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Impact of Worms and their Products on Eosinophils and Neutrophils in Experimental Asthma

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Abstract: Prolonged existence of helminth parasites in their mammalian hosts has led to the establishment of a very particular immunological microenvironment that supports the fitness of both the pathogen and the host. The modern way of living in developed societies has interrupted this tight relationship by almost completely removing helminths from the human population. It is believed that, as a consequence of this process, a rapid increase in the incidence of asthma and other inflammatory disorders has occurred. Data derived from experimental models clearly show that worms and their products can ameliorate asthma-like disease in mice. This review will concentrate on the effects of helminth-driven regulatory mechanisms on the function of eosinophils and neutrophils in experimental asthma. Eosinophils and neutrophils are major effector cells driving pathology in the lung, therefore learning how to control their exacerbated activation in asthma might aid in creating much needed novel therapeutics to combat this common inflammatory disorder.

Keywords: Helminths, eosinophils, neutrophils, asthma.

INTRODUCTION

Helminths are large, multicellular animals that can be found within two phyla: the Nematelminthes phylum containing the roundworms (nematodes) and the Platyhelminthes phylum containing the tapeworms (cestodes) and the flukes (trematodes). Most helminth parasites have coevolved with their mammalian hosts for countless generations e.g. the human hookworm *Necator americanus*, the filarial nematode *Brugia malayi* and the fluke *Schistosoma mansoni*. During this co-adaptation process a very finely tuned immunological microenvironment has developed that facilitates survival of the parasite within the host whilst protecting the host from immunopathologies that might occur as a consequence of the infection. Indeed, most humans chronically infected with helminths, regardless of the species, show little to no pathology, although considerable morbidity due to heavy and chronic exposure to worms does exist, especially in children born in endemic areas (reviewed in [1]).

Removal of helminths from human population in the Western countries, by means of increased hygiene and use of anthelmintic drugs, has been postulated to be one of the reasons accounting for the steep increase in the incidence of autoimmune diseases and allergies [2]. Indeed, a striking inverse distribution of these disorders and helminths is observed across the globe [3]. To address this phenomenon many epidemiologic studies, especially in the field of allergic disorders, have been undertaken to date (reviewed in [4-10]).

Noteworthy, human studies have failed to uniformly support a protective role of helminths in allergy probably due to the complexity of factors that determine the allergy outcome in the human population such as existence of additional environmental factors, genetic make up of the host, age- and intensity-dependent exposure to helminths and different asthma phenotypes.

Most of the field studies described to date rely on quantifying atopic responses e.g. measuring levels of allergen-specific IgE antibodies in skin prick test, which although very important for the development of asthma, are not the only determinant of the disease outcome. None of the field studies so far, at least to our knowledge, have addressed the impact of helminths on two potentially important inflammatory cells in allergic subjects – the eosinophil and the neutrophil. In contrast to mixed field data, experimental infections with certain species of helminth have shown very convincingly that such infections could confer a therapeutic effect in the course of inflammatory bowel disease (IBD) and asthma amongst other inflammatory conditions. In addition, although limited human trials using helminths to treat allergic conditions have perhaps produced disappointing results [11] more trials are planned and in addition, two species of parasitic helminth, the pig whipworm *Trichuris suis* and *Necator americanus* were successfully tested in a number of clinical trials against IBD and proved to be effective [12].

PRIMING OF TH2 RESPONSES BY HELMINTHS IS ACCOMPANIED BY CONCOMITANT INDUCTION OF VARIOUS IMMUNE SYSTEM REGULATORY ELEMENTS

Infections with different species of helminth almost uniformly stimulate induction of the Th2 type of

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adaptive immune responses characterized by production of various mediators such as interleukin-(IL-) 3, 4, 5, 9, 10 and 13 and antibodies of IgG1, IgG4 and IgE isotypes as well as increased numbers of eosinophils, basophils, mast cells and alternatively activated macrophages [13]. These varied immune system components that the host responds with to the helminth insult bear a great potential to significantly decrease parasite vitality or lead to its expulsion as shown in many experimental studies [14]. It is surprising therefore that helminth infections in humans are usually chronic, with very few people being able to completely resolve them [1]. One of the possible explanations for this phenomenon is that helminths in order to successfully colonize the human population have developed many strategies to subvert harmful host immune responses and a current paradigm is that we can exploit these essentially regulatory-inducing strategies to treat autoimmune diseases and allergies.

