



Eosinophils, the IL-5/IL-5R α axis, and the biologic effects of benralizumab in severe asthma

Andrea Matucci^{a,*}, Enrico Maggi^b, Alessandra Vultaggio^a

^a Immunology Unit, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy

^b IRCCS Pediatric Hospital Bambino Gesù, Rome, Italy

ARTICLE INFO

Keywords:

Bronchial asthma
Eosinophils
Interleukin 5
Monoclonal antibodies
Benralizumab

ABSTRACT

Bronchial asthma is a chronic inflammatory disease characterized, in a percentage of patients, as an eosinophilic inflammation of the airways. Eosinophils are recognized as a proinflammatory granulocyte playing a major role in the T2-high phenotype, which includes severe eosinophilic asthma. Eosinophilic asthma represents the majority of the phenotypic variants clinically characterized by severity and frequent exacerbations. For patients with severe uncontrolled asthma, monoclonal antibodies are used as add-on treatments. Among them, in addition to anti-immunoglobulin E therapy, biologic agents directed toward the interleukin (IL)-5/IL-5R α axis and, thus, interfering with the pathologic functions of eosinophils, are now available. Unlike the other anti-IL-5 monoclonal antibodies which exert an indirect effect on eosinophils, benralizumab, an afucosylated IgG₁ kappa antibody directed against the α subunit of IL-5R, directly depletes eosinophils and their associated bone marrow progenitor cells through induction of antibody-dependent cell-mediated cytotoxicity, through recruitment of natural killer cells. This article reviews the role of eosinophils in the pathogenesis of bronchial asthma and discusses the potential advantageous biologic effects of benralizumab in comparison with other monoclonal antibodies targeting the IL-5 ligand.

1. Introduction

Eosinophils are key cells in the inflammatory process of various clinical conditions, including bronchial asthma (BA) [1]. For this reason, therapies targeted at eosinophils are a good addition to the treatment options for patients with severe eosinophilic asthma.

Eosinophilopoiesis is the process whereby eosinophils differentiate from CD34⁺ progenitor cells in the bone marrow under the influence of a variety of cytokines, of which interleukin (IL)-5 is the major hemopoietic factor, and lineage-specific transcription factors, such as Stat-3. Under conditions other than homeostasis, such as severe forms of BA, eosinophil progenitors, characterized by the expression of CD34 and IL-5 receptor α (IL-5R α) have also been found in tissue and blood, supporting the hypothesis that eosinophilopoiesis may take place at a tissue level outside the bone marrow [2]. Eosinophils are released from the bone marrow into the peripheral blood in a phenotypically mature state and can undergo activation and recruitment into tissues under the influence of appropriate stimuli, mainly IL-5 and eotaxins. Eosinophils only remain for a short time in the peripheral blood (with a half-life of approximately 18 h) before migrating to the thymus or gastrointestinal

tract, where they reside and survive in homeostasis [3]. Although eosinophil cells express multiple receptors, the main types are the IL-5R α , CC chemokine receptor 3 (CCR3) and sialic acid-binding immunoglobulin-like lectin 8 (Siglec-8) [4]. In addition, eosinophils express receptors for IL-4, IL-13, IL-33, thymic stromal lymphopoietin (TSLP) and chemoattractant receptor-homologous molecule expressed on T helper type 2 cells (CRTH2) [5–8].

The effector functions of eosinophils are related to the release of substantial numbers of mediators stored in specific granules. These mediators include basic proteins, cytokines, chemokines, enzymes, etc., all capable of inducing inflammation and tissue damage. Granule contents are released via three different degranulation processes: 1) exocytosis (in which specific granules fuse with the eosinophil cell membrane); 2) cytolysis, determining the release of all contents; and 3) piecemeal degranulation (in which vesicles of specific granules are released in the cytosol of the cells and, travel through the eosinophil membrane, ultimately reaching the extracellular milieu) [9,10]. Of note, piecemeal degranulation has become accepted as the most commonly observed physiologic form of eosinophil degranulation.

The biologic functions of eosinophils are more complex and not

* Corresponding author. Largo Brambilla 3, 50134 Florence, Italy.

E-mail address: andrea.matucci@unifi.it (A. Matucci).

solely limited to the inflammatory response characteristic of various diseases, but rather exert a role in both innate and adaptive immune responses. In fact, eosinophils have been considered as effector cells during parasite infections and responsible for mucosal damage in allergic inflammation, but the role of these cells in homeostasis and immunoregulation has become increasingly recognized over the past decade [11–13]. Although not strictly considered “professional” antigen-presenting cells, eosinophils can nevertheless express cell surface molecules such as major histocompatibility complex (MHC) Class II molecules and the co-stimulatory molecules CD80 and CD86, which are required for antigen presentation to T cells [14]. Furthermore, eosinophils can process antigen and stimulate T cells in an antigen-specific fashion, resulting in T-cell proliferation and cytokine release [14]. More importantly, eosinophils promote maturation of monocyte-derived dendritic cells (DCs) *in vitro*, and, through the release of eosinophil-derived neurotoxin (EDN), are responsible for the accumulation of myeloid DCs observed in mouse models of allergic airway disease [15].

2. Factors influencing eosinophil differentiation, maturation, and survival

2.1. IL-5/IL-5R α axis

Interleukin-5, a cytokine produced as a dimer, is secreted by multiple cells, including T helper (Th)2 cells, mast cells, group 2 innate lymphoid cells (ILC2) and eosinophils [16,17]. Interleukin-5 is the most important factor influencing the differentiation, maturation and prolonged survival of eosinophils, and binds to the IL-5-specific α subunit of the receptor (CD125), part of a heterodimeric receptor with a common β subunit (CD131) [18]. CD131 is shared with the heterodimeric IL-3 receptor (CD123) and the granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor (CD116). The common β subunit, which does not express any ligand binding site, nevertheless confers high-affinity ligand binding to intracellular signaling [18].

The IL-5 receptor (R) is expressed on mature eosinophils, as well as their progenitors, including the eosinophil lineage-specific myeloblast. Interleukin-5 can be hypothesized to primarily influence eosinophil proliferation and the maturation of progenitors after the initial phase of the process in which other factors could be involved. This suggestion is derived from the observation that the anti-IL-5 monoclonal antibody, mepolizumab, induces substantial reductions of both mature and late immature eosinophils in the bone marrow while the number of early eosinophils both in blood and bone marrow are unaffected [19]. On the other hand, other cytokines, such as IL-3 and GM-CSF, may be responsible for this incomplete depletion of progenitors by mepolizumab [20]. In fact, IL-5 is not the only factor influencing eosinophil biology. Different subsets of eosinophils have been identified in experimental models and also in humans, which express different receptor markers, and are varying dependent on IL-5 [21].

