sensitization process by influencing the function of DCs. Clinically relevant allergens, as well as known environmental and genetic risk factors for allergy and asthma, often interfere directly or indirectly with the innate immune functions of airway epithelial cells and DCs [10]. The overlapping effects of adaptive and innate immunity will be discussed later.

2.2 Innate Immunity

The available epidemiological evidence suggests that the population-based proportion of asthma cases that are attributable to atopy is usually less than half [11]. According to some authors, too much emphasis may have been placed on the importance of atopy as a cause of asthma. The danger is that overemphasis on a particular theoretical paradigm for which the evidence is less substantial than is commonly assumed may have limited the research into other possible etiological mechanisms for the development of asthma [11].

Until recently, only the allergic cascade was considered relevant in asthma inflammatory reactions. There is now sufficient evidence to confirm that both arms of immunity are involved (Fig. 1; ESM 8, 9).

Epithelial cells are exposed to external environments on one side, and segregated from the other tissue components by the basement membrane on the other. When they receive extrinsic and intrinsic insults, they release soluble factors that act on stromal cells and immune cells. Damaged cells will stimulate adjacent epithelial or myeloid cells to secrete inflammatory mediators [12].

It is not yet known how *in vivo* administration with IL-25 or IL-33 leads to the enormous increase in type 2 innate lymphoid cells (ILC2) and the production of high levels of IL-4, IL-5, and IL-13 [13, 14].

ILC2 are known to produce IL-5 and IL-13 after stimulation with IL-33 and thymic stromal lymphopoietin (TSLP), but not IL-4 (Fig. 1; ESM 8, 9). In any case, under certain circumstances and with other stimulating molecules ILC2 can release IL-4. Pelly et al. [15] proposed a model in which mast cell-derived leukotriene D4 (LTD4), following the secretion of tissue damage-associated IL-33, stimulates IL-4 secretion by ILC2, supporting early T_H2

differentiation. ILC2 can also release IL-4 after prostaglandin D2 stimulation [16]. Recent evidence has identified ILC2 as antigen-presenting cells able to process and present antigens to CD4+ T cells and to relay signals to the adaptive immune system [17].

3 The Mediators: The Interleukins

The critical function for the T_H2 cytokines in the asthmatic response has been evident for some time. IL-4 is key in CD4+ T-cell commitment to a T_H2 phenotype and the induction of IgE production [18]. IL-5 regulates eosinophil development, activation, and tissue recruitment. IL-13 and IL-9 are important in mucus production and in airway hyper-responsiveness (AHR) [19].

3.1 Interleukin 5 (IL-5)

In humans the IL-5 receptor (IL-5R) is expressed exclusively on eosinophil and basophil progenitors in the bone marrow and on mature eosinophils and basophils [20].

In summary, the IL-5 cytokine is the main promoter of the production, maturation, and release of eosinophils from the bone marrow, and is also responsible for the increase in eosinophil levels in the blood [21]. IL-5 also activates eosinophils and prolongs their survival in the bloodstream, as well as providing an essential signal for their migration to tissues [21–23]. In the airways of asthmatic patients, the major IL-5-producing cells are T_H2 lymphocytes, natural killer T (NKT) cells, ILC2, mast cells, and the eosinophils themselves [24] (ESM 10).

3.2 Interleukin 33 (IL-33)

IL-33 is a cytokine that is released predominantly at the epithelial barrier when the epithelium is exposed to pathogens, allergens, or injury-inducing stimuli [25]. When epithelial cells receive extrinsic and intrinsic insults, they release or produce soluble factors that act on stromal and immune cells. Damaged cells passively release damage-associated molecular patterns (DAMPs) from the disrupted plasma membrane, which in turn stimulate adjacent epithelial or myeloid cells to secrete inflammatory mediators. Intracellular stores of IL-33 are released from epithelial cells upon infection or injury [25–27] (Fig. 1; ESM 8, 9).

Virtually all types of immune cells and numerous types of non-immune cells respond to IL-33 [28]. IL-33 receptor (IL-33R) is expressed on immune cells such as mast cells, basophils, eosinophils, and T_H2 cells, and has been shown to be upregulated on macrophages, neutrophils, and DCs. It

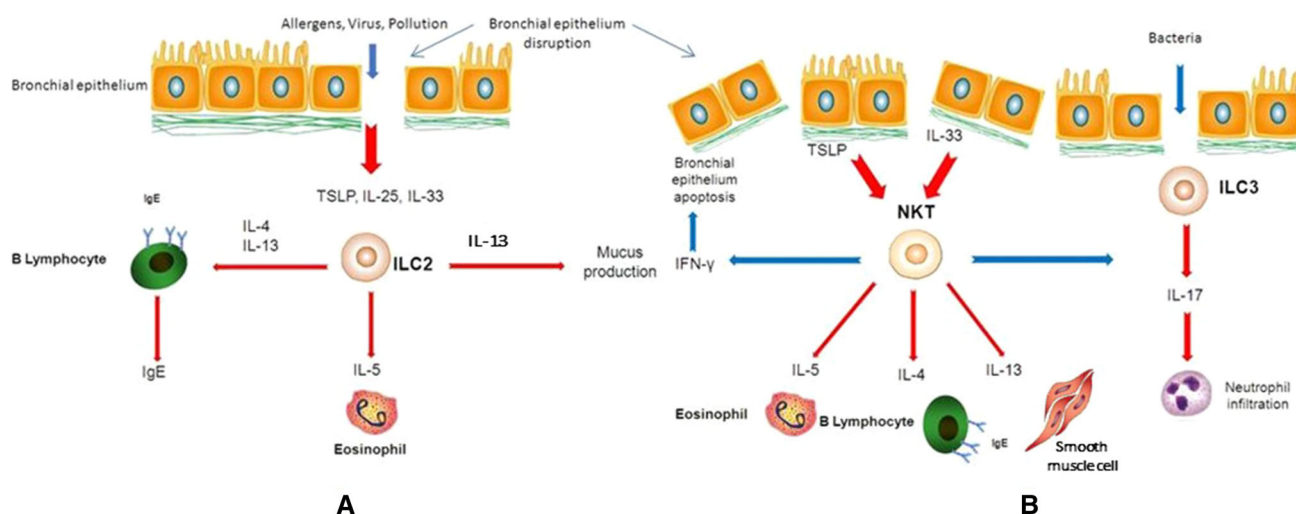


Fig. 1 Innate immunity in asthma. **a** The innate immunity in the lung was originally considered to consist of a barrier (the bronchial epithelium) and some cells, basically macrophages. The present interpretation of the pathophysiology differs notably. The disruption of the epithelium entails the destruction of its cells, which releases mediators. Although not being an active function (this is not properly a secretion of mediators), it confers on the epithelium a 'secretory function'. Allergens, viruses, and pollution can contribute or sometimes be the main cause of this bronchial epithelium disruption. Destruction of epithelial cells releases mediators (TSLP, IL-25, IL-

33) that stimulate type 2 innate lymphoid cells (*ILC2*), which subsequently release T_H2 cytokines, stimulating IgE production and eosinophil recruitment. **b** TSLP and IL-33 also stimulate natural killer T (*NKT*) cells. These *NKT* cells release T_H2 IL that favor IgE production and eosinophil recruitment. They also release IFN- γ which in turn contributes to bronchial epithelium apoptosis, creating a feedback loop. Innate lymphoid type 3 cells (*ILC3*) can be stimulated by bacteria; they release IL-17 which contributes to neutrophil infiltration. *IFN* interferon, *IgE* immunoglobulin E, *IL* interleukin, *TSLP* thymic stromal lymphopietin

is also expressed on non-immune cells such as endothelial, epithelial and smooth muscle cells, and fibroblasts.