Immuno-regulatory events associated with helminths are very complex, however based on the current literature it is possible to stratify them into the following categories: induction of lymphoid cells with a regulatory phenotype such as regulatory T cells [6] and regulatory B cells [15], induction of myeloid cells with an anti-inflammatory phenotype e.g. dendritic cells [9] and alternatively activated macrophages [16], inactivation of effector immune system cells such as: eosinophils, neutrophils, basophils and mast cells [17] as well as enhancement of mucosal barrier function [18].

The current review will provide analysis of how helminths can modulate functions of eosinophils and neutrophils in asthma and how this might relate to the identification of new biologic treatments to control this common chronic inflammatory disease.

INFLAMMATION IN ASTHMA AND ITS PARTIAL PARALLELISM TO HELMINTH-TRIGGERED IMMUNE RESPONSES

Allergic diseases are recognized as a global public health concern due to their extremely high prevalence worldwide. It has been estimated that about 30-40% of the world population is now affected by one or more allergic conditions e.g. asthma, rhinitis, anaphylaxis, eczema and drug, food and insect allergies [19]. Asthma is a chronic inflammatory disease with high morbidity and significant mortality, characterized by recurrent episodes of airway obstruction and wheezing. Typically in allergies the immune system reacts to non-infectious environmental substances known as allergens and mounts allergen-specific Th2 cell responses and IgE production. Repeated allergen exposure leads to chronic allergic inflammation that is a persistent inflammation involving many innate and adaptive immune system cells and is associated with changes in the structural cells at the affected sites, and in many cases with markedly altered function of the affected organs [20]. The key cytokines implicated in the process e.g. IL-4, IL-5 and IL-13 are all encoded in the Th2 cytokine gene locus on human chromosome 5q31 that acts as a unit and governs co-regulation and

co-expression of the member cytokines in response to T cell activation [21]. Such coordinated expression of Th2 cytokines has a detrimental effect on health, contributing to the pathogenesis in allergic inflammation. In the situation however when the immune system has to mount a complementary defense response against infections with helminths such coordination is paramount. The similarities by which the immune system reacts to control helminths and promote allergies have led to the idea that in the allergic disorders the immune system is "misguided" and reacts to otherwise non-pathogenic factors in the same manner that it reacts to products of helminths [22]. This parallelism between allergic conditions and parasitic infections is further highlighted by the fact that several genes carrying candidate targets for helminth-driven selective pressure e.g. IL-4, also harbor susceptibility alleles for asthma/allergy or are involved in airway hyper-responsiveness [23]. The apparent lack of Th2-driven pathology during chronic infections with helminths is due to the presence of a strong regulatory circuit in which regulatory T cells seem to play a major role [24]. In patients with asthma on the other hand, functional deficiency in regulatory T lymphocytes has been reported [25], and this is thought to facilitate disease progression and pathology due to an inability of the immune system to keep allergenic Th2 responses in check. Indeed, it was noted that the balance between allergen-specific regulatory T cells and Th2 cells might be decisive in the development of allergy [26].

COMPLEXITY OF IMMUNE RESPONSES IN ASTHMA

In the scientific community allergic disorders have been viewed as primarily Th2-type diseases, in which allergen-triggered production of IL-5 leads to an influx of eosinophils into the airways resulting in tissue pathology and organ dysfunction. This concept was reinforced by many immunologic studies carried out both in humans and experimental animals as well as being strengthened by substantial *in vitro* data. This view however is limited and does not account for the increasing evidence of heterogeneity in the inflammatory responses in asthma. Indeed, it has been shown that upon examination of sputum, bronchoalveolar lavage and lung biopsies of asthmatic patients, eosinophils as a major component of inflammation, are identified only in approximately 40–60% of studied cases [27]. In patients that display a non-eosinophilic type of inflammation the disease is associated mostly with an increased proportion of neutrophils or enhanced numbers of macrophages. In particular, the severe form of airway hyperreactivity has been attributed to patients with robust neutrophilia and enhanced levels of IL-17A. This finding of distinct phenotypes of asthma is also reflected in the way that treated patients react to commonly used drugs against asthma. Except for severe cases, eosinophilic asthma responds well to inhaled corticosteroids whereas neutrophilic asthma is refractory to this treatment. Interestingly, corticosteroids can actually accentuate