The biology of the IL-5/IL-5R α axis is also characterized by the fact that after eosinophils migrate to the tissue, IL-5R α is shed, as suggested by increased soluble IL-5R α at tissue after antigen challenge levels [22]. In agreement with these data is the finding that, *in vitro*, IL-5R α is shed from the surface of eosinophils obtained from healthy controls upon interaction with IL-5 [23]. The axis of IL-5/IL-5R α has recently been profoundly re-evaluated in considering the effects of biologics available for the treatment of severe forms of eosinophilic asthma [24]. The α -subunit, specific for IL-5, is the therapeutic target for benralizumab, a humanized monoclonal antibody indicated for the add-on treatment of severe, eosinophilic asthma [25].

2.2. Other cytokines and factors acting on eosinophil biology

In addition to IL-5, other cytokines such as IL-3 and GM-CSF may influence the maturation, survival, and function of eosinophils. The

expression of IL-3 and GM-CSF receptors by eosinophils and the presence of adequate amounts of these cytokines in the microenvironment may sustain eosinophil maturation [26]. The persistence of tissue eosinophilia in asthmatic patients treated with anti-IL-5 monoclonal antibodies may reflect the vicarious effects mediated by IL-3 and GM-CSF. In fact, although blocking IL-5 induces a very significant depletion of circulating eosinophils, at the bronchial tissue level eosinophilia is always persistent, reaching about 50% in comparison to the baseline level before treatment [27]. Recent data suggest that IL-33 may play a significant role in eosinophilopoiesis through its receptor ST2, in addition to inducing the activation of eosinophils [5]. The complex activity of IL-33 is suggested by the demonstration that its administration promotes eosinophil development both by increasing IL-5 production in the bone marrow and by expanding the number of IL-5R α on eosinophil precursors in mice [28]. In addition, at the bronchial level, IL-33 may induce IL-5 production by human ILC2, thus amplifying eosinophil inflammation [29,30]. Of note, ILC2s from patients with asthma, expressing the ST2 receptor appeared to be more sensitive to IL-33 when compared to those from control subjects [31,32].

Taking into account the sequence of these events, IL-33 seems to precede IL-5 in regulating lineage commitment in eosinophils and that it may be important in maintaining eosinophil homeostasis as well as inducing eosinophilia in pathological conditions. The role of IL-33 at the beginning of eosinophilopoiesis is suggested by the data indicating that IL-33 produced in the lungs of mice exposed to *Alternaria* induces the release of IL-5 associated with an increase in eosinophils and eosinophil progenitors in bone marrow [33].

For the activation and survival of eosinophils, we immediately think of cytokines. However, many other factors exert direct effects on these cell populations, influencing their activation, survival, and migration. The biology of eosinophils is deeply influenced by chemokines. Particularly, eotaxin 1 (CCL11) and eotaxin 3 (CCL26), ligands for CCR3, promote eosinophilia both cooperatively and via mechanisms that are independent of those utilized by IL-5 [34,35]. A number of new factors that promote eosinophilic inflammation *in vivo* have recently been identified. Among them, alarmin TSLP modulates eosinophil survival and activation [6,36].

Among cytokines actively involved in the pathogenesis of BA, tumor necrosis factor (TNF) (TNF- α is “TNF,” and TNF- β is lymphotoxin. Therefore, TNF exerts direct effects on human eosinophils. In fact, TNF inhibits eosinophil apoptosis, and this effect is not a result of the autocrine release of known survival-prolonging cytokines IL-3, GM-CSF, and IL-5, as their neutralization does not influence the effects of TNF. The anti-apoptotic signal was mediated mainly by the TNF-receptor 1 [37].

In addition to the role of cytokines and chemokines, it is important to remember the role of the traditional mediators, such as cysteinyl leukotrienes (CysLTs). CysLTs can have varying effects on eosinophils, ranging from intracrine regulators of secretion of granule-derived proteins to paracrine/autocrine roles in eosinophil chemotaxis, differentiation, and survival [38].

The expression of the proapoptotic receptor Siglec-8 on eosinophils in upper airway tissue could represent an endogenous pathway for controlling local eosinophilic inflammation. However, unlike the previous activating factors, the activation of Siglec-8 induces eosinophil apoptosis [39].

Fig. 1 represents a schematic view of the biology of eosinophils in bronchial inflammation.

3. Eosinophils as key cells in the pathogenesis of severe asthma

At present, there is a general consensus on the inflammatory nature of BA. Advances in the understanding of the pathogenetic mechanisms of BA are undoubtedly to be facilitated by the use of immunobiologic, molecular, and genetic approaches for the study of experimental models, as well as patients with BA. Even in mild and intermittent forms of BA, the histopathologic hallmark consists of the presence at the bronchial

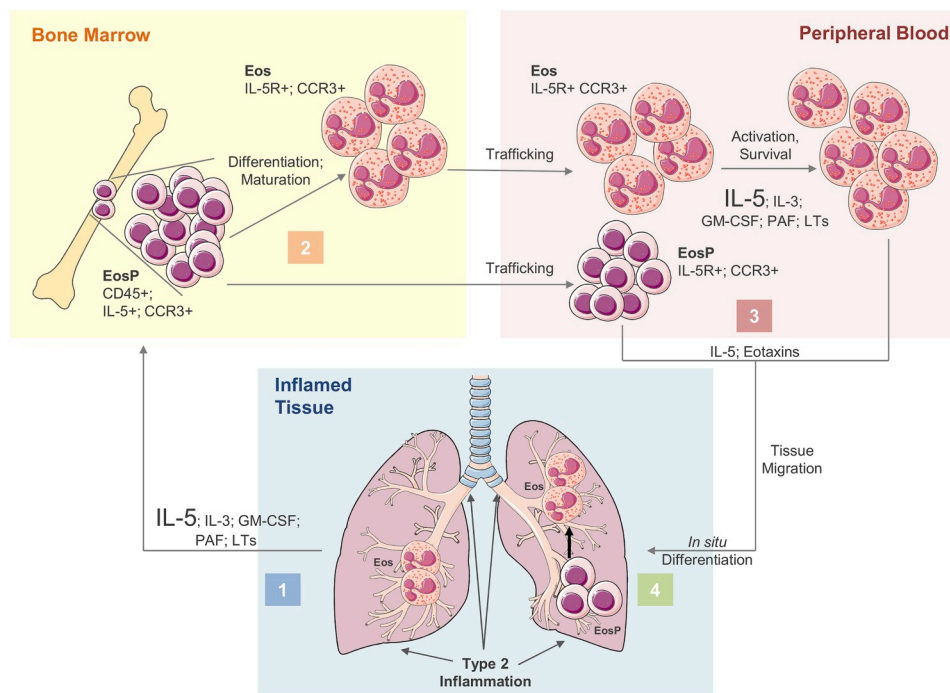


Fig. 1. Biology of eosinophils during bronchial inflammation. The diagram represents the differentiation of eosinophils (Eos) in bone marrow, under the effects of soluble factors produced in inflamed tissue. Both progenitors (EosP) and mature Eos migrate into the bronchial mucosa, where Eos sustain tissue inflammation and EosP undergo local differentiation (*in-situ* eosinophilopoiesis). IL, interleukin; CCR3, C-C chemokine receptor type 3; PAF, platelet-activating factor; GM-CSF, granulocyte macrophage colony-stimulating factor; LTs, leukotrienes; CD, cluster of differentiation.