The effects of IL-33 differ depending on the concomitant environment. It can stimulate conventional T_H2 cells to release IL-4, IL-5, and IL-13. However, in combination with signal transducer and activator of transcription 5 (STAT5)-activating cytokines (IL-2, IL-7, and TSLP), IL-33 can induce the production of IL-5 and IL-13, but not IL-4 by effector T_H2 cells [28] (Fig. 2; ESM 11).

It regulates DCs either positively or negatively. Cultured bone-marrow-derived DCs produce proinflammatory cytokines and upregulate cell surface molecules in response to IL-33. IL-33-primed DCs can efficiently induce allergic airway inflammation [29, 30]. It may also stimulate the activation of eosinophils to secrete various cytokines and chemokines, upregulate cell adhesion molecules, promote degranulation, and increase cell survival [31].

IL-33R signaling causes downstream production of type 2 cytokines. The engagement of IL-33R with its ligand IL-33 contributes to T_H2 -mediated pathologies and allergic responses [32, 33], but has also been shown to promote T_H1 - and T_H17 -mediated responses [32, 33]. The types of cytokines and chemokines produced by basophils are similar to those released from *ILC2* (i.e., basically IL-4, IL-5, IL-13, and others) [32]. It seems that the action of IL-33 on basophils is to prime them to respond through main receptors such as IL-3, IgE, or chemokine receptors. Inhibition of IL-33 signaling via blockade of the IL-33R may

result in downregulation of immune cell responses and therefore presents a potential novel treatment for severe asthma in addition to standard of care [31]. As a result, the framework for the function of IL-33 has expanded beyond its well documented role in T_H2 immunity. In particular, IL-33 plays a pivotal role in the link between injury at the mucosal barrier caused by extrinsic or intrinsic factors resulting in the release or secretion of IL-33 from the epithelial barrier.

The IL-33R gene codes for both a soluble form (sST2) and a membrane-bound 'long' form (ST2L or IL-33R). Soluble ST2 exists in the serum and is elevated in severe asthmatics during an exacerbation [32].

3.3 Interleukins 4 and 13 (IL-4 and IL-13)

There are two overlapping receptors for IL-4 and IL-13, each of which contains the α -subunit of the IL-4 receptor (IL4R α). Type I receptors, found on B and T lymphocytes, monocytes, eosinophils, and fibroblasts, control the differentiation of T_H2 cells and are activated by IL-4 alone. Type II receptors, found on epithelial cells, smooth muscle cells, fibroblasts, monocytes, and activated B cells, are activated by both IL-4 and IL-13 [34]. Because of this overlap, antibodies that target the IL4R α chain inhibit both cytokine pathways.

Representation of the interrelations between innate and adaptive immunity in asthma

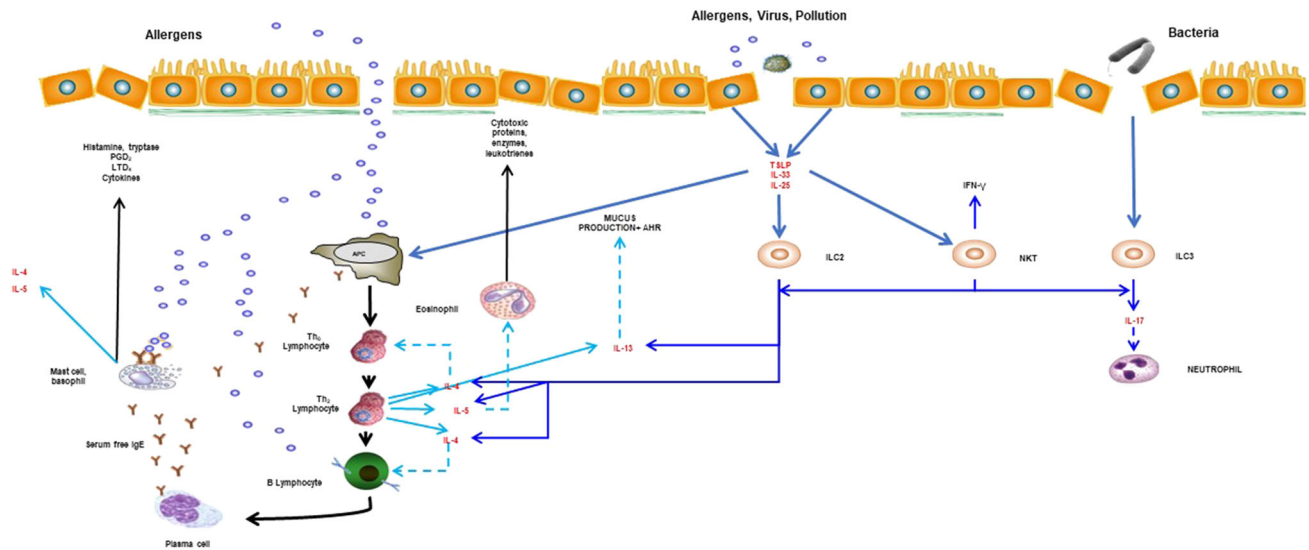


Fig. 2 Representation of the interrelations between the innate and adaptive immunity systems in asthma. The allergic cascade is represented on the left hand side and the innate immunity on the right. As the figure shows, both arms can result in the production of the same interleukins. The adaptive immunity is activated by allergen exposure. The bronchial epithelium disruption can cause the release of alarmins, which activate the innate lymphoid cells. Thus, since the adaptive immunity is subordinated to the innate immunity, it can also be activated by the innate immunity. APC antigen-presenting cell, *IFN* interferon, *IgE* immunoglobulin E, *IL* interleukin, *ILC2* type 2 innate lymphoid cells, *LTD₄* leukotriene D₄, *NKT* natural killer T cells, *PGD₂* prostaglandin D₂, *TSLP* thymic stromal lymphopoietin

The function of IL-4, initially considered to be the most important, was to promote the adhesion between B and T cells [35] (Fig. 2; ESM 11).

The increase in T-cell adhesion induced by IL-4 was shown to be due to a dose-dependent [36] up-regulation of class II major histocompatibility complex (MHC) molecules [35]. IL-4 also promotes the differentiation of T_H0 into T_H2 in an autocrine manner. After prostaglandin stimulation, ILC2 are also able to release IL-4 [4]. Later, IL-4 was found to participate in cell traffic [37]. Transmigration from blood across the postcapillary venules is a multistep process in which the first element is the adhesion of the blood cells to the endothelial cells (EC). The selective expression of the vascular cell adhesion molecules (VCAM) by microvascular EC modulates this traffic in a site-directed manner. During both acute and chronic inflammation, microvascular EC at other sites can be induced to support traffic of various leukocyte subtypes [38]. IL-4 is associated with allergic conditions, and it has been shown to selectively increase expression of VCAM-1 on EC in vivo, suggesting that it could be responsible for VCAM-1 expression in allergic disease (Fig. 3; ESM 12).

However, IL-4 alone induces only a low level of VCAM-1 expression and has only a limited effect on VCAM-1 gene promoter activity. Tumor necrosis factor (TNF)- α is also associated with allergic diseases and has been found to rapidly increase transcription of the VCAM-1 gene. In combination with TNF- α , IL-4 synergistically increases the level of VCAM-1 on the cell surface. This synergy results

from a combination of transcriptional activation by TNF- α and the stabilization of the resulting transcripts by IL-4 (since it increases the half-life of VCAM-1 mRNA in EC). Thus, IL-4 allows subthreshold concentrations of TNF- α (concentrations that would not normally activate expression of adhesion molecules on the endothelium) to selectively increase VCAM-1 expression and to prolong its appearance on the surface of cells in allergic disease [39].

