Chapter

# Navigating the Terrain: Type 2 Cytokines and Biologic Intervention in Severe Eosinophilic Asthma

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## Abstract

Asthma is a chronic respiratory disease characterized by bronchial hyperreactivity. There are several endotypes of which allergic asthma is the most common. Severe eosinophilic asthma is prevalent in approximately 5% of asthmatics and its phenotype overlaps with allergic asthma and type 2 inflammation. Patients with refractiveness to corticosteroids underline the difficulty in controlling persistent inflammation in severe eosinophilic asthma. The focus of biological therapies is geared towards the understanding of the intricate interplay of the cytokines that drive the eosinophil's ability to induce chronic inflammation with airway obstruction. This chapter takes the reader down a historical journey of initial studies that were performed using mouse helper T cell clones for reconstitution experiments to unravel the mechanism of the role T helper 2 cytokines play in allergic asthma. We then reviewed the classic in vivo experiments that demonstrated how antibodies to IL5 can down regulate eosinophils in the blood and their progenitors in the bone marrow of mice. We also delve into the complex interaction of the alarmins on the cytokines triggers of allergic inflammation with elevated eosinophils. Finally, we review the clinical literature on the beneficial effects of humanized monoclonal antibodies in use for treatment of patients suffering from severe eosinophilic asthma.

**Keywords:** cytokine, antibody, alarmin, asthma, eosinophil, inflammation

## 1. Introduction

Asthma is a chronic respiratory disease characterized by bronchial hyperreactivity (narrowing of the airways), wheezing and tightness of the chest [1, 2]. It affects more than 300 million people worldwide with approximately 25 million in the United States [3–6]. It is one of the most chronic diseases in children with the morbidity and mortality rates highest among African American children in the United States [7]. They are at least 10 times more likely to die from asthma than their white counterparts [7–9]. It is

a complex and heterogenous disease with varying severity that has a great spectrum of symptoms and wide differences in treatment efficacy [10]. The future in asthma management is to classify its many patterns known as endotypes, that connect recognizable characteristics with immunological mechanisms [5]. These endotypes can range from allergic bronchopulmonary aspergillosis, viral-exacerbated asthma, exercise-induced asthma to allergic and eosinophilic asthma [5, 10, 11].

Allergic asthma is the most common endotype as it affects approximately 66% of patients with asthma and more than half of the patients with severe asthma [12]. It is characterized by elevated levels of serum IgE (>0.35kU/L) with specificity for allergens in sensitized individuals [13]. These allergens are often innocuous and ubiquitous such as the antigens in house dust mites, pollen, dog and or cat dander. Atopic individuals diagnosed with allergic asthma show a positive skin prick test to allergens along with bronchial hyper-responsiveness and elevated levels of blood eosinophils [13].

Eosinophilia of the airways is observed in greater than 50% of all asthmatics. Those patients with sputum eosinophilia (≥3%) and blood eosinophil levels of ≥300cells/μl are designated as having severe eosinophilic asthma [13, 14]. High blood eosinophil count is a risk factor for asthma [15, 16]. Individuals exposed to tobacco smoke as well as ex-smokers have significantly higher levels of eosinophils when compared to non-smokers [17]. Indeed, in a murine model of asthma, mice exposed to environmental tobacco smoke had significantly higher levels of blood eosinophils and increases in bronchial hyperreactivity when compared to those exposed to filtered air [18]. Patients with prolonged eosinophilic asthma will have an accumulation of eosinophils not only in the blood and sputum but also in the bronchial tract. These patients will have a basement membrane zone of the airways that is thickened. Here, eosinophils will produce cytokines, chemokines and other mediators of inflammation [19] resulting in airway obstruction and airway remodeling [20]. This remodeling results in subepithelial fibrosis, thickening of the sub-basement membrane, increase airway smooth muscle mass and mucous gland hyperplasia with consequences of asthma exacerbation [19]. The Global Initiative for Asthma (GINA) describes asthma exacerbation as the inability of an asthmatic to respond to inhaled corticosteroids (ICS) due to high levels of inflammation in the airways with consequences of tightness of the chest, wheezing and decrease in lung function [21].

The European Academy of Allergy and Clinical Immunology Biological Guidelines from 2020 states that allergic asthma and eosinophilic asthma are subtypes of a type 2 (T2)—high inflammation [22]. A T2 high phenotype is characteristic of patients with high levels of blood eosinophil and IL5 in bronchial biopsies. A common biomarker for a T2-high inflammation is a fractional exhaled nitric oxide (FeNO) of ≥35 ppb [13]. Clinical studies show that many of these patients met the definition of both allergic and eosinophilic asthma demonstrating overlapping characteristics between both groups [13, 23]. Severe eosinophilic asthma patients will express a T2-high asthma phenotype with a biomarker of FeNO of ≥35 ppb [13]. These patients also exhibit a T helper (Th)2 cytokine profile (IL4, IL5, IL9, IL13) with IL5 significantly elevated in those with severe asthma [5, 24].

Asthma in many patients is controlled by treatment using standard protocol which includes ICS and beta2 adrenergic bronchodilators [25]. However, 5–10% of patients on corticosteroids do not respond to these medications resulting in persistent inflammation [14]. At center stage of patients with persistent inflammation is the eosinophil and the major cytokine-IL5. IL5 plays the most important role in the growth, differentiation, activation, recruitment and survival of eosinophils [26–28]. Excessive amount of IL5 in the bronchial region is believed to be responsible for the persistent

inflammation and the ineffectiveness of corticosteroids in patients with severe eosinophilic asthma [29].