mucosa level of epithelial lesions, thickening of the basement membrane, and inflammatory infiltration comprising activated eosinophils, Th2 lymphocytes, and degranulated mast cells. However, based on the more recent observations of the inflammatory process at the level of bronchial mucosa of asthmatic patients, at least two different endotypes could be proposed (T2 “high” and T2 “low”), depending on the degree of Th2 inflammation [40]. Eosinophils are recognized as a proinflammatory granulocyte that plays a major role in the T2-high phenotype, which includes the sub-group of severe eosinophilic asthma. This asthma subgroup is characterized by elevated sputum eosinophils (>3%) that frequently requires high dosages of oral steroids to achieve disease control [41]. A correlation has been described between the number of infiltrating/blood eosinophils and the clinical severity of asthma [42,43]. Specifically, patients with a blood eosinophil count greater than 400 cells/ μ L are at risk of future exacerbations.

The amplification and maintenance of eosinophilic airway inflammation and the related clinical exacerbations and chronicity of BA depend on complex mechanisms involving cytokines and chemokines. Eosinophils migrate through the endothelium toward the epithelium in sequential steps in which the cells are initially lightly tethered to the endothelium and roll along its surface. Although IL-5 is a relatively weak chemoattractant, it effectively and specifically primes eosinophils for enhanced chemotactic responsiveness and prolongs their survival in target tissues by inhibiting their apoptosis. Moreover, chemokines and their receptors are receiving considerable attention for their fundamental role in leukocyte migration. Chemokines, such as eotaxins, RANTES (regulated upon activation normal T cells expressed and secreted) (CCL5), monocyte chemoattractant protein (MCP)-3 (CCL7), and MCP-4 (CCL13) strongly induce chemotaxis of eosinophils [44]. Only eosinophils primed by IL-5 as well as by IL-3 and GM-CSF can express all of the membrane receptors and integrins. They, therefore, become able to bind to adhesion molecules overexpressed on the mucosal microvascular endothelial cells and can be selectively attracted by a particular subgroup of β -chemokines, which bind to their membrane CCR3. Accumulation of eosinophils at the bronchial level causes damage mediated by degranulation and the release of toxic proteins such as MBP, ECP, EDN as well as lipid mediators, oxygen free radicals. Eosinophil-derived cationic granule proteins can function by disrupting

the integrity of lipid bilayers, exhibit neurotoxic properties and RNase activities, and/or participate in generating reactive oxidants and radical species capable of damaging the lung epithelium [45]. Eosinophils also produce chemokines and cytokines amplifying the recruitment and activation of other inflammatory cells. In addition, during asthmatic bronchial inflammation, epithelial cells undergo reparation and regeneration favored by transforming growth factor (TGF)- β released by eosinophils participating in the so-called remodeling process [46].

4. Anti-IL-5R α monoclonal antibody strategy: mechanisms of action

4.1. Effects on tissue and circulating eosinophils

In recent years, monoclonal antibodies have become available for the treatment of severe forms of asthma, defined as a subset of difficult-to-treat asthma, defined as uncontrolled despite adherence with maximal optimized therapy and treatment of contributory factors, or that worsens when treatment is decreased [47]. Benralizumab is a humanized, afucosylated IgG1k monoclonal antibody directed against the α subunit of IL-5R, via recognition of an epitope located close to the IL-5 binding site [48]. For this reason, the interaction between benralizumab and IL-5R α allows the inhibition of IL-5 binding to its specific receptor, thus preventing the functions of IL-5 on its target cells (Fig. 2, mechanism 1: IL-5 inhibition).

Interleukin-5 is the main regulator of the biology of eosinophils, including their differentiation, maturation, activation, and survival. Thus, by competing with IL-5, benralizumab modulates all of these biologic steps [49].

More importantly, due to its unique engineering (being an afucosylated monoclonal antibody), benralizumab is capable of markedly reducing eosinophils and other IL-5R + cells, such as eosinophil progenitors and basophils, by inducing antibody-dependent cell-mediated cytotoxicity (ADCC) by natural killer (NK)-cells and/or macrophages (Fig. 2, mechanism 2: marked reduction of IL-5R + cells) [48]. The high capacity of benralizumab to induce ADCC is the consequence of the lack of fucosylation of the CH2 domain of the Fc constant segment, which induces a remarkable enhancement (5–50 times) of the affinity of