ILC2 were initially reported to produce high levels of IL-5 and IL-13 and very low levels of IL-4 in response to IL-33 in vitro. Interestingly, this cytokine pattern (high IL-5 and IL-13 and low IL-4) may be more related to the effects of IL-33 because conventional T cells stimulated with IL-33 develop into IL-5 producing cells that do not produce IL-4. Subsequently, studies have shown that ILC2 stimulated with TSLP and leukotriene D₄ produces IL-4, suggesting that, under certain conditions, they may be a source of IL-4 [16].

ILC2 are the main source of IL-13 (Fig. 1), although basophils and mast cells are also known to be capable of producing IL-13 [40]. The receptor for IL-13 consists of IL-4R α and IL-13R α [41]. DCs express IL-13R α 1, which is undetectable on naive CD4⁺ T cells. IL-13 also has no effect on T_H2 cell differentiation in vitro [42], indicating that it is unlikely to act directly on naive CD4⁺ T cells. Instead, DCs seem to be the main target of ILC2-derived IL-13. ILC2-derived IL-13 has a critical role, as it promotes the migration of activated lung DCs into the draining lymph node where they prime naive T cells to differentiate into T_H2 cells [43].

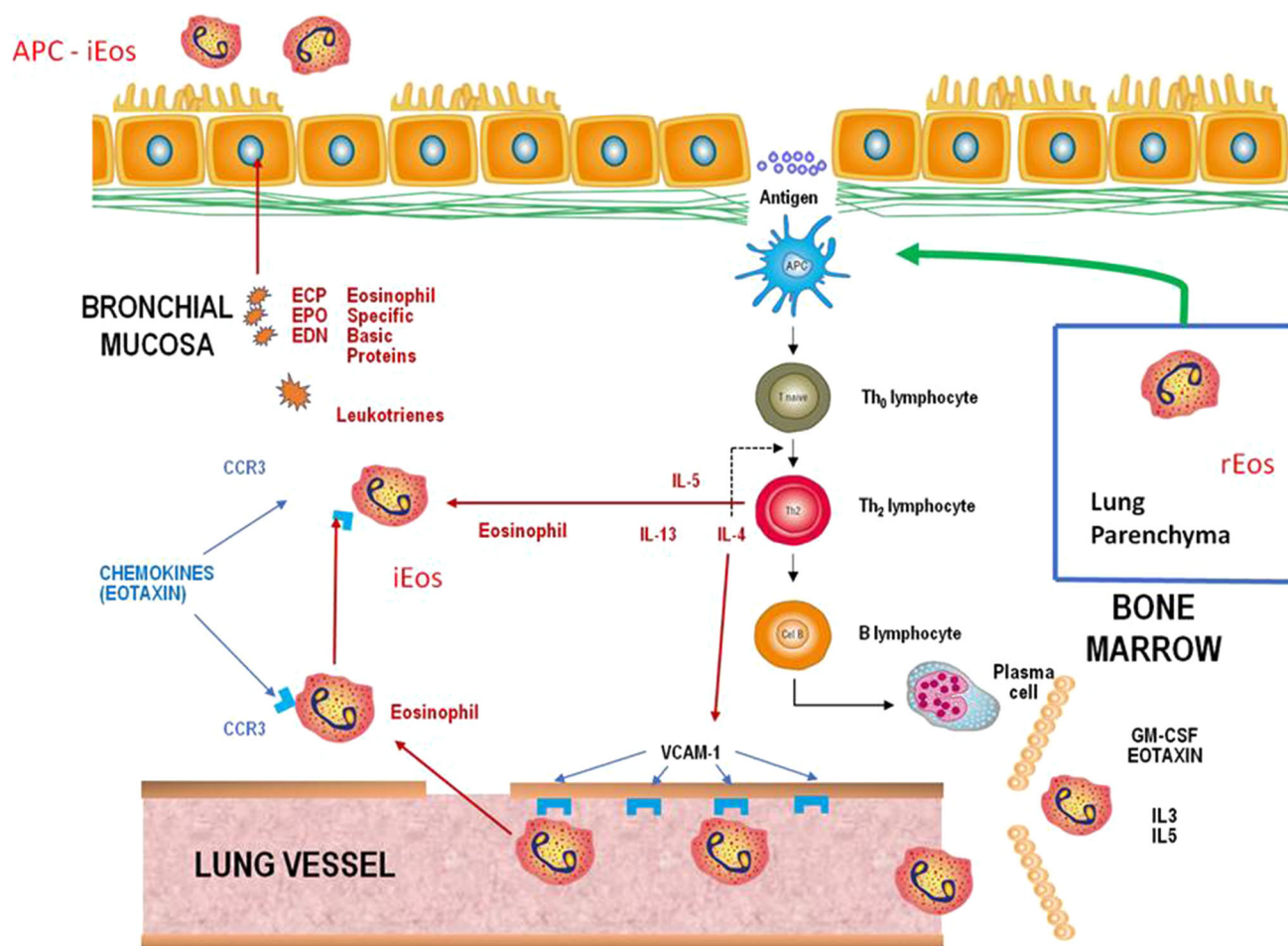


Fig. 3 Basic description of eosinophil biology relevant to the lung. This figure outlines the first part of the allergic cascade and the main site where eosinophils are synthesized (the bone marrow). For differentiation into eosinophils, the eosinophil progenitor (EosP) cells require the presence of IL-5. Eosinophils are released from the bone marrow into the blood stream. Eosinophils are retained in the target organ, in this case the bronchial mucosa, via capture by vascular adhesion molecules (VCAM-1) whose expression on the membrane surface of the endothelial cells is facilitated by IL-4, one of the T_H2 cytokines. Once in the bronchial mucosa, the eosinophils need to be activated to start releasing mediators that have toxic effects for the

bronchial mucosa. Eosinophils have also been found on the lumen of the airways, its origin being obviously the bronchial mucosa. Since all these eosinophils come from the blood stream after being produced (or induced) at the bone marrow level, they are called induced eosinophils (*iEos*). Recently, a population of eosinophils that reside in the lung parenchyma have been described, termed resident eosinophils (*rEos*). *APC* antigen-presenting cell, *ECP* eosinophil cationic protein, *EDN* eosinophil-derived neurotoxin, *EPO* eosinophil peroxidase, *GM-CSF* granulocyte-macrophage colony-stimulating factor, *IL* interleukin, *VCAM* vascular cell adhesion molecule

4 Effector Cells

Our greater understanding of human immunity has increased the number and types of cells in the immune system considered ‘effector cells’ or cells located at the end of a process whose actions are directly responsible for clinical symptoms. From an educational perspective, this section will differentiate between the classically described and recently emerged effector cells.

4.1 Classical Effector Cells: Mast Cells and Basophils

Mast cells and basophils are white blood cells (granulocytes) derived from the myeloid stem cell and are a part of the immune system. The similarities in their appearance and function meant that initially mast cells were considered to be tissue-resident basophils, but later it was shown that the two cells develop from different hematopoietic lineages and are thus different cells.

In their membrane, mast cells contain the high affinity receptor for IgE (FcεRI). When this receptor binds to IgE, the molecule is able to recognize the antigen and the acute phase of the allergic reaction occurs (Fig. 2). This acute phase occurs very fast and lasts approximately an hour. These cells were considered to be the end-stage of the allergic cascade, which in turn was considered to be the end of the physiopathological problem. Progressive improvement in the knowledge of the adaptive arm of the immunity reaction has shown that both mast cells and basophils are the cells responsible for releasing important mediators not only for the acute phase of the allergic cascade but also for feeding it back and stimulating other linked reactions [6].