neutrophilic inflammation as they have been shown to promote neutrophil survival [28]. Recognition of different phenotypes of asthma is therefore pivotal for better asthma management. To address this issue many recent studies have aimed at better asthma classification based on the type of cellular composition, clinical manifestation and gene expression profiling. The study of Baines *et al.* [29] for example, used unsupervised hierarchical clustering of induced sputum gene expression profile to stratify the studied population of patients with recognized asthma. Three distinct phenotypes were identified using this approach and they related to both the nature of the clinical asthma and the type and degree of inflammation (Fig. 1). Recognition of different asthma phenotypes in humans should now lead to development of appropriate mouse models that can recapitulate major disease features observed in different strata. However, this poses a difficulty, as the molecular and cellular mechanisms underlying different phenotypes are not fully understood at present. Nevertheless, researchers have already started addressing this issue and working with animal models that offer the possibility to study the disease beyond exclusive activation of Th2 cells and eosinophil-biased inflammation. For example, it was reported that A/J mice develop severe asthma

associated with production of IL-17A and Th2 cytokines, whereas C3H/HeJ mice manifest less severe airway hyperresponsiveness and produce only Th2 cytokines with little to no IL-17A [30].

Eosinophils and neutrophils in asthma are not only useful markers for delineation of different disease phenotypes but also major effector cells driving pathology of afflicted tissue. Learning how to control the inappropriate activation of both types of granulocytes is therefore particularly interesting from a therapeutic point of view. Eosinophils and neutrophils are among the most important types of cells activated upon infection with helminths. Historically they were known as the pivotal effectors having an instrumental role in parasite damage induction and/or elimination. It is not surprising therefore that helminths in order to persist in the host evolved many strategies to avoid the destructive action of granulocytes.

EXPERIMENTAL MODEL TO STUDY MODULATION OF ASTHMA BY HELMINTHS AND THEIR PRODUCTS

The majority of studies investigating the impact of worms on allergic airway disease are carried out in the mouse, which offers a unique opportunity to study the