Severe eosinophilic asthma has now become the focus of many new therapies due to the eosinophil's ability to induce chronic airway inflammation, leading to edema, mucus plugging and ultimately airway remodeling and its refractiveness to corticosteroids [30, 31]. The presence of IL5 has been implicated in the prolonged inflammation and remodeling of the airways as shown by several investigators [32–34]. The production of IL5 in this capacity involved a series of complex interactions involving type 2 innate lymphoid cells (ILC2), Th2 cells, the cytokines produced by them and other cells of the immune system. In this chapter, we delve into the early studies (in vitro and in vivo) that paved the way to the understanding of the cytokine functions particularly IL4 and IL5 and the series of intricate interactions that lead to severe eosinophilic asthma. We conclude by discussing the efficacy of biologics in use to treat severe eosinophilia in patients with T2-high asthma.

## 2. Early in vitro studies helped to unravel the functions of IL4 and IL5

In the early 1980s, investigators from the laboratories of Vitetta used supernatants from a specific T helper cell clone in culture which when added to liposaccharide (LPS) activated murine B cells caused enhancement of IgG1 while inhibiting the production of IgG3 and IgG2b [35]. When the supernatant was analyzed using biochemical procedures, it was found that it contained the B cell stimulatory factor-1 which today is known as IL4 [36]. Indeed, when anti- IL4 antibodies were added to these cultures, this enhancing effect was abrogated [36]. Later, Coffman et al. observed that LPS stimulated murine splenic B cells in culture produced elevated levels of IgG1, IgG2a, IgG2b, IgM but no IgE [37]. However, when IL4 was added to the cultures of splenic B cell, IgE was produced along with the other isotypes mentioned above showing that IL4 was responsible for the production of IgE [37]. When IL5 was added to the LPS stimulated B-cell cultures, there was a significant increase in the production of IgA and it was markedly increased when the combination of IL4 and IL5 were added.

Soon after the discovery by Mosmann et al. [38] that T helper cells can be of 2 types; namely Th1 and Th2, immunologists began the tedious process of functionally characterizing the roles of these cells. With the use of mouse T helper clones, scientists were able to decipher the functions of the cytokines secreted by these T cell clones. Coffman et al. performed in vitro reconstitution experiments in which they used Th1 and Th2 clones to show how T cell can help B cells produce various isotypes [39]. The major cytokines produced by Th1 clones are IL2 and IFN-γ while Th2 clones produced IL4 and IL5. Studies revealed that when Th2 clones were stimulated in 7-day culture with mouse B cells, they produced elevated levels of the IgE, IgG1, IgM and IgA. Removal of IL4 with the use of anti-IL4 antibodies, significantly blocked the IgE response while only slightly reducing the other isotype responses.

In these early studies, Coffman et al. were also able to reconstitute the Th2 response using the Th1 clones [39]. Here, an autoreactive mouse T cell clone called H66-61 Th1 cell line was used to reconstruct a Th2 type response. Irradiated H66-61 Th1 cells were placed in 7-day culture with mouse B-cells. Therefore, these irradiated T cells cannot proliferate but can provide help to B cells. Since Th1 clones secrete IFN-γ and this cytokine can inhibit the function of IL4 in culture, anti-IFN-γ was added to all cultures. Recombinant (r) IL4 and or IL5 cytokines were added to the

| H66-61 Cells + B Cells + Anti-IFN-γ + | IgE | IgG1 | IgM | IgA |
|---|---|---|---|---|
| Medium | <1 | 227 | 1590 | 30 |
| r IL4 | 610 | 4160 | 11,100 | 41 |
| r IL5 | <1 | 1380 | 15,200 | 208 |
| r IL4 + r IL5 | 333 | 12,300 | 64,300 | 281 |
| D9-Sup | 577 | 20,700 | 91,900 | 407 |

*All additions were added on day 0 of the cell cultures.*

**Table 1.**
*The effects of recombinant (r) IL4 and IL5 on H66-61-stimulated B cell cultures rIL4 was added at a concentration of 500 U/ml, rIL5 at 5 U/ml, D9 supernatant at 3%, anti-IFN- at 5 g/ml.*

cultures yielding the isotypes as shown in **Table 1**. The results demonstrated that Th1 clone was able to provide help to the B-cells for the secretion of the isotypes that are characteristics of a Th2 response. The similarity in isotype production was observed when supernatant from a Th2 clone (D9-sup) was added to B cell cultures. Furthermore, these results showed that IL4 triggered a significant increase in the isotypes IgM, IgG1 and especially IgE. The addition of IL5 to the cultures showed a significant increase in IgA but not IgE. Previously, Sanderson et al. in 1985 used a bone marrow culture system to show that a cytokine from a specific T cell clone that is not IL2 or IL3 is an eosinophil differentiating factor [40]. Molecular cloning and genetic characterization revealed that this factor is indeed IL5 which is a growth factor for eosinophils [41, 42]. These early in vitro studies revealed that Th2 cells and their cytokines (mainly IL4 and IL5) played a prominent role in allergic responses.