4.2 Cells with a Recently Identified Effector Role: Eosinophils and Natural Killer T Cells

4.2.1 Eosinophils

Eosinophils were initially considered end-stage cells involved in host protection against parasite infection. Later their role in immunopathology was discovered, and today they are considered multifaceted leukocytes that contribute to various physiological and pathological processes depending on their location and activation status. They represent a small proportion of the total white cell count.

The life cycle comprises the bone marrow, blood, and tissue phases (Fig. 3; ESM 12). Eosinophils are mainly produced in bone marrow from pluripotential stem cells. The stem cells differentiate into a progenitor, which is able to produce mixed colonies of basophils and eosinophils. Among various hematopoietic factors, the most important trigger of eosinophil production is IL-5. IL-5 is responsible for the differentiation at the bone marrow level of stem cells into eosinophils and for their subsequent maturation. This process increases the concentration of eosinophils in peripheral blood. These eosinophils travel through the bloodstream and eventually pass through the pulmonary vessels. In asthma patients, eosinophils migrate from the bloodstream to the pulmonary parenchyma where they develop cytotoxic activity (Fig. 3; ESM 12).

Eosinophils are resident in various organs such as the gastrointestinal tract, mammary glands, and bone marrow, and they may play roles in tissue and immune homeostasis of these organs. It is generally considered that they are not present in healthy lungs. However, a recent article [44] described a subpopulation of eosinophils that may reside in the lung. So at present, we have two populations of eosinophils in the lung: residents (rEos) located in the lung parenchyma and that have receptors for IL-5 but appear to respond either little or not at all to increases in IL-5, and induced eosinophils (iEos) that play an essential role in

maintaining airway inflammation in chronic asthma located near the airway (Fig. 3) [45, 46]. The iEos may be localized in the bronchial mucosa, in the bronchial lumen, or in the lymph nodes, and their function depends on their location (Fig. 3; ESM 12).

Knowledge of the different functions of the eosinophils has progressively improved. They were first identified as a source of a number of regulatory or pro-inflammatory cytokines and chemokines [47], sometimes producing cytokines (autocrine cytokines) such as IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF) that are able to act on eosinophils themselves. Clinical studies have demonstrated the central role of eosinophils in the pathogenesis of asthma, including induction of AHR, increased mucus production, and airway narrowing and restructuring [48, 49]. All of these are characteristic signs and symptoms that eventually damage the tissues and cause organ dysfunction, so that the severity of the disease correlates with the levels of eosinophils in the peripheral blood [48, 49]. Due to these activities, they are considered effector cells.

Eosinophils have also developed other abilities which contribute indirectly to these effector characteristics. They are able to release IL-4 [50, 51], and the IL-4 protein has been localized to eosinophils in the airways [52] of patients with allergic diseases. When appropriately stimulated with eotaxin, human eosinophils rapidly release stored IL-4. In mice, eosinophils seem to play a role in long-term maintenance of plasma cells in bone marrow [53].

Although the presence of eosinophils in respiratory secretions is a hallmark of allergic airway disease, little attention has been paid to the potential physiological function of intraluminal eosinophils, or to whether eosinophils might migrate back across the epithelium to re-enter submucosal tissues (Fig. 3; ESM 12).

Many cell types express class I MHC molecules for the presentation of intracellular antigens. These molecules may stimulate a cytotoxic T-cell immune response, eventually destroying the cell and the pathogen within. This is especially relevant in the case of the most common class of intracellular pathogens, the virus. Viruses infect nearly every tissue of the body, so all these tissues must necessarily be able to express class I MHC. On the other hand, class II MHC molecules are expressed only in the cells of the immune system, specifically cells that affect other arms of the immune response. Thus, these cells are called 'professional' antigen-presenting cells to distinguish them from the ones that bear class I MHC [54]. The three types of professional antigen presenters are macrophages, DCs, and B cells. If eosinophils within the airways are to be able to present inhaled antigens to T cells, several attributes are necessary: migration from airways, the ability to express class II MHC proteins by effectively internalizing,

processing, and presenting airway-derived antigens to T cells, and homing to lymph nodes. Eosinophils have been recognized as potential antigen-presenting cells under certain conditions [55, 56] (Fig. 3; ESM 12). Although circulating eosinophils are generally devoid of surface MHC II expression, they are induced to express MHC II [57, 58] after appropriate cytokine stimulation. Once recruited into the airways, in allergic diseases they are exposed to inhaled allergens. Within the airway lumen they can process inhaled antigens, travel to regional lymph nodes, and function in vivo as antigen-presenting cells to stimulate responses of CD4⁺ T cells [59, 60]. For their part, rEos seem to play a homeostatic or immunomodulatory role; the effect of drugs with eosinophil-depleting action is not clear [25] (Fig. 3; ESM 12).

4.2.2 Natural Killer Cells

Many NKT cells express a highly restricted T-cell receptor (TCR) repertoire and are called invariant NKT cells (iNKT) [61]. Previously, it has been reported that T_H2 cytokines such as IL-4 and IL-13 derived from NKT cells were indispensable for the development of antigen-induced asthma (Fig. 1; ESM 9). Akbari et al. [62] reported that the development of antigen-induced AHR and T_H2 immune responses (airway eosinophilia and antigen-specific IgE production) was significantly impaired in NKT cell-lacking mice. These authors also reported that direct activation of pulmonary NKT cells could induce pulmonary eosinophilia, serum IgE production, and AHR [63]. In their study, even in the absence of conventional CD4 T cells, the activation of pulmonary NKT cells induced eosinophilia and severe AHR. Thus, it was suggested that NKT cells might function as effector cells for inducing and amplifying allergen-induced asthma.

Nagata et al. [64] observed that T_H2 responses were severely induced even if NKT cells were lacking. The reason for the retained T_H2 responses might be the fact that the IL-7-like cytokine thymic stromal lymphopoietin (TSLP) can affect conventional T cells and DCs and thus enhance T_H2 responses [65]. In spite of the retained T_H2 responses, however, the development of AHR was almost completely eliminated. These results indicate that T_H2-derived eosinophilia and IgE production are not necessary for upregulating AHR. On the other hand, IL-13, known to be able to directly induce airway contraction and mucus secretion [66] and is produced by NKT cells, seemed responsible for the exacerbation of AHR since TSLP mainly induced IL-13 production by NKT cells [67]. It is therefore accepted that TSLP has the potential to induce T_H2 immune responses. DCs activated by TSLP are responsible not only for T_H2 priming but also for the maintenance and further polarization of T_H2 central

memory cells in allergic diseases [63]. AHR seems to be controlled by NKT cells (presumably due to their production of IL-13), even in the situation of TSLP elevation and T_H2 exacerbation.

5 The Relationship between Adaptive and Innate Immunity

The innate immune system evolved long before the adaptive immune system, and in many aspects, the older system supports the function of the newer one. Without the vital antigen-presenting function of innate immune cells, and without the production of cytokines of innate immune origin, the adaptive immune responses would be ineffectual. In this respect, while the two forms work hand in hand, adaptive immunity is subordinated to innate immunity [68]. We now have enough information to describe the collaborative effects of innate immunity on the allergic cascade as well as its own inflammatory effects (Fig. 2; ESM 11).

The TSLP, a 'stromal lymphopoietin' (or tissue-derived cytokine), has been shown to induce the production of T_H2-attracting chemokines and to prime T_H2 cell development [65, 69]. TSLP is of particular interest because it appears to be produced at the epithelial interface [69] and may represent a mechanism through which environmental stimuli initiate T_H2 responses to the allergen and, through chemokine production, retain or attract T_H2 cells in asthmatic airways.