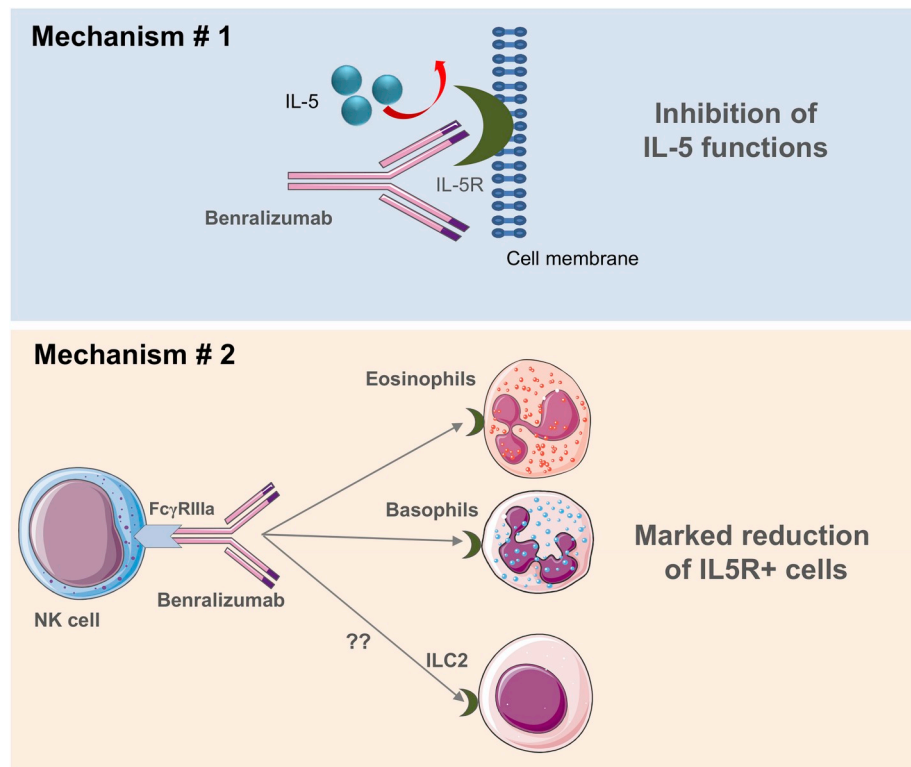


Fig. 2. The two mechanisms of action of benralizumab. Panel A: the interaction between benralizumab and IL-5R α allows the inhibition of IL-5 binding to its specific receptor, thus preventing the functions of IL-5 on its target cells. Panel B: benralizumab markedly depletes eosinophils and other IL-5R + cells, such as eosinophils progenitors and basophils, by inducing antibody-dependent cell-mediated cytotoxicity (ADCC) by natural killer (NK)-cells. FcγRIIIa, crystalloid fragment receptor type IIIa; NK, natural killer; ILC2, innate lymphoid cells type 2; IL, interleukin.

benralizumab for the FcγRIIIa on NK cells compared to the fucosylated parent molecule [48]. This additional activity of benralizumab toward both eosinophils and their progenitors differentiates it from the other IL-5 ligand targeted therapies.

While in clinical practice the concentration of blood eosinophils is typically measured in asthmatic patients, airway bronchial eosinophilia holds greater clinical relevance. Unlike anti-IL-5 monoclonal antibodies, which result in incomplete clearance of eosinophils at the tissue level [27], benralizumab is capable of dramatically reducing eosinophils and eosinophil progenitors at the bronchial level (sputum and bronchial mucosa), even if the number of mucosal and/or submucosal airway eosinophils is found to be less affected by treatment than the number of circulating cells [50]. Towards explaining this concept, it could be speculated that the residual proportion of tissue eosinophils could likely be caused by a lesser ability of the administered drug dose to effectively neutralize airway IL-5 and to mediate ADCC at the tissue level. In addition, the reduced expression of membrane IL-5R on eosinophils [22, 23] described in humans might make eosinophils less susceptible to the treatment, even if the reduction of expression of IL-5R on tissue eosinophils is not a “complete” phenomenon and a residual expression of IL-5R occurs. Of note, the molecular mechanism of ADCC is relatively independent on the level of expression of the target receptor [51]. In other words, benralizumab works well, even in the presence of low levels of IL-5R on target cells. The downregulation of IL-5R might also occur in eosinophil progenitors; however, this point has never been investigated. Finally, it could be speculated that the residual proportion of tissue eosinophils are homeostatic eosinophils, even though this has not yet been proven. The persistence of homeostatic eosinophils may derive from insensitivity to the drug and, specifically, from their supposed independence from IL-5, even though they express IL-5R [21]. In addition, the preferential localization of eosinophils in the parenchymal area could be the reason why they persist despite treatment with both anti-IL-5 or anti-IL-5R, taking into account that this specific site may be reached with difficulty by mAbs.

The modes of action of benralizumab (mechanism 1 + mechanism 2) (Fig. 2) and particularly the ADCC-induced eosinophils apoptosis results

in near complete depletion of these cells in the blood, bone marrow, and lung tissue and therefore result in attenuation of eosinophilic inflammation [50].

4.2. Effects on other IL-5R + cells

Basophils share many features with mast cells, including expression of FcεRI and release of histamine after activation. A notable feature of basophils is their ability to secrete cytokines, specifically their rapid and potent expression of Th2 cytokines such as IL-4 and IL-13. Although basophils circulate in the periphery (<1% of peripheral blood leukocytes), they can infiltrate inflamed tissues (skin and airways), because they express integrins and chemokine receptors [52]. Basophils are well-known IL-5R α -expressing cells. However, the clinical benefits of their almost total depletion after benralizumab administration remain to be defined.

Group 2 innate lymphoid cells, producing a panel of Th2 cytokines, particularly IL-5, but also IL-4 and IL-13, seem to play a role in bronchial inflammation in asthmatic patients, because it has been demonstrated that a significantly greater number of ILC2 are detectable in blood and sputum of patients with severe asthma [53,54]. ILC2 lacking T-cell receptors are directly activated by specific cytokines, including IL-25, IL-33, and TSLP (now called alarmines), which are produced by epithelial cells following activation by allergens, pollutants, and viruses [55]. Some data in the literature regarding the expression of IL-5R on ILC2 are available, but not conclusive [54,56]. Of note, recent data from a sputum sub-study of patients from the ZONDA study with benralizumab showed that there was a reduction in IL-5R α expressing ILC2 cells [57]. Regarding mast cells, evidence of the expression of IL-5R α has been obtained *in vitro* experiments, but data in humans are lacking [58].

5. Eosinophil depletion and safety

The fact that the eosinophil lineage has been maintained throughout evolution suggests a role in supporting good health, although the role of these cells in normal conditions and diseases is still incompletely

defined. Several lines of evidence suggest that the deficiency of eosinophils is not associated with any health problems [59].

The roles of eosinophils in homeostasis and maintenance of an efficient natural immune response have gained more attention after the description of the regulatory population of eosinophils [21]. Treatment with benralizumab, and, to a lesser extent with anti-IL-5 monoclonal antibodies mepolizumab and reslizumab, which reduce but do not deplete eosinophils, have raised the question about the safety of these approaches. Particularly, the question remains as to the pivotal role of eosinophils in protection against parasites and viruses as well as their role in tumor immunology.

Regarding the potential risk of parasitic infestations, we have to underline that the immune response toward these microorganisms includes the intervention of several cells other than eosinophils [60]. Data to define the primary function of eosinophils in protecting against parasites are still lacking, despite many studies having been performed in animals and humans [61,62]. Results obtained in animal models do not strongly support an indispensable role for eosinophils in immunity to most helminth parasites *in vivo*. To this point, eosinophils may contribute to the pathogenesis of helminth infections, due to their ability to cause tissue damage [63]. Finally, even if parasitic infections are a minor issue in industrialized countries, at present, no data about the safety of IL-5/IL-5R α targeted therapies in populations at high risk of helminthic infestations are available.

In addition to their anti-parasitic activity, eosinophils may participate in the host defense against other microbial pathogens, including fungi, bacteria, and viruses, due to their expression of innate receptors (pathogen-associated molecular patterns [PAMPs]), as well as their content in mediators (e.g., ribonucleases) that have anti-viral and anti-bacterial activity. Of note, an eosinophil antiviral response has been demonstrated against respiratory viruses that are mainly involved in asthma exacerbations [64,65]. For this reason, the impaired number of eosinophils might affect the virus-induced loss of asthma control, although data are lacking.