## 3. Murine models of parasitic infection, allergy and asthma helped to demonstrate the in vivo functions of the allergic cytokines -IL4 and IL5

The role of IL4 and IL5 was further defined in experimental models using rodents infected with the nematode *Nippostrongylus brasiliensis* (Nb) larvae [43, 44]. Nb larvae, upon infection in mice pass through the lung where they molt during the first few days triggering an immune response with blood and lung eosinophilia and elevation of IgE antibodies. Coffman et al. performed an elegant study with the use of monoclonal antibodies (mAb) to IL4 and IL5 to functionally characterize the role of these cytokines in vivo [43]. BALB/c mice were injected subcutaneously (SC) with 750 third stage Nb larvae. On the same day, they were treated with 2 mg per mouse of an anti-IL5 (TRFK-5) mAb. Another infected group received 10 mg/mouse of an anti-IL4 (11B11) mAb instead of TRFK-5 mAb. Nb infected BALB/c mice showed a 25- to 100-fold increase in total IgE levels and a 4- to 8-fold increase in eosinophils. However, the group that was treated with anti-IL5 showed no increase in blood and lung tissue eosinophils but made the normal increases in serum IgE as expected from the infection. This inhibition of eosinophils by TRFK-5 also blocked the development of eosinophils from the progenitors in the bone marrow [44]. The infected group that was treated with 11B11 made significantly reduced levels of total serum IgE (**Table 2**) but made elevated levels of eosinophils as characteristic of an Nb-infected mouse. The inability of TRFK-5 to block the IgE response was consistent with previous in vitro work by Coffman et al. [39]. This was the first in vivo model to demonstrate the

| Treatment | Total serum IgE (μg/ml) on days | | |
|---|---|---|---|
| | -7 | 11 | 14 |
| No antibody | 0.87 (0.43) | 32.4 (9.7) | 31.1 (11.2) |
| Anti-IL-5 | 0.53 (0.34) | 34.3 (19.9) | 34.9 (20) |
| Anti-IL-4 | 1.85 (0.83) | 2.5 (1.6) | 3.5 (2.4) |
| IgG1 Control | 1.15 (0.23) | 12.0 (6.7) | 24.8 (7.7) |

**Table 2.**
*Total serum IgE in BALB/c mice after subcutaneous injection of Nippostrongylus brasiliensis.*

ability of antibodies to IL5 to block eosinophilia. This seminal study on the independent regulation of IgE and eosinophils was later confirmed by others using mouse models of allergy, asthma and allergic aspergillosis [45, 46].

Savelkoul et al. further clarified the necessity of IL4 for an IgE response from the in vivo model of inflammation using Nb infected SJA/9 mice [47]. SJA/9 mice genetically have an SJL background ($H\text{-}2^s$) and bear the BALB/c H chain allotype [$Ig^a$] [48]. They are unable to mount IgE responses to allergenic substances and helminthic parasites [48]. When the T cells of Nb- infected SJA/9 mice were placed in culture with the mitogen concanavalin A, IL4 was produced in the supernatant. Furthermore, when B cells from Nb infected SJA/9 mice were placed in culture with IL4 and LPS, IgE was produced. Similarly, the administration of rIL4 to Nb infected SJA/9 mice resulted in IgE production as they made significant levels of serum IgE which was comparable to strains that are normal IgE responders. These Nb infected mice made eosinophils that were blocked when these mice were treated with anti-IL5. Thus, IgE defect was restored by administration of IL4 to these SJA/9 mice. This study further confirmed that the contribution of IgE and eosinophil to immune responses are controlled independently by IL4 and IL5 respectively. Today, it is known that IL4 is a switch factor for the production of IgE [49] and IL5 does not act on human B cells but is the major cytokine responsible for eosinophil maturation, differentiation and survival [19].

## 4. How strain variations in mice influenced the allergic response to the allergenic substance ovalbumin (OVA)

A study was done to examine the allergic response from various strains of mice that were classified by the quantity of allergic antibodies (lgE) they produced after immunization with the allergenic substance- ovalbumin (OVA) [50]. These classifications were termed "high," "low," and "non" responders according to the levels of IgE mice produce upon stimulation with allergens. Therefore, experiments were performed on SJA/9, BALB/c, C57BL/6 and 129SvEv mice to examine their abilities to make an allergic response. Except for the IgE non responder strain SJA/9, all strains are capable of mounting IgE responses to allergenic substances. The aim of this study was to understand the genetics of these strains of mice with respect to IgE production to gain an understanding of the immunogenicity of individuals with a genetic predisposition to allergy and asthma.

Mice were immunized by intraperitoneal (IP) injection with 1O μg OVA in aluminum hydroxide (OVA/AL) on day O followed by exposure to a 1% aerosolized OVA which was done on day 27. Using this experimental protocol, OVA-specific lgE was elevated in all groups except in the SJA/9 strain in which it was undetectable (**Figure 1**).

It was hypothesized that this lack of lgE secretion in SJA/9 mice is due to a mechanism which is similar to that which is responsible for the nonallergic state in some individuals. OVA specific IgE peaked in all IgE responsive groups at about day 8 after the challenge with aerosolized OVA. The largest amount of lgE was seen in the 129SvEv mice while the lowest detectable amount was from the C57BL/6. After the peak response, OVA specific-lgE remained detectable in the last collection of serum which was on day 100.

Eosinophils in the blood were quantified after the first inhalation of aerosolized OVA (data not shown). All mice had elevated levels of blood eosinophils 5 days after this inhalation. Although, there were no lgE in the blood of SJA/9, this group had the highest level of blood eosinophils. Two weeks after the aerosol challenge, eosinophils remained significantly elevated in the 129/SvEv group when compared to the others.