The TSLP receptor (TSLPR) has been found on DCs, T cells, B cells, mast cells, NKT cells, and monocytes. Indeed, TSLP has an important role in the maturation of T-cell populations through activation of antigen-presenting cells. The ability of myeloid dendritic cells (mDCs) to respond to TSLP is consistent with the finding that they seem to express the highest levels of TSLPR [65, 70]. TSLP expression is linked to many disease states including asthma. Studying tissue samples obtained from bronchial biopsies, Ying et al. [71] showed that TSLP mRNA-expressing cells were identifiable in the bronchial epithelium and submucosa in both asthmatics and controls but were significantly higher in asthmatics.

TSLP has several functions. In the innate immunity arm, TSLP produced by epithelial cells may rapidly induce an innate phase of allergic inflammatory response by activating mDCs, mast cells, and NK cells to produce T_H2 cytokines, chemokines, and proinflammatory cytokines. The role of TSLP in the triggering of an early innate phase of allergic inflammation is supported by an in vivo observation that it can induce moderate airway inflammation in B- and T-cell-deficient mice [72]. The TSLP-induced innate phase of allergic immune response has four

important consequences: it induces a transient inflammatory response via IL-6 and IL-13; it recruits eosinophils via IL-5 and eotaxin-2, as well as neutrophils via IL-8; it prepares for the local adaptive T_H2 responses by activating TSLP-mDCs (TSLP-DC) during the adaptive phase of allergic immune responses; and finally it teaches a unique population of mDCs to induce naïve $CD4^+$ T cells to differentiate into inflammatory T_H2 cells [73].

In adaptive immunity, TSLP strongly upregulates the expression of MHC class II on human mDCs, favors the maturation of T-cell populations through activation of antigen-presenting cells, and potently activates human mast cells [74].

So, it is clear that the two arms of immunity are inter-related. As stated above, until recently innate immunity was basically considered as a ‘barrier’ (formed by the bronchial ciliated epithelium) accompanied by some macrophage-like cells with a capacity for nonspecific phagocytosis. But now this concept has evolved because it has been observed that the bronchial ciliated epithelium, when damaged by external agents, undergoes a disruption accompanied by the rupture of cells which release protein substances contained in their cytoplasm and which, on reaching the extracellular medium, become mediators of immune reactions. This ‘barrier’ epithelium now behaves like a ‘secretory’ epithelium. Mediators of these bronchial epithelial cells act quickly on the innate immunity arm, stimulating type I innate lymphoid cells (NKT cells), ILC-2 and ILC-3. These cells will finally release T_H2 interleukins (IL-4, IL-5, and IL-13). For their part, macrophages considered to be capable of exerting only a phylogenetically very primary phagocytic function, contribute to the chronicity of the process through the adaptive immunity. Both arms are ultimately responsible for eosinophil recruitment.

This concept of T_H2 -high asthma has evolved quickly and is now called type-2 cytokine ‘high’ asthma, given the ability of non- T_H2 cells to express these cytokines, and now includes many further biomarkers. Interestingly, although atopy and IgE have been traditional markers of an ‘allergic phenotype’, many atopic individuals do not demonstrate T_H2 -like inflammation and many with increases in type-2 cytokines and eosinophils are not atopic [34, 75].

6 Pharmacological Effects of Monoclonal Antibodies for Asthma

This section discusses mAbs already commercialized (omalizumab, mepolizumab, and reslizumab) and those in late stage of clinical development (dupilumab and benralizumab). Several other molecules are under development.

6.1 Omalizumab

Omalizumab became commercially available for severe asthma treatment more than a decade ago. This drug is an anti-IgE monoclonal antibody that is approved for use in patients >12 years of age with moderate-to-severe persistent asthma that is uncontrolled with ICS+LABA therapy. Omalizumab’s structure allows it to inhibit IgE interactions for both the high- and low-affinity IgE receptors [76]. Additionally, due to homeostatic mechanisms, omalizumab causes the dissociation of high-affinity IgE receptors from IgE.

Many trials [77–80] and real-life studies [81–88] with omalizumab have demonstrated reductions in asthma exacerbations and improved asthma control. Initially, omalizumab was thought to exert its effect by blocking free IgE, so that after sensitization, by binding to the free IgE to form trimers or hexamers it prevented them from binding to their receptors on the surface of mast cells and basophils. If the story ended here, by blocking all the IgE the problem would be solved. However, although 99% of the free IgE is blocked at 4–6 months of treatment, not all patients can be considered responders; in addition, those treated with suboptimal doses would not be expected to respond to treatment.

The Australian Xolair Registry revealed that in some patients with weight >150 kg and/or a blood IgE concentration >1500 IU/mL, the administration of the highest recommended dose of omalizumab in the dosing tables also obtained significant improvements in symptom control, quality of life, and forced expiratory volume in one second (FEV_1) [89].

It was therefore suggested that the drug might have an immunomodulatory effect [6], exerted in different ways: first, by the internalization of receptors on the cell surface of distinct cells involved in adaptive immunity as the concentration of free IgE decreased; and second, by exerting a downstream effect in the allergic cascade, thus reducing the release of T_H2 cytokines. Finally, the internal feedback of the allergic cascade underwent a downregulation effect due to the decreased stimulation of DCs by free IgE. The effect of internal feedback was recently endorsed after the observation that in chronic severe asthma, the allergic cascade activity is maintained to some extent even in the absence of the allergen that triggers the event; a recent pilot study showed that omalizumab was equally effective in severe allergic asthma whether the asthma was due to perennial or seasonal allergens [90] (ESM 13).

It has recently been suggested that omalizumab may modulate the innate immunity in different ways. It was initially shown that the production of interferon- α (IFN- α) after a viral infection was lower in asthma patients

compared with healthy subjects and that there was an inverse correlation between IgE and FcεR1α expression on plasmacytoid dendritic cells (pDCs) and blood IgE concentration [91]. It is known that pDCs involved in the allergic immune response play a crucial role in innate immune defenses against (predominantly viral) infections. In healthy individuals, pDCs recognize an invading respiratory virus through a Toll-like receptor and rapidly produce vast amounts of type I and III IFN. IFN secretion by pDCs acts in a paracrine manner to establish the immediate phase of the antiviral state in non-specialized antiviral cells.

In contrast, in a genetic or functional defect, pDCs fail to induce the antiviral state in airway epithelial cells and facilitate injury to the airway epithelium; this releases T_H2 cytokines which positively feed the allergic cascade, recruit eosinophils, and promote the recruitment and expansion of type-2 innate lymphoid cells, which support eosinophil survival through the production of interleukin [92]. DCs of patients with asthma display an increased expression of the high-affinity IgE receptor. Omalizumab seems to favor the anti-viral immune responses of the pDCs by reducing the FcεRI on their surface, and by preventing the IgE from binding to these FcεRI [92]. Omalizumab thus helps to prevent exacerbations of airway diseases triggered by viral infections [93], as the Inner-City Anti-IgE Therapy for Asthma (ICATA) [94] and Preventative Omalizumab or Step-up Therapy for Severe Fall Exacerbations (PROSE) studies showed [95]. Thus, pDCs play a role in both innate and adaptive immunity; both pathways seem to influence each other.