Consideration of cancer is important. First, to avoid an oversimplification of the concepts of tumor immunosurveillance, we must consider that several cells, particularly CD4⁺ and CD8⁺ T lymphocytes, and cytokines such as interferon- γ (IFN- γ) are involved [66]. The role of eosinophils in tumor development (immunosurveillance) and in the immune response in established cancer disease (specific anti-tumor response) is poorly understood. No data are available about the effects of the absence of eosinophils in tumor immunosurveillance. Whereas CD8⁺ T cells and NK cells are key players in facilitating the elimination of cancer cells, the ability of eosinophils to participate in tumor clearance is controversial, because the results in the literature are inconsistent. Specifically, in a model of lung metastasis, the presence of eosinophils in the tumor microenvironment, through the production of chemokines (CCL22), facilitate the recruitment of regulatory T cells that suppress the beneficial anti-tumor specific response [67]. In contrast, in a melanoma tumor model, it has been demonstrated that eosinophils are important in anti-cancer immunity, playing a protective role by inducing the infiltration of CD8⁺ T cells [68]. Overall, we suggest that the role of eosinophils in established cancer disease likely depends on the tumor type, although this is still controversial and, to date, the data has been generated in mouse models, and therefore it is uncertain whether it will extrapolate to human disease.

Another important issue regarding the safety profile of benralizumab is related to the speculative depletion of homeostatic IL-5R + eosinophils. Even if, in mice models, the regulatory function of this subpopulation of eosinophils (rEOS) has been demonstrated [21], no conclusive data are available in humans. Furthermore, specifically, the effect of benralizumab on this rEOS has never been investigated, despite their IL-5R expression.

A hypothetical risk connected to the use of benralizumab and its safety is related to the possible release of the content of granules, in consideration of mechanisms of ADCC. However, *in vitro* studies have

demonstrated that benralizumab induces apoptosis of eosinophils (assessed by means of Annexin V staining) and furthermore this phenomenon is not associated with the release of inflammatory mediators such as eosinophil cation protein (ECP) and EDN stored in the granules [48]. More importantly, *in vivo* data demonstrate that treatment with benralizumab causes a decreased concentration of ECP and EDN in serum, and, thus, despite ADCC, there is no evidence that granule proteins are released in the blood [69]. On the other hand, ADCC is, in any case, a natural process, used by the body's host defense mechanism to target infected cells and cancer cells and, therefore, is a controlled mechanism for clearing cells (i.e., apoptosis) without inducing cell necrosis. Additionally, long-term analysis of the possible adverse effects observed in patients who had received benralizumab treatment for up to 2 years demonstrated a safety profile similar to that reported in randomized controlled trials of up to approximately 1 year's duration [70].

Finally, even if over the last year the role of autoimmune responses in asthma patients has been revised, no conclusive data have been obtained about it to date. Specifically, much more data are needed to clarify both the positive impact (modulation) or negative impact (promotion) of biologicals, including benralizumab, on the autoimmune response in lung inflammation [71].

6. Conclusions

Eosinophils and the IL-5/IL-5 α axis play a key role in the pathogenesis of several inflammatory diseases, including bronchial asthma. Eosinophilic asthma phenotype is common in BA and constitutes the indicated patient population of benralizumab. Its unique double mechanism of action, characterized by the functional inhibition of IL-5 and by the enhanced antibody-dependent cellular cytotoxicity on IL-5R α -expressing cells, sustains its direct, anti-eosinophilic therapeutic effects (Fig. 3). Unlike the IL-5-targeted therapies, the biologic activity of benralizumab influences the total life cycle of eosinophils, from their development in the bone marrow to their migration, survival, and differentiation in inflamed mucosa. Immunosuppression is a potential risk of all eosinophil lowering agents. Despite direct and near complete effects, there has been no evidence to date of any meaningful imbalance in adverse events that would suggest any particular liability for benralizumab. Real world evidence, including post-marketing reports and prospective data from severe-asthma registries, will further inform the

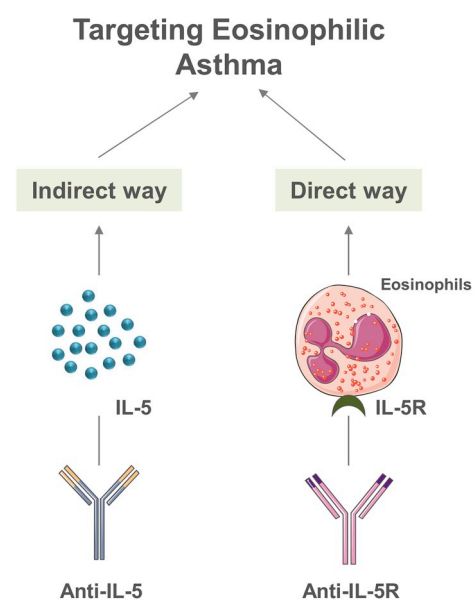


Fig. 3. Benralizumab as a unique anti-eosinophilic biological agent. Eosinophils can be targeted indirectly by anti-IL-5 monoclonal antibodies, and directly by anti-IL-5R α therapy.

safety profile of benralizumab and other eosinophil lowering modalities.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: AM and AV have received payment for lectures from Novartis, GlaxoSmithKline, Teva, AstraZeneca and have consultant arrangements with Novartis, GlaxoSmithKline, Teva, AstraZeneca. EM has received research support from Novartis, and has consultant arrangements with Novartis, AstraZeneca; Teva, Sanofi.

Acknowledgments

The authors thank Ray Hill, an independent medical writer, for English-language editing assistance. This assistance was funded by AstraZeneca Italy.