At this point, it was necessary to examine the cytokine profile in the lung of each strain of mice. Thus, on day 150, all mice were rechallenged with a 1% aerosolized OVA and their lung cytokines assessed 4 days after this tertiary challenge. **Table 3** shows that despite the high levels of OVA-specific lgE from the 129/SvEv group, IL4 was very low in this group. OVA-specific lgE and IL4 were undetectable in SJA/9 mice. The highest level of IL4 was seen in BALB/c mice with 888 ± 301 ng/ml in the lung

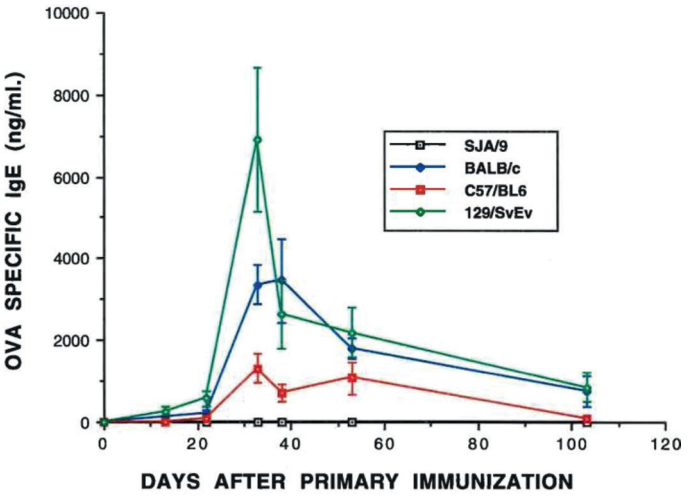


**Figure 1.**
*lgE responses from various strains of mice after exposure to OVA.*

| Strain | IL3 (ng/ml) | IL4 (pg/ml) | IL5 (ng/ml) | IFN-γ (ng/ml) |
|---|---|---|---|---|
| SJA/9 | <0.156 | <1.56 | 1.11 ± 0.41 | <0.156 |
| C57/BL9 | <0.156 | 70 ± 24 | 3.09 ± 1.00 | <0.156 |
| BALB/c | 0.556 ± 0.0830 | 888 ± 301 | 8.94 ± 2.09 | <0.156 |
| 129/SvEv | <0.156 | 34 ± 10 | 3.56 ± 1.24 | <0.156 |

*All mice received 10 ug OVA/AL IP on day 0 and 1% aerosolized OVA for 20 minutes on day 27 and day 150. In vitro restimulation of unseparated lung cells were done on day 154.*

**Table 3.**
*Lung cytokines from OVA immunized strains of mice.*

of these mice. IFN-γ was not detected in any of the groups demonstrating that the immune response was not a Th1 type response. IL3 was detected in the BALB/c mice but not in the other groups.

The result of this experiment confirms previous studies that IL4 is a necessary cytokine for the production of lgE [47]. That is, SJA/9 mice did not produce detectable levels of lgE and did not produce any IL4 after the OVA specific in vitro restimulation of homogenized lung cells. This study also shows that IL4 is necessary but not fully responsible for the production of lgE since the highest levels of lgE came from the 129SvEv mice even though IL4 in this group was very low compared to the BALB/c group. Presently, it is known that IL13 also plays a role in the production of IgE [51]. Low levels of IL5 were seen in the SJA/9 mice when compared to the BALB/c mice (1.11 ± 0.41 vs. 8.94 ± 2.09 respectively). However, the eosinophils in the SJA/9 strains were highest when compared to the other groups. Despite the absence of IL4 and lgE from the SJA/9 strain, a Th2 response (eosinophilia and Th2 cytokines) was seen in this group. The lack of IFN-γ from all the strains revealed that these animals were not responding with a Th 1 type response, instead they are capable of producing an allergic type of response even in the SJA/9 strain that lacked detectable lgE and IL4. The level of the response from each strain may represent the atopic state of a particular strain. The SJA/9 strain represents the low responders while the 129/SvEv with their high levels of lgE may be mice which can be comparable to atopic individuals.

## 5. The complex role of the cytokines and inflammatory mediators in severe eosinophilic asthma

### 5.1 Damaged epithelial cells release alarmins to initiate the innate immune response

Numerous studies have been performed which have recognized the major regulatory mechanisms in allergic airway diseases [4, 52, 53]. There are many multifactorial events that are necessary for a non-allergic individual to develop allergic airway disease such as severe eosinophilic asthma (**Figure 2**). It begins with damaged lung epithelial cells after an atopic individual is exposed to aeroallergens such as pollen and/or environmental irritants such as tobacco smoke [54]. The damaged epithelium secretes the alarmins -IL33, IL25 and TSLP [55, 56]. These cytokines can cause a shift in the immune response towards an allergic phenotype with pathological consequences related to airway allergic disease [55]. For example, in a mouse model of asthma, mice treated intranasally with IL25, developed epithelial cell hyperplasia, mucus hyper-secretion and bronchial hyperreactivity [57]. Indeed, severe asthmatic patients with fixed airflow limitation show an increase in IL25 [58]. Others have shown that the level of circulating fibrocytes (progenitor cells that enter the circulation and inflame the bronchial epithelium) bearing the receptor for IL25 correlates with the severity of asthma [59].

IL33 is a member of the IL1 family which participates in the polarization of the immune response towards a T2 type inflammation. It is expressed not only by epithelial cells but also by vascular endothelia cells. Atopic patients chronically exposed to environmental pollutants or allergens containing serine proteases will show an early increase in IL33 from their damaged epithelial cells. This cytokine is present in the bronchoalveolar lavage and airway smooth muscles, and they activate dendritic