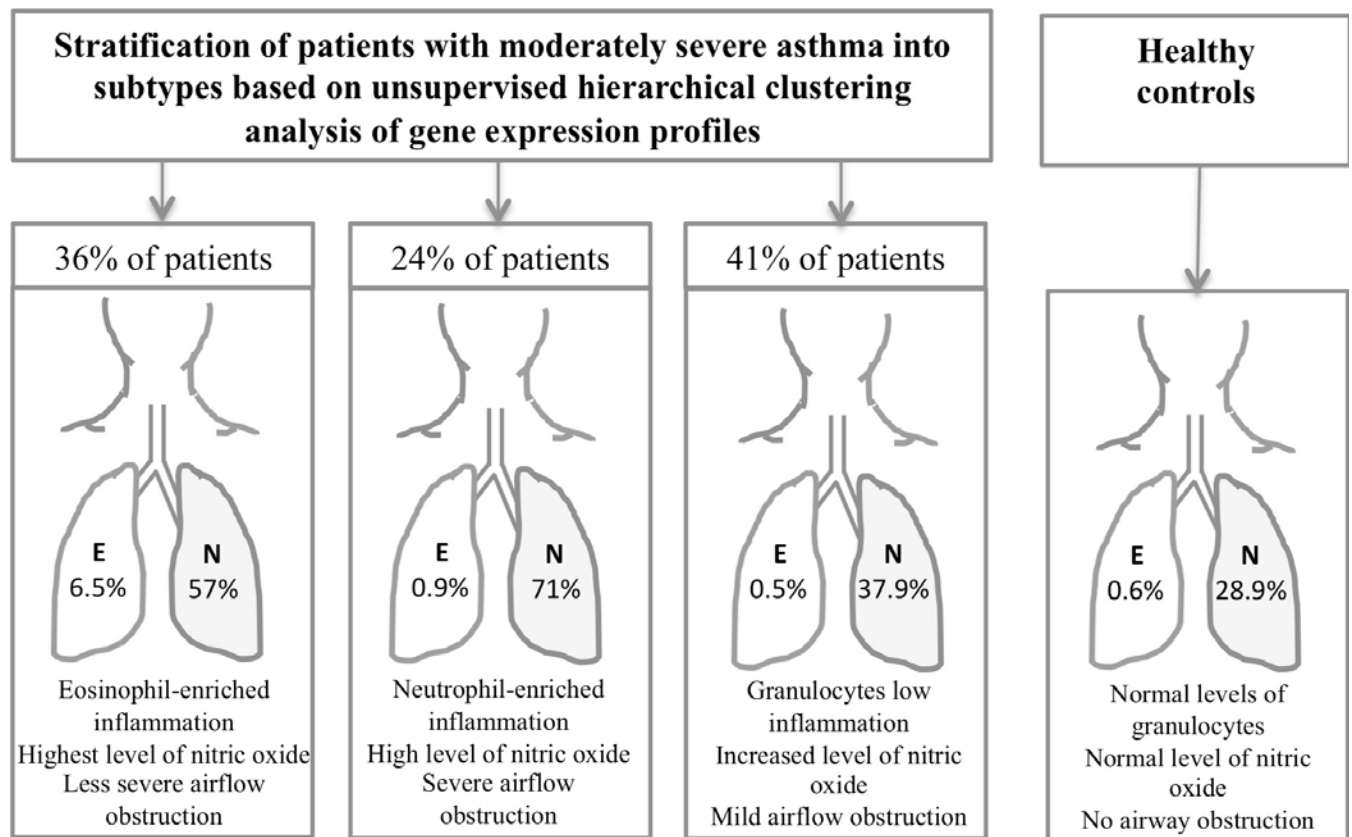


Fig. (1). Graphic representation of results from studies by Baines *et al.*, 2010. Induced sputum samples were collected from 59 people with asthma and 13 healthy controls. RNA was extracted from selected sputum and transcriptional profiles were generated and analyzed. Differentially-expressed genes between transcriptional asthma phenotypes were related to inflammatory and immune response. Unsupervised hierarchical clustering of gene expression profiles in asthma revealed 3 different groups as shown. These distinct asthma phenotypes related to both clinical and inflammatory parameters. E (eosinophils), N (neutrophils).

mechanisms of allergic reactions in detail (reviewed in [31-34]). The researchers use the OVA/Alum model in which a surrogate allergen, ovalbumin (OVA) is injected into the mouse peritoneal cavity in combination with the Th2 skewing adjuvant aluminium hydroxide (alum) to prime for OVA-specific T cell responses. The most widely used strains are BALB/c and C57BL/6 due to their well-characterized immune responses and good predisposition to mount Th2 responses (BALB/c) as well as availability of genetically engineered mice (C57BL/6). Despite the wide use of the OVA/Alum model, including by pharmaceutical companies to screen for novel drugs with anti-allergic properties, the system does possess pitfalls that are summarized in Table 1.

Up until recently, little has been known regarding innate immune responses triggered by the injection of alum and OVA into the mouse. Elegant studies performed by Kool *et al.* in 2008 [35, 36] revealed that application of alum induces endogenous danger signals such as uric acid and release of host DNA, that stimulates production of early IL-1 β from peritoneal macrophages and dendritic cells and the influx of inflammatory cells via the NALP3 inflammasome pathway. Furthermore, in the presence of alum, OVA was shown to be taken up, processed, and presented by inflammatory monocytes that migrated from the peritoneum, thus becoming inflammatory DCs that induce persistent Th2 responses [36]. This initial step in the development of the allergic reaction under experimental conditions is known as a “sensitization phase”.

During a “challenge phase”, a series of intranasal OVA applications provoke localized allergic inflammation characterized by influx of inflammatory cells into the bronchial spaces, pathological changes in the lungs and impaired airflow. Termination of the model usually occurs 24 hrs after the last intranasal OVA challenge and changes in the degree of lung inflammation are

measured. The analysis typically involves quantification of leukocytes especially eosinophils and neutrophils infiltrating bronchoalveolar space during local allergen provocation and measurement of Th2 type cytokines and chemokines such as IL-5 and eotaxin, respectively.