Eosinophilic asthma has been considered a phenotype of severe asthma. Allergic asthma can present with normal or increased numbers of eosinophils. The guidelines generally do not distinguish between the pathway responsible for the eosinophilia (i.e., atopic or non-atopic). Thus, there seems to be some degree of laboratory overlap. Omalizumab can decrease the number of eosinophils in sputum and the bronchial mucosa and to a lesser extent in peripheral blood [96]. When in the bloodstream, eosinophils are not harmful and so the slight decrease in eosinophil concentration after omalizumab administration does not reduce the molecule's pharmacological benefits. By exerting a downstream effect on the allergic cascade, omalizumab decreases the release of IL-4 by T_H2 cells. By reducing the blood free IgE concentration, it also decreases the number of activated basophils, which are the cells responsible for a notable amount of the IL-4 release. As a consequence, it notably reduces the traffic of eosinophils from peripheral blood to bronchial mucosa. In the innate arm, the situation is slightly confusing because ILC2 only release IL-4 under certain situations, such as stimulation with leukotriene D4 and prostaglandin D2 released by mast cells [16, 97]. By

modulating the number and activity of mast cells, ILC2 are expected to release lower amounts of IL-4. As for IL-5, omalizumab can reduce its release from both of its origins. In adaptive immunity, omalizumab has a similar effect as on IL-4; it decreases its release by T_H2 lymphocytes and activated eosinophils as well as by activated mast cells (the most important releaser of IL-5). In summary, omalizumab downregulates the activity of the 'classic' effector cells, mast cells and basophils (ESM 14).

In innate immunity, by improving the balance between allergy and the antiviral effect of pDCs on the one hand and avoiding epithelial cell damage on the other, omalizumab also tends to reduce IL-5 concentration and thus modulate eosinophil recruitment. Moreover, the control over the release of eosinophil toxic molecules and mediators will avoid the progressive deterioration of the bronchial epithelium and then downregulate its secretor activity, which will in turn decrease the release of mediators stimulating innate lymphoid cells. In this arm of immunity, omalizumab also exerts an effect on the newly identified effector cells, eosinophils and ILCs. The effect on the innate arm of immunity helps to explain why omalizumab is helpful in allergic eosinophilic asthma. By decreasing the number of sputum eosinophils, it also decreases the antigen-presenting cell activity of some of these eosinophils. No information is available regarding the drug's possible effect on rEos.

Omalizumab exerts a downstream effect on both the adaptive and innate cascades and thus reduces IL-5 release (ESM 14). There are several possible explanations for the benefits of this drug in eosinophilic asthma patients. At the bone marrow level, the low concentration of IL-5 may reduce the expression of its receptor on the surface of CD34+ cells. In the bloodstream, it may mitigate eosinophil progenitor (EoP) traffic much as it does with eosinophils, and in the bronchial mucosa, the low concentration of IL-5 in the media can downregulate the maturation process of EoP into eosinophils. These benefits may occur in both allergic and non-allergic patients.

In some cases, omalizumab fails to improve allergic asthma. This is probably due to the fact that the predominant physiopathological dysregulation comes initially from adaptive immunity, probably as a consequence of the highly intense activity of the allergic cascade, which severely damages the bronchial epithelium and causes it to release other mediators (IL-25, IL-33, TSLP) that strongly stimulate IL-5 release (Fig. 2; ESM 11). Then, the amount of IL-5 coming from the innate arm is high enough to maintain persistently high eosinophil recruitment. Eosinophils will act as effector cells that will damage the epithelium, thus causing a loop that only drugs with a highly selective anti-eosinophilic recruitment effect or toxic activity against activated eosinophil (anti-IL-5 or

anti-IL-5R) can mitigate. In these cases in which allergic eosinophilic patients do not respond clinically to omalizumab, the switch to an anti-IL5 is indicated. An alternative in the future might be a combination of both mAbs to interfere in both pathways at the same time, although there is no information about this possibility at present.

Finally, in a post-hoc analysis (EXTRA [Study of Omalizumab (Xolair) in Subjects with Moderate to Severe Persistent Asthma]), Hanania et al. [98] observed that patients who responded the best to omalizumab were those with periostin ≥ 50 ng/mL, exhaled nitric oxide (FeNO) values ≥ 19.5 ppb, and $\geq 260/\mu$ eosinophils. Their study was designed to identify possible biomarkers but probably the most relevant conclusion would be that the allergic patients who respond best to omalizumab are those with eosinophilia. This is why some authors (myself included) advocate prescription of omalizumab as the first mAb in severe eosinophilic asthma due to allergy [99].

Omalizumab has also been found to be useful in non-atopic patients [100–102]. The rationale for the use of omalizumab in non-allergic patients is based on the finding that IgE can be produced locally in mucosal tissue without any increase in IgE levels in the blood. In allergic patients, a significant decrease in surface Fc ϵ RI expression caused by omalizumab is also seen in DCs, which are potent antigen-presenting cells. In a randomized study, García et al. [103] evaluated the changes in the expression of the Fc ϵ RI in non-atopic patients. The results clearly showed that, compared with placebo, omalizumab decreased the concentration of Fc ϵ RI on basophils and plasmacytoid DCs to the same extent as in severe atopic asthma.

At present, three hypotheses have been proposed to explain the possible mechanisms of anti-IgE treatment in intrinsic asthma [104–106]. The first one proposes that patients with intrinsic asthma have a localized allergy with high concentrations of allergen-specific IgE antibodies in the airways (the concept of entropy) [104]. Interestingly, an alternative or additional explanation may be provided by Kalesnikoff et al. [105] who observed that by binding to its high-affinity receptor (Fc ϵ RI), IgE was able to induce intracellular signaling pathways, resulting in the production of cytokines (e.g., IL-4, IL-6, IL-13, TNF- α) and the enhancement of mast-cell survival without cross-linking by allergens. In addition, IgE can directly bind and activate receptors present on eosinophils, neutrophils, and monocytes. The third hypothesis is related to the above-mentioned activity of omalizumab favoring the immune response to viral infections [93].

As a result of all these different modes of action, omalizumab has also been shown to interfere in certain stages of the remodeling process [106–108]. We will not develop these points further here as this is not the purpose of the paper.

The issue of whether treatment can be withdrawn after a certain period remains unclear. After the Lowe et al. [109] model that postulated that after 5 years of treatment there was an underproduction of IgE during a period of 15 years, several studies were published. The studies by Nopp et al. [110, 111] seem to be in this line and the recent XPORT (Evaluating Xolair Persistency of Response After Long-Term Therapy) [112] study showed that, after a minimum of 5 years of treatment, some patients tolerated omalizumab withdrawal without exacerbation during the first year after withdrawal. There are no clear conclusions on this issue at present, but it seems that at least some patients do tolerate omalizumab withdrawal.

6.2 Anti-IL-5: Mepolizumab and Reslizumab

IL-5 is a critical cytokine for eosinophil maturation and is responsible for the increased levels of eosinophils in the blood [22, 113]. It also activates eosinophils, prolongs their survival in the bloodstream, and provides an essential signal for their migration to tissues [23, 24, 113–115]. In the airways of asthmatic patients, the major IL-5-producing cells are T_H2 lymphocytes, NKT cells, ILC-2, mast cells, and the eosinophils themselves [25]. Blocking the IL-5 allows this process to be stopped; this is the basis of treatment with anti-IL-5 monoclonal antibodies (mAb) in eosinophilic asthma [116].

Many attempts to target eosinophils for the treatment of asthma by means of IL-5 neutralization have been made over the years. Administration of anti-IL-5 antibodies demonstrated a reduction in eosinophilic airway inflammation and in the response to methacholine challenge [117]. In addition, mice with the inactivated IL-5 gene did not show an increase in eosinophil levels in the blood and the airways, AHR, or lung injury compared with control animals in a murine asthma model [118]. These observations demonstrated the significant role played by IL-5 and eosinophils in the pathogenesis of asthma. Preclinical studies demonstrated that asthma improved significantly when IL-5 signaling was deficient or in the absence of eosinophils [119].

Mepolizumab and reslizumab are humanized anti-IL-5 antibodies (IgG₁ and IgG₄, respectively) that neutralize circulating IL-5 by binding to the same epitope that IL-5 binds to its receptor on the eosinophil cell membrane.