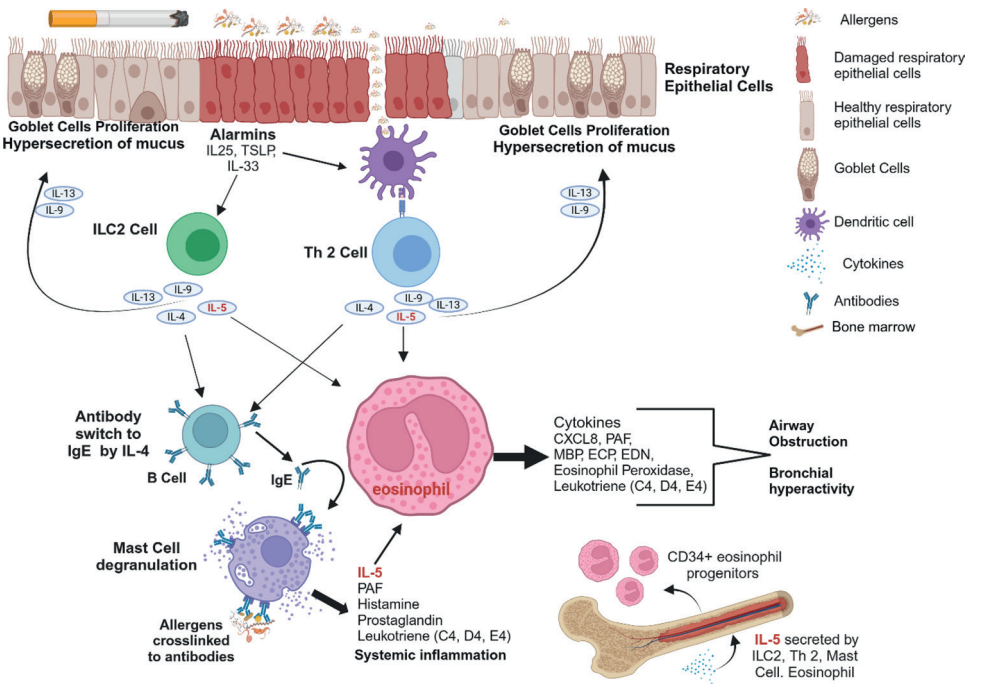


**Figure 2.**
*The interaction of the alarmins with the T2 cytokines and the prolongation of eosinophilia from damaged epithelial cells of the airways (image created with BioRender.com).*

cells - the major antigen presenting cells [60]. Thus, the expression of IL33 in humans can lead to atopic diseases with elevated levels of IgE, hyper eosinophilia and asthma.

The thymic stromal lymphopoietin (TSLP) is another cytokine that is secreted by the damaged epithelial cell in response to proteases [61, 62]. TSLP is a member of the IL2 cytokines family, and its gene can be found on the human chromosome at 5q22.1 which is close to the cluster of cytokines- IL4, 5 and 13 located at 5q3185 [63]. TSLP is also expressed in lung fibroblast, and smooth muscle. Its receptor is expressed on innate lymphoid cell type 2 (ILC2), dendritic cells, T cells, B cells, mast cells, basophils, monocytes and eosinophils [61]. It is involved in dendritic cells maturation and the skewing of T helper cells to a Th2 phenotype particularly during an allergic response [64]. Indeed, TSLP's mRNA and protein are elevated in asthmatic patients and correlate with airway hyperresponsiveness regardless of the levels of eosinophilia and T2 inflammation [52, 65].

Collectively, the alarmins work together to activate the ILC2 cell during the innate phase of the allergic response by contributing to the initiation of the inflammatory response. ILC2 cells are the early producers of several T2 cytokines including IL4, IL5, IL9, IL13 [66]. The ILCs which arise from the common lymphoid progenitor are closely related to the Natural Killer (NK) cells. They reside in peripheral tissues and are categorized into 3 groups namely 1, 2 and 3 according to the cytokines they produce and their surface characteristics [67].

It was found that when ILC cells were stimulated with IL25 and IL33, the neuropeptide receptor Nmur1 was expressed preferentially by ILC2 cells. Furthermore, when neuromedin U (NMU), the ligand for Nmur1 was co administered with IL25, they amplified the allergic inflammation with increases in eosinophils in the lung and

broncho alveolar lavage [68]. TSLP and to a lesser extent IL25 and IL33 from epithelial cells causes the release of IL5 from ILC2 cells. Also, studies have shown that when TSLP was added to IL33-stimulated ILC2 human cells, the production of IL4, IL5 and IL13 was enhanced along with increase expression of the transcription factor GATA3 [69]. This transcription is required for the expression of IL5 from Th2 cells [69]. IL13 produced by ILC2 cells is necessary for the migration of dendritic cells to the draining lymph nodes where they induce naïve T cells to become Th2 cells during the process of T cell activation. TSLP stimulated dendritic cells has been shown to lead to the production of chemokines CCL17 and CCL21 and ultimately to the priming of Th2 cells [64]. In chronic conditions, TSLP downregulates the development of Tregs while maintaining the Th2 type inflammation [64]. Indeed, TSLP is increased in the airway walls of patients with severe asthma. Thus, the ILC2 cell, due to its early secretion of cytokines, participates in the innate phase and is necessary for the adaptive phase of the allergic response as naïve T cells differentiate into effector Th2 cells [70, 71].

IL3, IL5 and GM-CSF participate in the development of eosinophils however, IL5 is the major cytokine for its development, and it is at center stage in patients with severe eosinophilic asthma [31]. Though the main cellular sources of IL5 are Th2 and ILC2 cells, it is also secreted by other cells such as mast cells, basophils, NKT cells and eosinophils themselves pointing to the increase in eosinophils as largely due to the many sources of IL5 [26, 72–74]. Meanwhile, the damaged epithelial cells also secrete chemokines CCL5 (RANTES) and CCL11 (eotaxin-1) that can bind to a receptor called CCR3. Also, IL4 and IL13 can stimulate epithelial cells in the lungs to produce eotaxins [75]. The CCR3 receptor is expressed on Th2 cells, macrophages, eosinophils, basophils and mast cells [31, 76]. While eotaxin-1 is required for the initial steps in the inflammatory response, eotaxin-2 and eotaxin-3 participate in the prolongation of eosinophil survival [31]. Thus, there will be an influx of these cells from the blood to the respiratory tract which contributes to the obstruction of the airways.