References

- [1] J.A. O'Sullivan, B.S. Bochner, Eosinophils and eosinophil-associated diseases: an update, *J. Allergy Clin. Immunol.* 141 (2) (2018) 505–517.
- [2] R. Sehmi, S.G. Smith, M. Kjarsgaard, K. Radford, L.P. Boulet, C. Lemiere, C. M. Prazma, H. Ortega, J.G. Martin, P. Nair, Role of local eosinophilopoietic processes in the development of airway eosinophilia in prednisone-dependent severe asthma, *Clin. Exp. Allergy* 46 (6) (2016) 793–802.
- [3] N. Farahi, C. Loutsios, R.P. Simmonds, L. Porter, D. Gillett, S. Heard, A.M. Peters, A.M. Condliffe, E.R. Chilvers, Measurement of eosinophil kinetics in healthy volunteers, *Methods Mol. Biol.* 1178 (2014) 165–176.
- [4] B.S. Bochner, Siglec-8 on human eosinophils and mast cells, and Siglec-F on murine eosinophils, are functionally related inhibitory receptors, *Clin. Exp. Allergy* 39 (3) (2009) 317–324.
- [5] W.B. Cherry, J. Yoon, K.R. Bartemes, K. Iijima, H. Kita, A novel IL-1 family cytokine, IL-33, potentially activates human eosinophils, *J. Allergy Clin. Immunol.* 121 (6) (2008) 1484–1490.
- [6] C.K. Wong, S. Hu, P.F. Cheung, C.W. Lam, Thymic stromal lymphopoietin induces chemotactic and pro-survival effects in eosinophils: implications in allergic inflammation, *Am. J. Respir. Cell Mol. Biol.* 43 (3) (2010) 305–315.
- [7] E.A. Jacobsen, A.D. Doyle, D.C. Colbert, K.R. Zellner, C.A. Protheroe, W.E. LeSuer, N.A. Lee, J.J. Lee, Differential activation of airway eosinophils induces IL-13-mediated allergic Th2 pulmonary responses in mice, *Allergy* 70 (9) (2015) 1148–1159.
- [8] A.M. Dvorak, P. Estrella, T. Ishizaka, Vesicular transport of peroxidase in human eosinophilic myelocytes, *Clin. Exp. Allergy* 24 (1) (1994) 10–18.
- [9] V.S. Muniz, P.F. Weller, J.S. Neves, Eosinophil crystalloid granules: structure, function, and beyond, *J. Leukoc. Biol.* 92 (2) (2012) 281–288.
- [10] K. Nagata, H. Hirai, K. Tanaka, K. Ogawa, T. Aso, K. Sugamura, M. Nakamura, S. Takano, CRTH2, an orphan receptor of T-helper-2-cells, is expressed on basophils and eosinophils and responds to mast cell-derived factor(s), *FEBS Lett.* 459 (2) (1999) 195–199.
- [11] T. Wen, M.E. Rothenberg, The regulatory function of eosinophils, *Microbiol. Spectr.* 4 (5) (2016).
- [12] C. Berek, Eosinophils can more than kill, *J. Exp. Med.* 215 (8) (2018) 1967–1969.
- [13] H. Abdala-Valencia, M.E. Coden, S.E. Chiarella, E.A. Jacobsen, B.S. Bochner, J. J. Lee, S. Berdnikov, Shaping eosinophil identity in the tissue contexts of development, homeostasis, and disease, *J. Leukoc. Biol.* 104 (1) (2018) 95–108.
- [14] H.B. Wang, I. Ghiran, K. Matthaie, P.F. Weller, Airway eosinophils: allergic inflammation recruited professional antigen-presenting cells, *J. Immunol.* 179 (11) (2007) 7585–7592.
- [15] D. Yang, H.F. Rosenberg, Q. Chen, K.D. Dyer, K. Kurosaka, J.J. Oppenheim, Eosinophil-derived neurotoxin (EDN), an antimicrobial protein with chemotactic activities for dendritic cells, *Blood* 102 (9) (2003) 3396–3403.
- [16] M. Ricci, A. Matucci, O. Rossi, [Bronchial asthma: pathogenetic mechanisms and genetic aspects] [Italian], *Recent. Prog. Med.* 88 (11) (1997) 530–540.
- [17] B.W. Li, R.W. Hendriks, Group 2 innate lymphoid cells in lung inflammation, *Immunology* 140 (3) (2013) 281–287.
- [18] S.E. Broughton, T.L. Nero, U. Dhagat, W.L. Kan, T.R. Hercus, D. Tvorogov, A. F. Lopez, M.W. Parker, The β c receptor family - structural insights and their functional implications, *Cytokine* 74 (2) (2015) 247–258.
- [19] A. Menzies-Gow, P. Flood-Page, R. Sehmi, J. Burman, Q. Hamid, D.S. Robinson, A. B. Kay, J. Denburg, Anti-IL-5 (mepolizumab) therapy induces bone marrow eosinophil maturational arrest and decreases eosinophil progenitors in the bronchial mucosa of atopic asthmatics, *J. Allergy Clin. Immunol.* 111 (4) (2003) 714–719.
- [20] C. Yoshimura-Uchiyama, M. Yamaguchi, H. Nagase, K. Matsushima, T. Igarashi, T. Iwata, K. Yamamoto, K. Hirai, Changing expression of IL-3 and IL-5 receptors in cultured human eosinophils, *Biochem. Biophys. Res. Commun.* 309 (1) (2003) 26–31.
- [21] C. Mesnil, S. Raulier, G. Paulissen, X. Xiao, M.A. Birrell, D. Pirotton, T. Janss, P. Starkl, E. Ramery, M. Henket, F.N. Schleich, M. Radermecker, K. Thielemans, L. Gillet, M. Thiry, M.G. Belvisi, R. Louis, C. Desmet, T. Marichal, F. Bureau, Lung-resident eosinophils represent a distinct regulatory eosinophil subset, *J. Clin. Invest.* 126 (9) (2016) 3279–3295.
- [22] L.Y. Liu, J.B. Sedgwick, M.E. Bates, R.F. Vrtis, J.E. Gern, H. Kita, N.N. Jarjour, W. W. Busse, E.A. Kelly, Decreased expression of membrane IL-5 receptor alpha on human eosinophils: I. Loss of membrane IL-5 receptor alpha on airway eosinophils and increased soluble IL-5 receptor alpha in the airway after allergen challenge, *J. Immunol.* 169 (11) (2002) 6452–6458.
- [23] L.Y. Liu, J.B. Sedgwick, M.E. Bates, R.F. Vrtis, J.E. Gern, H. Kita, N.N. Jarjour, W. W. Busse, E.A. Kelly, Decreased expression of membrane IL-5 receptor alpha on human eosinophils: II. IL-5 down-modulates its receptor via a proteinase-mediated process, *J. Immunol.* 169 (11) (2002) 6459–6466.
- [24] G. Garcia, C. Taille, P. Laveneziana, A. Bourdin, P. Chanez, M. Humbert, Anti-interleukin-5 therapy in severe asthma, *Eur. Respir. Rev.* 22 (129) (2013) 251–257.