The DREAM (Mepolizumab for Severe Eosinophilic Asthma) [120], MENSA (Mepolizumab as Adjunctive Therapy in Patients with Severe Asthma) [121], and SIRIUS (Steroid Reduction with Mepolizumab Study) [122] studies were pivotal to mepolizumab development. A post-hoc analysis from the MENSA and SIRIUS trials revealed an overall 47% reduction in asthma exacerbation rate calculated versus placebo; the reduction was up to 52% in

patients with eosinophil counts of at least 150 cells/mL and 70% in patients with eosinophil counts of at least 500 cells/mL. However, the predicted efficacy of mepolizumab was reduced in patients with baseline eosinophil counts of 150 cells/mL or less [123].

As for reslizumab, Castro et al. [124] observed that the decrease in peripheral blood eosinophils was accompanied by an improvement in FEV₁ and quality of life (Asthma Control Questionnaire-5 [ACQ-5]), especially in the group of patients with nasal polyposis. Phase III of the drug's development consisted of five large studies [124–128]. The first two studies by Corren et al. [125] and Bjermer et al. [126] defined a peripheral blood eosinophil value ≥ 400 cells/ μ L at the time of recruitment as eosinophilia. In contrast to the mepolizumab trials, these studies included patients who were receiving medium-range doses of ICS. Both studies showed that reslizumab at doses of 3 mg/kg obtained significant improvements compared with placebo, in terms of both lung function and asthma symptoms. Corren et al. [125] observed a mean FEV₁ increase of 270 mL and Bjermer et al. [126] an increase of 160 mL after 16 weeks of treatment.

These excellent results have recently been corroborated by two large phase III studies by Castro et al. [127]. Patients with uncontrolled asthma treated with high doses of ICS and ≥ 400 eosinophils/ μ L in peripheral blood were recruited. After a year, reslizumab showed a 54% reduction in exacerbations as well as a delay in the onset of the first exacerbation. It is striking that the greatest benefits were observed in the group of patients receiving treatment with oral corticosteroids. In this population, the reduction of exacerbations compared with the placebo group was 68%.

Both drugs thus demonstrate a greater effectiveness in patients with higher values of eosinophils in blood (>400). This is not surprising since the drugs act by inhibiting IL-5, which exerts its fundamental effect on eosinophils. Open-label long-term studies have demonstrated that the clinical benefits of both drugs were maintained [128, 129].

Eosinophil differentiation and maturation from CD34+ hematopoietic progenitors occurs mainly in the bone marrow stimulated by IL-5 [130]. Eosinophil recruitment to the airways, including EoP, is also primarily regulated by IL-5 and eotaxin [130].

In mild allergic asthmatics, bone marrow-derived IL-5 responsive EoP are elevated in blood, in the mucosa of the upper and lower airways, and in the airway secretions of allergic asthmatics [131]. Menzies-Gow et al. [132, 133] found that treatment with an anti-IL-5 caused a complete maturational arrest of eosinophils in the bone marrow and blood but only partially depleted mature eosinophils in the bronchial mucosa; this was associated with a significant but incomplete attenuation of EoP numbers within the bronchial mucosa, suggesting that local IL-5-driven

differentiation may play an important contributory role in the development of airway eosinophilia. Sehmi et al. [131] observed a marked luminal increase in EoP in severe asthmatics compared with patients with mild atopic asthma.

A logical interpretation would be that if IL-5 can upregulate its own receptor expression on CD34+ cells, an anti-IL-5 treatment would attenuate EoP numbers. However, the IL-5R expression on CD34+ cells is controlled by a delicate combinatory balance between the threshold expression levels of different transcription factors that serve as master regulators for eosinophil-lineage commitment from common myeloid precursors [134]. The expression of IL-5R on the surface of CD34+ cells regulates eosinophilopoiesis. This receptor provides a permissive rather than an active signal for IL-5-mediated terminal differentiation of EoP, which may explain the increased EoP numbers in the blood.

These findings are in line with studies that have shown significant modulation of local airway eosinophilia in asthmatics using biological agents targeting IL-5 [132, 135], while those that targeted the recruitment of mature eosinophils by chemoattractants such as eotaxin were ineffective [136]. The dissociation observed between the systemic and local airway effects of mepolizumab therapy suggests that local maturation rather than systemic recruitment of mature cells may be the predominant process promoting persistent airway eosinophilia in severe asthmatics.

Sehmi et al. [131] showed that patients with severe eosinophilic asthma present an exaggerated eosinophilopoietic process in their airways. Treatment with subcutaneous mepolizumab 100 mg significantly attenuated the systemic differentiation of eosinophils, but did not suppress local airway eosinophil differentiation to mature cells. Targeting IL-5-driven eosinophil differentiation locally within the lung may be of relevance for the optimal control of airway eosinophilia and asthma.

However, as in the case of omalizumab, within 3 months of cessation of mepolizumab, Haldar et al. [137] found that eosinophil blood and sputum counts had risen once again to pretreatment levels. The increase was also associated with a return of frequent asthma exacerbations, and ultimately a greater need for asthma control. The data from Kim et al. [138] suggest that anti-IL-5 therapy had significantly increased IL-5 levels 1 month after therapy stopped and that this increase in serum IL-5 was responsible for the rebound eosinophilia observed in some patients.

As noted above, prolonged airway eosinophilia is at least partly dependent upon continuous de novo eosinophil production. Significantly, this is attenuated by the in vivo blockade of IL-5, both in the airways and in the bone

marrow [139]. In allergic asthmatics, bone marrow-derived IL-5 responsive EoP present high levels in the blood, in the mucosa of the upper and lower airways, and in airway secretions. In allergic rhinitis patients, local IL-5-dependent differentiation of EoP was observed when nasal biopsies were performed. This showed that local differentiation (i.e., in situ eosinophilopoiesis) can rapidly increase mucosal levels of mature eosinophils during an inflammatory response.

6.3 Agents in Late Stage of Clinical Evaluation: Benralizumab and Dupilumab

Attempts to target eosinophils for the treatment of asthma by means of IL-5 neutralization have resulted in only partial removal of airway eosinophils, and this warrants the development of more effective interventions to explore further the role of eosinophils in the clinical expression of asthma. In humans, the IL-5 receptor (IL-5R) is expressed exclusively on eosinophil and basophil progenitors in the bone marrow and on mature eosinophils and basophils. A drug with a higher anti-eosinophilic effect might be developed by targeting not the IL-5 but its receptor. Benralizumab is an anti-IL-5R antibody that targets blood and airway eosinophils in patients with uncontrolled eosinophilic asthma [140].

Certain aspects deserve to be emphasized. By blocking the receptor for IL-5, benralizumab avoids the binding of the ligand to its receptors and thus prevents its direct cell effect on eosinophils without modifying free IL-5 concentration. This can offer some additional benefits which are outlined below. Benralizumab is a humanized afucosylated mAb designed to target IL-5R α expressed on eosinophils and basophils. The lack of a fucose sugar moiety on the oligosaccharide core enhances the drug's binding affinity to Fc γ RIII α and augments antibody-dependent cell-mediated cytotoxicity (ADCC), inducing apoptosis of target cells, a property that is not observed in anti-IL-5 mAbs [20]. In vitro studies have demonstrated that basophils constitutively express IL-5R α but do not require the cytokine IL-5 for survival. In vivo basophils play a critical role in the development of IgE-mediated chronic allergic inflammation in the skin, and increase in numbers in the airways of asthmatic subjects on allergen provocation [141]. Benralizumab potently induces basophil apoptosis in vitro (its ability to deplete basophils in vivo and its downstream consequences remain to be demonstrated) [140].

Benralizumab's ability to block the IL-5R may prevent several problematic situations. For example, the formation of cytokine/anti-cytokine immune complexes does not guarantee cytokine neutralization, as these complexes can actually increase the potency as well as the concentration

of a cytokine. It has been shown that subjects treated with mepolizumab display higher levels of IL-5 in the circulation, the majority of them in IL-5/anti-IL-5 complexes [142].