### 5.2 Th2 type allergic response is responsible for the adaptive phase and participates in the prolongation of the chronic inflammation in severe eosinophilic asthma

After the initial phase of the allergic response, Th2 cells with the series of cytokines they produce which are mainly IL4, IL5, and IL13 play a prominent role in propagating the chronic inflammation seen in severe eosinophilic asthma. IL4, a switch factor for IgE, is largely responsible for the increase in allergic antibodies. IgE attaches to mast cells via the high affinity Fc epsilon receptor. If crosslinking of antigen occurs via the fragment antigen-binding (FAB) region, then the mast cell becomes degranulated as shown in **Figure 2** with the release of inflammatory mediators such as histamine, chemotactic factors, cytokines, metabolites of arachidonic acid. These mediators act on vasculature, goblet cells, smooth muscles and inflammatory cells in the airways to bring about bronchoconstriction [77]. IL4 also promotes the commitment for the differentiation of naïve T cells to Th2 cells. The IL4 receptor activates STAT6 from the naïve T cell which promotes the expression of the transcription factor -GATA3. The B lymphocyte-induced maturation protein-1 (Blimp-1) enhances the GATA3 expression in T helper cells causing them to differentiate to Th2 cells with amplification of the inflammatory allergic response in atopic individuals exposed to aeroallergens [78]. Therefore, IL4 with its ability to cause naïve T cells to commit to a Th2 pathway, plays a prominent role in the prolonged elevation of IL5 in severe eosinophilic asthma. Epithelial cell activation occurs due to the presence

of IL-5, IL-13, and periostin, (a protein upregulated by IL-13) which also causes the biomarker FeNO for a Th2 inflammation to be expressed [79]. Together, IL13 and IL9 have a role in bronchial hyperreactivity and the remodeling of the airway epithelium by causing epithelial cells to differentiate into goblet cells [4]. The increase in goblet cells can lead to a hyper secretion of mucus in an asthmatic individual [4, 80, 81].

The high levels of IL5, particularly in the bronchial tracts, in patients with severe eosinophilic asthma can hinder the pro-apoptotic effect from the treatment with corticosteroids [82]. This refractory state is defined as severe T2-high eosinophilic asthma in which eosinophils accumulate and proliferate throughout the airways. The IL5 receptor alpha chain is expressed on eosinophils, airway epithelial cells and lung fibroblast [78, 83]. The elevated presence of IL5 and eosinophils can lead to the development of co-morbidities such as chronic rhinosinusitis and nasal polyps due to the continuous production and survival of eosinophils in the bronchial tracts [79]. These patients experience frequent exacerbation of their allergic phenotype and difficulty controlling their respiratory conditions. The unraveling of the function of Th2 cytokines, particularly IL5, in maintaining the elevated levels of eosinophils has aroused the interest of researchers to explore the use of biological markers for therapeutic intervention in severe eosinophilic asthma [84, 85]. This development of biologics can be the key to control severe eosinophilic asthma when it is uncontrollable with the use of corticosteroids due to the high levels of IL-5.

## 6. The use of biologics for the treatment of eosinophilic asthma

The first published report that antibodies to IL5 can inhibit eosinophils in vivo was done using a mouse model of *Nippostrongylus brasiliensis* infected parasitized mice in 1989 [43]. Later scientists used a monkey model of asthma and a mouse model of pulmonary inflammation to also demonstrate the inhibitory effect of anti-IL5 on eosinophils [86, 87]. These preclinical studies paved the way for the use of anti-IL5 biologicals in the treatment of severe eosinophilic asthma. Over the last decade, biologics that target cytokines and cytokine receptors, have been developed to be used as an adjuvant therapy in the management of severe eosinophilic asthma [88–91]. Several clinical trials have evaluated the efficacy of anti-IL5 or anti-IL5 receptor antibodies in patients with asthma [92, 93]. These biologics are: Mepolizumab, Reslizumab and Benralizumab.

Mepolizumab is a humanized mAb (IgG1 k type), originated by binding anti human IL5 antigen recognition sites from murine origins into a human IgG1 heavy chain [94]. It has a high affinity in binding free IL-5 (a 134 amino acid dimeric glycoprotein with 4 helix bundle mortif that has a 52-KDa homodimer) resulting in IL-5 inability to bind to the receptor IL5Rα (**Figure 3**) [94, 95]. This binding causes a reduction of eosinophils in both the blood and airways [96]. Its very specific binding ability may explain the lack of relatively significant side effects of Mepolizumab as it does not seem to interfere with the biological activities of other cytokines [94]. However, early studies with Mepolizumab showed mixed response from clinical trials conducted in several asthmatic patients' population where there was documented reduction in circulating eosinophils without much significant clinical response in the severity of the disease [92, 97]. These early investigations included a small population study that was conducted on difficult to treat asthmatic patients whose treatment included high dose ICS and/or oral corticosteroid (OCS). This study also showed a similar outcome in reduction of blood eosinophils with little effect on the clinical outcome, except for a small improvement in lung FEV1 (forced expiratory volume in 1 second) function [98]. Many of these early studies were done in which a specific