- [25] M. Hassani, L. Koenderman, Immunological and hematological effects of IL-5 (Ralpha)-targeted therapy: an overview, *Allergy* 73 (10) (2018) 1979–1988.
- [26] S. Esnault, E.A. Kelly, Essential mechanisms of differential activation of eosinophils by IL-3 compared to GM-CSF and IL-5, *Crit. Rev. Immunol.* 36 (5) (2016) 429–444.
- [27] P.T. Flood-Page, A.N. Menzies-Gow, A.B. Kay, D.S. Robinson, Eosinophil's role remains uncertain as anti-interleukin-5 only partially depletes numbers in asthmatic airway, *Am. J. Respir. Crit. Care Med.* 167 (2) (2003) 199–204.
- [28] L.K. Johnston, C.L. Hsu, R.A. Krier-Burris, K.D. Chhiba, K.B. Chien, A. McKenzie, S. Berdnikov, P.J. Bryce, IL-33 precedes IL-5 in regulating eosinophil commitment and is required for eosinophil homeostasis, *J. Immunol.* 197 (9) (2016) 3445–3453.
- [29] M.C. Larose, A.S. Archambault, V. Provost, M. Laviolette, N. Flamand, Regulation of eosinophil and group 2 innate lymphoid cell trafficking in asthma, *Front. Med.* 4 (2017) 136.
- [30] J.V. Fahy, Type 2 inflammation in asthma—present in most, absent in many, *Nat. Rev. Immunol.* 15 (1) (2015) 57.
- [31] P. Licona-Limon, L.K. Kim, N.W. Palm, R.A. Flavell, TH2, allergy and group 2 innate lymphoid cells, *Nat. Immunol.* 14 (6) (2013) 536–542.
- [32] K.R. Bartemes, G.M. Kephart, S.J. Fox, H. Kita, Enhanced innate type 2 immune response in peripheral blood from patients with asthma, *J. Allergy Clin. Immunol.* 134 (3) (2014) 671–678 e4.
- [33] E.L. Anderson, T. Kobayashi, K. Iijima, K.R. Bartemes, C.C. Chen, H. Kita, IL-33 mediates reactive eosinophilopoiesis in response to airborne allergen exposure, *Allergy* 71 (7) (2016) 977–988.
- [34] V. Provost, M.C. Larose, A. Langlois, M. Rola-Pleszczynski, N. Flamand, M. Laviolette, CCL26/eotaxin-3 is more effective to induce the migration of eosinophils of asthmatics than CCL11/eotaxin-1 and CCL24/eotaxin-2, *J. Leukoc. Biol.* 94 (2) (2013) 213–222.
- [35] P.D. Collins, S. Marleau, D.A. Griffiths-Johnson, P.J. Jose, T.J. Williams, Cooperation between interleukin-5 and the chemokine eotaxin to induce eosinophil accumulation in vivo, *J. Exp. Med.* 182 (4) (1995) 1169–1174.
- [36] W. Wang, Y. Li, Z. Lv, Y. Chen, Y. Li, K. Huang, C.J. Corrigan, S. Ying, Bronchial allergen challenge of patients with atopic asthma triggers an alarmin (IL-33, TSLP, and IL-25) response in the airways epithelium and submucosa, *J. Immunol.* 201 (8) (2018) 2221–2231.
- [37] H. Kankaanranta, P. Ilmarinen, X. Zhang, I.M. Adcock, A. Lahti, P.J. Barnes, M. A. Gienbycz, M.A. Lindsay, E. Moilanen, Tumour necrosis factor- α regulates human eosinophil apoptosis via ligation of TNF-receptor 1 and balance between NF-kappaB and AP-1, *PLoS One* 9 (2) (2014), e90298.
- [38] G.A. Thompson-Souza, I. Gropillo, J.S. Neves, Cysteinyl leukotrienes in eosinophil biology: functional roles and therapeutic perspectives in eosinophilic disorders, *Front. Med.* 4 (2017) 106.
- [39] G. Kano, M. Almanan, B.S. Bochner, N. Zimmermann, Mechanism of Siglec-8-mediated cell death in IL-5-activated eosinophils: role for reactive oxygen species-enhanced MEK/ERK activation, *J. Allergy Clin. Immunol.* 132 (2) (2013) 437–445.
- [40] P.G. Woodruff, B. Modrek, D.F. Choy, G. Jia, A.R. Abbas, A. Ellwanger, L.L. Koth, J. R. Arron, J.V. Fahy, T-helper type 2-driven inflammation defines major subphenotypes of asthma, *Am. J. Respir. Crit. Care Med.* 180 (5) (2009) 388–395.
- [41] P. Nair, M.M. Pizzichini, M. Kjarsgaard, M.D. Inman, A. Efthimiadis, E. Pizzichini, F.E. Hargreave, P.M. O'Byrne, Mepolizumab for prednisone-dependent asthma with sputum eosinophilia, *N. Engl. J. Med.* 360 (10) (2009) 985–993.
- [42] J. Bousquet, P. Chanez, J.Y. Lacoste, G. Barneon, N. Ghavanian, I. Enander, P. Venge, S. Ahlstedt, J. Simony-Lafontaine, P. Godard, F.B. Michel, Eosinophilic inflammation in asthma, *N. Engl. J. Med.* 323 (15) (1990) 1033–1039.
- [43] D.B. Price, A. Rigazio, J.D. Campbell, E.R. Bleeker, C.J. Corrigan, M. Thomas, S. E. Wenzel, A.M. Wilson, M.B. Small, G. Gopalan, V.L. Ashton, A. Burden, E. V. Hillyer, M. Kerkhof, I.D. Pavord, Blood eosinophil count and prospective annual asthma disease burden: a UK cohort study, *Lancet Respir Med* 3 (11) (2015) 849–858.
- [44] H. Tachimoto, M. Ebisawa, B.S. Bochner, Cross-talk between integrins and chemokines that influences eosinophil adhesion and migration, *Int. Arch. Allergy Immunol.* 128 (Suppl 1) (2002) 18–20.
- [45] C.N. McBrien, A. Menzies-Gow, The biology of eosinophils and their role in asthma, *Front. Med.* 4 (2017) 93.
- [46] A. Januskevicius, S. Vaitkiene, R. Gosens, I. Janulaityte, D. Hoppenot, R. Sakalauskas, K. Malakauskas, Eosinophils enhance WNT-5a and TGF-beta1 genes expression in airway smooth muscle cells and promote their proliferation by increased extracellular matrix proteins production in asthma, *BMC Pulm. Med.* 16 (1) (2016) 94–105.
- [47] Global Initiative for Asthma (GINA), Global strategy for asthma management and prevention, Updated 2018, <http://www.ginasthma.org/>, 2018.
- [48] R. Kolbeck, A. Kozhich, M. Koike, L. Peng, C.K. Andersson, M.M. Damschroder, J. L. Reed, R. Woods, W.W. Dall'acqua, G.L. Stephens, J.S. Erjefält, L. Bjerner, A.