A phase IIb dose-ranging study of benralizumab revealed that a dose of 100 mg reduced asthma exacerbations in patients with eosinophilic asthma. Additionally, patients with blood eosinophil levels of at least 300 cells/mL had lower *exacerbation* rates on benralizumab therapy [140]. More recently, two phase III trials of severe, uncontrolled, eosinophilic asthma, the SIROCCO (efficacy and safety of benralizumab for patients with severe asthma uncontrolled with high-dosage inhaled corticosteroids and long-acting β 2-agonists) [143] and CALIMA (benralizumab, an anti-interleukin-5 receptor α monoclonal antibody, as add-on treatment for patients with severe, uncontrolled, eosinophilic asthma) [144] studies, confirmed the previous findings: patients with add-on therapy with benralizumab were noted to have a significantly lower exacerbation rate and improved FEV₁.

The benefits, risks, and therapeutic limitations of benralizumab appear to be very similar to the other two anti-IL-5 drugs, except for the fact that ADCC in some cases may eliminate all the eosinophils in the blood. More time is needed to assess this possible side effect in depth, and at present we do not know what effect this drug may have on rEos.

Dupilumab is a human monoclonal antibody to the α unit of IL-4 receptor, inhibiting binding and downstream signaling of both IL-4 and IL-13, and thereby indirectly inhibiting IgE production by preventing B-cell IgE class switch [145].

It thus exerts an indirect effect on the classic effector cells (masts cells and basophils). Since it also decreases the release of IL-5 by T_H2 lymphocytes and mast cells it reduces eosinophil traffic and recruitment. In the innate cascade, the drug blocks the effects of IL-4 as well as IL-13 and thus deactivates one of the newly identified effector cells, the NKT cells. It does not seem to affect IL-5 release by ILC2 and so probably does not downregulate local eosinopoiesis.

From a clinical point of view, the study by Wenzel et al [146] revealed that dupilumab reduced serum IgE levels in the treatment group and also other biomarkers of T_H2 inflammation, including FeNO. That early study demonstrated the efficacy of dupilumab in moderate-to-severe persistent asthma in patients with elevated eosinophil levels (blood eosinophil level >300 cells/mL or sputum eosinophils of 3%). In a more recent phase IIB trial [147], these same authors showed improvements in lung function and reductions in exacerbations, irrespective of patients' baseline eosinophil levels. Dupilumab is not currently

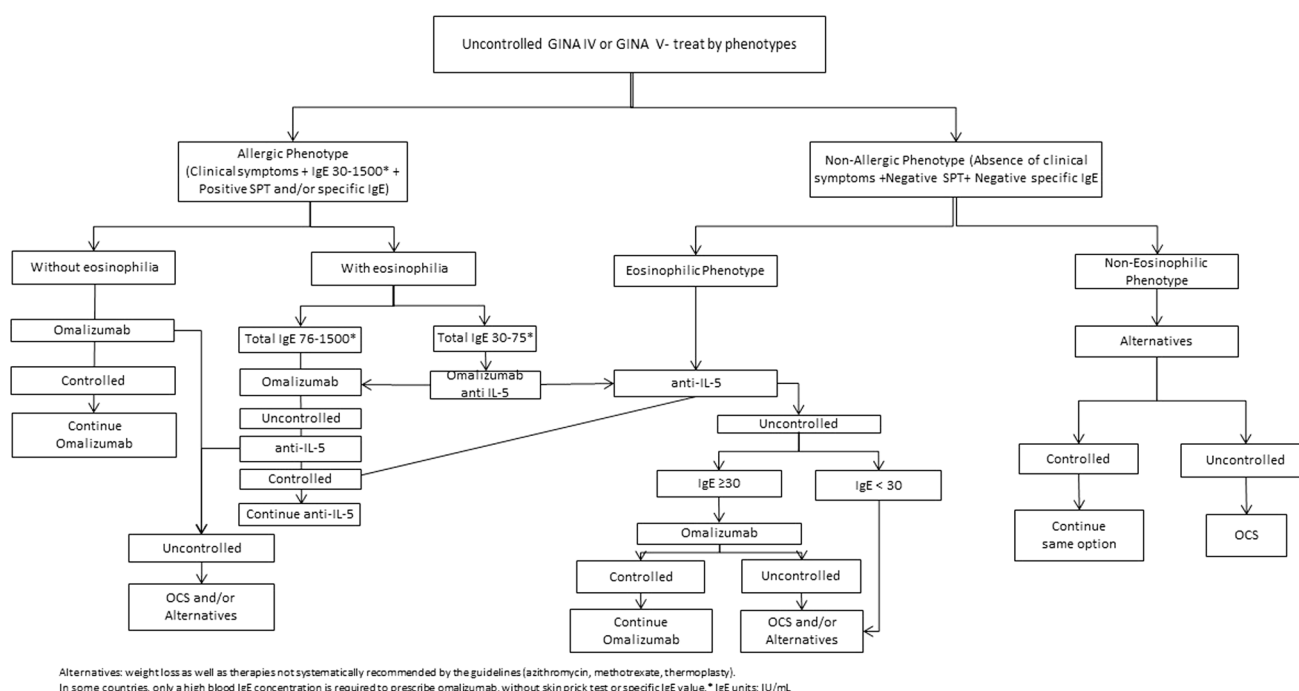


Fig. 4 Decision tree for the treatment of uncontrolled severe asthma. *GINA* Global Initiative for Asthma, *IgE* immunoglobulin E, *OCS* oral corticosteroids, *SPT* skin prick test

approved by medical agencies for asthma, and is still undergoing clinical trials.

7 Conclusions

This article has summarized the immunology and pathophysiology of adaptive and innate immunity, as well as the possible effects of the different antibodies. Despite all the available information, consensus documents do not establish the order to follow when deciding which mAbs should be considered a first option or an alternative [148]. Based on the mechanistic information that has been extensively reviewed, I propose a series of strategies for deciding which mAb to use (Fig. 4; ESM 15).

This decision tree is based upon the different phenotypes patients may have. It should be noted that the allergic phenotype is not defined only by a positive skin prick test, nor is the eosinophilic phenotype defined by an elevated blood eosinophil concentration (the number of peripheral blood eosinophils is frequently high in allergic patients too). The phenotype is a clinical description that includes clinical symptoms plus laboratory test results.

The first general criterion used in this algorithm is the differentiation between allergic and non-allergic phenotypes. In allergic patients, omalizumab will be the first option, regardless of the blood eosinophil concentration. Anti-IL5 may be an alternative in those patients in which omalizumab has failed and the blood eosinophil

concentration is elevated. Since omalizumab has shown to be definitively effective when blood IgE concentration is >76 IU/mL, in allergic patients with a high number of eosinophils and relatively low blood IgE concentration (IgE between 30 and 75 IU/mL), either omalizumab or anti-IL-5 can be prescribed. If the calculated dose of omalizumab exceeds the dose advised in the dosing tables (due to weight or blood IgE concentration >1500 IU/mL), a trial should be performed with the highest authorized dose. In the non-allergic phenotype arm, specifically in the eosinophilic phenotype, when anti-IL-5 has failed, omalizumab can be tried if blood IgE concentration is ≥30 IU/mL (compassionate use).

The alternative treatments include weight loss as well as therapies not systematically advised by the guidelines which may be tested by experienced research groups. In some cases (neutrophilic asthma), oral corticosteroids are not expected to work although a trial course may be recommended.

Drugs currently not marketed have not been included. In all probability, these strategies will be modified and new alternatives will appear in the near future, but the rationale proposed in this paper is unlikely to change significantly.

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