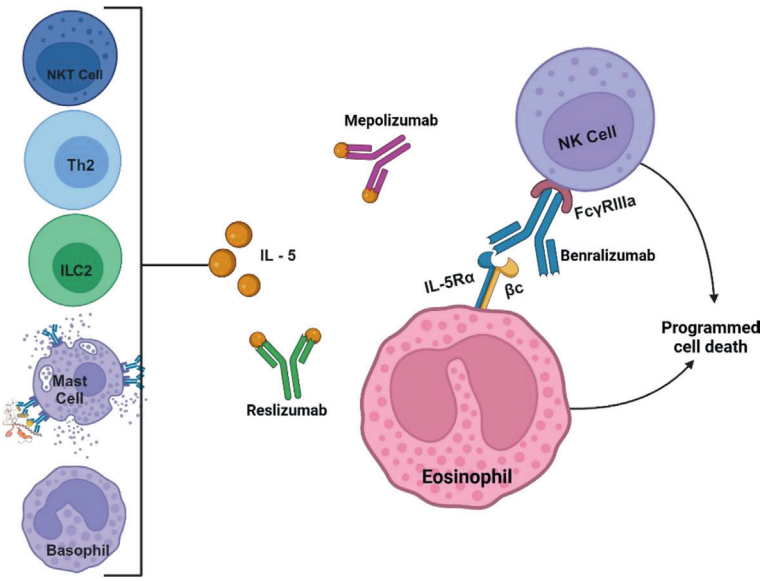


**Figure 3.**
*The mechanisms of action of reslizumab, mepolizumab and benralizumab on eosinophils. Reslizumab and mepolizumab use different idiotypic regions to bind to the IL5 molecule. The FAB region of Benralizumab attaches to the IL5 receptor on the eosinophil and the Benralizumab's Fc region attaches to the Fcγ receptor as shown on the NK cell to induce ADCC. The sources of IL5 are also shown (image created with BioRender.com).*

participant group consisted of various asthma phenotypes ranging from mild to moderate chronic asthma. Despite the reduction of eosinophils, the quality of life for these patients was not improved with respect to asthma exacerbation rates [93].

Eventually, investigators examined patients suffering solely from severe chronic asthma [99]. This small study involved patients with bronchial eosinophilia who were not responsive to treatment with corticosteroids. The result of this study showed reduction not only in eosinophil levels but also in asthma exacerbations after treatment with Mepolizumab. Later, a 52-weeks study termed; the DREAM (Dose Ranging Efficacy And safety with Mepolizumab in severe asthma) study was conducted as one of the largest asthma studies (621 participants) to examine the effect of Mepolizumab in participants with severe asthma [100]. The study had strict criteria that required at least 2 exacerbation which included the use of OCS or a visit to an emergency room or hospitalization and signs of eosinophilic inflammation (either sputum eosinophils >3%, peripheral blood eosinophils >300 x $10^6$/L, FeNO>50 ppb or loss of asthma control after <25% reduction in either ICS or OCS dose). Participants received 13 infusions of either 75, 250 or 750 mg of intravenous (IV) mepolizumab. Results showed all three doses significantly reduced asthma exacerbation equally and reduced both blood and sputum eosinophils [100].

Mepolizumab as Adjunctive Therapy in Patients with Severe Asthma (MENSA) was a study of 576 asthmatic patients over 52 weeks who were treated with ICS with or without OCS [101]. They were randomized to receive either 75 or 100 mg mepolizumab SC every 4 weeks or a placebo. The inclusion criteria were asthmatic with at least 2 exacerbations requiring systemic corticosteroids the previous year with evidence of eosinophilic inflammation (eosinophil count 150 cells/uL at screening or above 300 cells/μL at some point in the previous year). Results showed a 47 and 53% respectively in IV and SC mepolizumab associated reduction in asthma exacerbations [101].

Mechanistically, investigators have shown that after treatment with Mepolizumab, there is a decrease in PD-1 expression on Tregs hereby allowing this subset of cells to perform its immunomodulatory duty [102]. TGF-β is also reduced after severe asthmatics with eosinophilia are treated with Mepolizumab. This cytokine is secreted by many cells in the respiratory tract but is mainly liberated by eosinophils [103]. It is believed to play a role in the differentiation of fibroblast to myofibroblasts which participates in airway remodeling. Thus, with the treatment of severe asthmatics with Mepolizumab, there was a reduced expression of some extracellular matrix proteins and consequently the reduction of airway remodeling [31, 102]. Corticosteroids have been shown to be ineffective in reducing airway remodeling in patients with chronic asthma particularly those with severe eosinophilic asthma. Therefore, Mepolizumab is a necessary add-on therapeutic for the inhibition of fibrosis and airway remodeling of individuals with severe eosinophilic asthma which accounted for approximately 5% of all asthmatics [14, 31]. This treatment also reduces the severity of exacerbation even in patients with comorbidity such as bronchiectasis [104]. The FDA and the European Medicines Agency both eventually approved Mepolizumab as an adjuvant treatment for severe eosinophilic asthma [93].

Reslizumab, another anti-IL5 mAb showed significant improvement in lung function in a study ($P = 0.002$ vs. placebo) with trend towards improved asthma score and reduction in sputum eosinophils [96]. It is an IL-5 neutralizing IgG4K mAb, that is currently approved for adjunct therapy for adults with severe eosinophilic asthma [91]. Data from the BREATH phase 111 clinical trial which involved three double -blinded studies in patients 12–75 year with eosinophilic asthma not controlled on an ICS used reslizumab 3 mg/kg once every 4 weeks for 52 weeks. The results in various subgroups showed a significant reduction in asthma exacerbation frequencies, lung function and improved quality of life [105]. Ibrahim et al., explored the clinical efficacy of Reslizumab in patients with inadequately controlled asthma, elevated blood eosinophils, taking high dose ICS, a second controller with at least 4 exacerbations and one hospitalization [106]. The results were statistically significant through the use of a validated asthma control questionnaire. Of note, patients had a decrease in maintenance steroid usage while taking Reslizumab [106]. In comparison of severity, patients with late-onset eosinophilic asthma were noted to have a greater response to Reslizumab in reductions of exacerbation and improvement to lung function than those with early-onset asthma [91, 107].