- A. Humbles, D. Gossage, H. Wu, P.A. Kiener, G.L. Spitalny, C.R. Mackay, N. A. Molfino, A.J. Coyle, MEDI-563, a humanized anti-IL-5 receptor alpha mAb with enhanced antibody-dependent cell-mediated cytotoxicity function, *J. Allergy Clin. Immunol.* 125 (6) (2010) 1344–1353 e2.
- [49] W.W. Busse, R. Katial, D. Gossage, S. Sari, B. Wang, R. Kolbeck, A.J. Coyle, M. Koike, G.L. Spitalny, P.A. Kiener, G.P. Geba, N.A. Molfino, Safety profile, pharmacokinetics, and biologic activity of MEDI-563, an anti-IL-5 receptor alpha antibody, in a phase I study of subjects with mild asthma, *J. Allergy Clin. Immunol.* 125 (6) (2010) 1237–1244 e2.
- [50] M. Laviolette, D.L. Gossage, G. Gauvreau, R. Leigh, R. Olivenstein, R. Katial, W. W. Busse, S. Wenzel, Y. Wu, V. Datta, R. Kolbeck, N.A. Molfino, Effects of benralizumab on airway eosinophils in asthmatic patients with sputum eosinophilia, *J. Allergy Clin. Immunol.* 132 (5) (2013) 1086–1096, e5.
- [51] T. van Meerten, R.S. van Rijn, S. Hol, A. Hagenbeek, S.B. Ebeling, Complement-induced cell death by rituximab depends on CD20 expression level and acts complementary to antibody-dependent cellular cytotoxicity, *Clin. Cancer Res.* 12 (13) (2006) 4027–4035.
- [52] K.D. Stone, C. Prussin, D.D. Metcalfe, IgE, mast cells, basophils, and eosinophils, *J. Allergy Clin. Immunol.* 125 (2 Suppl 2) (2010) S73–S80.
- [53] L. Cosmi, F. Liotta, L. Maggi, F. Annunziato, Role of type 2 innate lymphoid cells in allergic diseases, *Curr. Allergy Asthma Rep.* 17 (10) (2017) 66.
- [54] S.G. Smith, R. Chen, M. Kjarsgaard, C. Huang, J.P. Oliveria, P.M. O’Byrne, G. M. Gauvreau, L.P. Boulet, C. Lemiere, J. Martin, P. Nair, R. Sehmi, Increased numbers of activated group 2 innate lymphoid cells in the airways of patients with severe asthma and persistent airway eosinophilia, *J. Allergy Clin. Immunol.* 137 (1) (2016) 75–86 e8.
- [55] B.N. Lambrecht, H. Hammad, The immunology of asthma, *Nat. Immunol.* 16 (1) (2015) 45–56.
- [56] A.K.A. Wright, C. Weston, B.M.J. Rana, C.E. Brightling, D.J. Cousins, Human group 2 innate lymphoid cells do not express the IL-5 receptor, *J. Allergy Clin. Immunol.* 140 (5) (2017) 1430–1433 e4.
- [57] R. Sehmi, H.F. Lim, M. Mukherjee, C. Huang, K. Radford, P. Newbold, L.P. Boulet, D. Dorscheid, J.G. Martin, P. Nair, Benralizumab attenuates airway eosinophilia in prednisone-dependent asthma, *J. Allergy Clin. Immunol.* 141 (4) (2018) 1529–1532 e8.
- [58] C. Dahl, H.J. Hoffmann, H. Saito, P.O. Schiotz, Human mast cells express receptors for IL-3, IL-5 and GM-CSF; a partial map of receptors on human mast cells cultured in vitro, *Allergy* 59 (10) (2004) 1087–1096.
- [59] G.J. Gleich, A.D. Klion, J.J. Lee, P.F. Weller, The consequences of not having eosinophils, *Allergy* 68 (7) (2013) 829–835.
- [60] C.C. Motran, L.F. Ambrosio, X. Volpini, D.P. Celias, L. Cervi, Dendritic cells and parasites: from recognition and activation to immune response instruction, *Semin. Immunopathol.* 39 (2) (2017) 199–213.
- [61] R.F. Sturrock, R. Kimani, B.J. Cottrell, A.E. Butterworth, H.M. Seitz, T.K. Siongok, V. Houba, Observations on possible immunity to reinfection among Kenyan schoolchildren after treatment for *Schistosoma mansoni*, *Trans. R. Soc. Trop. Med. Hyg.* 77 (3) (1983) 363–371.
- [62] P. Hagan, H.A. Wilkins, U.J. Blumenthal, R.J. Hayes, B.M. Greenwood, Eosinophilia and resistance to *Schistosoma haematobium* in man, *Parasite Immunol.* 7 (6) (1985) 625–632.
- [63] A.D. Klion, T.B. Nutman, The role of eosinophils in host defense against helminth parasites, *J. Allergy Clin. Immunol.* 113 (1) (2004) 30–37.
- [64] A.S. Flores-Torres, M.C. Salinas-Carmona, E. Salinas, A.G. Rosas-Taraco, Eosinophils and respiratory viruses, *Viral Immunol.* 32 (5) (2019) 198–207.
- [65] Y.S. Sabogal Pinos, S.M. Bal, A. Dijkhuis, C.J. Majoor, B.S. Dierdorp, T. Dekker, E.P. Hoefsmit, P.I. Bonta, D. Picavet, N.N. van der Wel, L. Koenderman, P.J. Sterk, L. Ravanetti, R. Lutter, Eosinophils Capture Viruses, a Capacity that Is Defective in Asthma, 2019. *Allergy*.
- [66] O.J. Finn, Cancer immunology, *N. Engl. J. Med.* 358 (25) (2008) 2704–2715.
- [67] R. Zaynagetdinov, T.P. Sherrill, L.A. Gleaves, A.G. McLoed, J.A. Saxon, A. C. Habermann, L. Connelly, D. Dulek, R.S. Peebles Jr., B. Fingleton, F.E. Yull, G. T. Stathopoulos, T.S. Blackwell, Interleukin-5 facilitates lung metastasis by modulating the immune microenvironment, *Cancer Res.* 75 (8) (2015) 1624–1634.
- [68] R. Carretero, I.M. Sektioglu, N. Garbi, O.C. Salgado, P. Beckhove, G.J. Hammerling, Eosinophils orchestrate cancer rejection by normalizing tumor vessels and enhancing infiltration of CD8(+) T cells, *Nat. Immunol.* 16 (6) (2015) 609–617.
- [69] T.H. Pham, G. Damera, P. Newbold, K. Ranade, Reductions in eosinophil biomarkers by benralizumab in patients with asthma, *Respir. Med.* 111 (2016) 21–29.
- [70] W.W. Busse, E.R. Bleeker, J.M. FitzGerald, G.T. Ferguson, P. Barker, S. Sproule, R. F. Olsson, U.J. Martin, M. Goldman, BORA Study Investigators, Long-term safety and efficacy of benralizumab in patients with severe, uncontrolled asthma: 1-year results from the BORA phase 3 extension trial, *Lancet Respir Med* 7 (1) (2019) 46–59.
- [71] M. Mukherjee, P. Nair, Autoimmune responses in severe asthma, *Allergy Asthma Immunol Res* 10 (5) (2018) 428–447.