Benralizumab, is an anti-IL5-receptor cytolytic mAb that binds directly to the IL-5 receptors on eosinophils thus enabling the immune system to remove them [108]. It is a humanized IgG1k, afucosylated mAb, (lacking oligosaccharides in the Fc region) which works by inducing apoptosis in target cells via antibody-dependent cellular cytotoxicity (ADCC) (**Figure 3**). In comparison to the other anti -IL-5 biologics, it has the most efficiency in the depletion of eosinophil in peripheral blood [96]. Others have observed clinically and statistically significant reduction in asthma exacerbations with the addition of Benralizumab with current treatment protocols. Some patients were able to discontinue systemic steroid therapy and became exacerbation-free by this adjunct therapy [108, 109]. However, studies have shown bronchial eosinophils remained after treatment with Benralizumab [85]. Investigators have shown that there exists a subset of lung-resident eosinophils with regulatory functions which are different from the inflammatory eosinophils that participate in the Th2 responses [110–112]. These lung resident homeostatic eosinophils are IL5 independent while the inflammatory eosinophils are IL5 dependent [107]. Indeed, IL5 deficient mice have reduced level of basal eosinophils and are unable to produce an eosinophilic inflammation as in the context of the Th2 response [110]. Therefore, the eosinophils in the bronchial tree that remained

after treatment with anti-IL5 biologics may be the protective homeostasis eosinophils as those remaining may be responsible for the lack of increased risk of infection after treatment [113]. Overall, the therapeutic effect of anti-IL5 demonstrates that IL5 is the major cytokine responsible for the activation and survival of eosinophils [114]. Presently, the GINA recommendations for anti IL5 therapy are on the final Stepwise (step 5) approach to the management of severe asthma [21].

Beside targeting IL5 and its receptor for the treatment of severe eosinophilic asthma, other T2 cytokines have been targeted successfully and are currently approved. Dupilumab is a fully human mAb that blocks the alpha receptor subunit of IL-4, blocking both IL-4 and IL-13 since they share a common subunit [115]. The first clinical trial involved 52 patients with severe eosinophil asthma who were given dupilumab at a dose of 300 mg SC while 52 with similar baseline characteristic were given a placebo. The results showed that only 6% of participants from the dupilumab group continued to have asthma exacerbation versus 44% in the placebo group. This study was a 12-week provocative method where long-acting β2 agonist (LABA) was discontinued at week 4 and ICS at weeks 6–9. The results showed an 87% reduction in asthma exacerbation, improved lung function and Th2 inflammatory markers when compared to the placebo [116]. Several other studies have concluded that Dupilumab was effective in decreasing asthma exacerbation, improving lung function and quality of life in patients with asthma [117, 118].

Omalizumab, an anti-IgE mAb, downregulates IgE receptors by inhibiting IgE binding to mast cells, dendritic cells and basophils which results in the inhibition of IgE mediated inflammation. Consequently, Omalizumab treatment decreased eosinophils and multiple inflammatory mediators. It is one of the biologics currently being utilized for the treatment of moderate to severe allergic asthma with a positive treatment response [119].

Tezepelumab is a human mAb that binds to TSLP. In a randomized, double blind, placebo-controlled 52 weeks trial in patients with uncontrolled asthma who were on LABA and medium to high dose ICS, 3 doses of Tezepelumab were compared to placebo [31]. Asthma exacerbation rates were significantly reduced irrespective of the baseline eosinophil blood count or despite the dose (low, medium, or high) given. However, health related quality of life was only noted to be improved in the high dose group [31]. It has been FDA approved for children ages 12 years and older and adults with severe eosinophilic asthma.

## 7. Concluding remarks

The use of mAb to treat severe eosinophilic asthma has been effective as observed in clinical trials and real-world clinical efficacy studies [101–103, 120–121]. Biologic therapy as an adjunct treatment has decreased the number of asthma exacerbations, reported symptom days, and improved lung function. Across the studies these improvements have been particularly notable in those with the highest blood eosinophil counts when anti-IL5 biologics were compared to omalizumab and dupilumab [118, 122–124]. This treatment works for asthmatics with severe eosinophilia [118]. However, the benefits of biologics in moderate asthma are still an ongoing area of study.

The main limitations of biologics in asthma treatments are their high cost and the tedious ongoing research into the pathogenesis of the subtypes of severe asthma [107]. The variations in endotypes pose challenges to its application in various patient populations. The utilization of biomarkers such as IgE, eosinophil count and FeNO

have been shown to be beneficial for identification of endotypes especially for severe eosinophil asthmatic patients at increased risk of exacerbations [89, 119, 125, 126]. However, their identification and treatment can also be challenging due to the varying amounts of overlap of eosinophilic asthma with other endotypes particularly T2 and allergic asthma. Biomarkers such as eosinophil counts can be misleading because environmental factors can cause natural fluctuation in baseline count as observed in some healthy individuals. For example, healthy individuals exposed to tobacco smoke have a significantly higher median eosinophil count compared to nonsmokers making it difficult to determine the necessity for the use of biologics as add on in treatment [17]. Also, race should be considered as a factor when implementing treatment for vulnerable demographics. African Americans are at a higher risk for morbidity and mortality from severe asthma and studies show that those on ICS exhibit higher levels of eosinophilic inflammation than their white counterparts [127]. Finally, early identification of at-risk individuals can be instrumental in the prevention and treatment of severe eosinophilic asthma as it would potentially reduce the need for healthcare resources as medicine becomes more personalized.

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