Undoubtedly allergens are the most important components of allergy induction, however other molecules known to be ubiquitous in living environments might be involved in the modulation of allergenic immune responses. Among these factors lipopolysaccharides (LPS, endotoxin) has been shown to be particularly relevant in precipitating and altering the course of asthma as suggested by numerous epidemiological and experimental data [37]. LPS is also known to pose a problem in the laboratory as commercially available preparations of OVA contain varied amounts of LPS and its quantification is rarely performed and disclosed in publications. One might therefore speculate that the differentially increased numbers of lung neutrophils in most of the OVA/Alum models simply reflect the different LPS content of OVA preparations. Indeed, recent data showed that the presence of LPS in an OVA preparation used to prime allergen-specific Th2 responses and allergic lung inflammation accounted for increased influx of neutrophils into lungs and enhanced local numbers of macrophages [38]. This type of inflammation therefore bears a strong resemblance to the non-eosinophilic type of inflammation recorded in a substantial fraction of patients. Interestingly, instillation of LPS without the presence of any allergen induces lung injury due to infiltration of alveolar space with neutrophils [39].

REGULATION OF EOSINOPHIL FUNCTION BY HELMINTHS

Eosinophil recruitment is a typical feature of helminth infections and allergies. These granulocytes

Table 1. Pitfalls Associated with the OVA/Alum Model Used to Study Impact of Helminths on Allergic Airway Inflammation

Model Component	Problem
Mouse strain	Routine use of BALB/c and C57BL/6 strains that offer only limited representation of genetic factors involved in asthma; use of other mouse strains e.g. A/J, C3H/HeJ or C3H/HeN might increase the number of observed disease phenotypes.
Allergen	OVA is a biochemically simple surrogate allergen with weak properties to induce immune responses unless co-administered with an adjuvant; use of a potent allergen such as house dust mites (HDM; <i>Dermatophagoides</i> sp.) that can stimulate allergic airway disease when administered by means of inhalation without prior systemic priming or use of adjuvant offers an attractive alternative.
Adjuvant	Alum induces endogenous danger signals that activate the inflammasome complex and induce subsequent release of bioactive IL-1 β . Even though a natural sensitization to air-born allergens doesn't occur in the presence of alum <i>per se</i> , there is growing evidence indicating that the inflammasome plays a key role in the pathogenesis of acute and chronic respiratory diseases.
LPS contamination of OVA	LPS is a powerful immunomodulator of allergic reactions both in natural and experimental settings. Its effects depend most probably on the timing and pattern of exposure (transient high exposure versus chronic moderate exposure, present at the sensitisation phase or challenge). It is therefore pivotal to determine contamination of LPS in different allergen preparations (commercially obtained OVA is notoriously associated with variable amounts of LPS, however other allergens such as HDM extract prepared from mites can contain endotoxin and Gram-negative <i>Bartonella</i> species that are thought to be the source of LPS).
Phase of reaction	More studies using helminths and their products should focus on their therapeutic potential i.e. testing their ability to suppress established disease when given at the challenge.

can degranulate in the presence of worm antigen or allergen and release toxic granules containing e.g. eosinophil peroxidase and major basic protein that can either facilitate destruction of parasites or cause tissue damage and associated pathologies like in asthma [40]. In addition to their effector functions, eosinophils can also produce various cytokines and chemokines that modulate the functions of other immune system cells and shape on-going inflammation. Eosinophils can also present antigen to naïve and memory T cells especially in the context of Th2 immunity [41] and as such initiate and maintain inflammatory immune responses (summarized in Table 2).

Down-Regulation of IL-5/Eotaxin Axis by Active Worm Infections

One way to modulate the function of eosinophils and other granulocytes is to regulate the number of cells in the afflicted tissue *via* targeting their proliferation, migration and the rate at which they undergo cell death/apoptosis. Eosinophils, as with other granulocytes, are produced in the bone marrow after receiving appropriate proliferative and differentiation signals from the periphery. Their trafficking from the place of birth to the site of inflammation is a multi step process that involves egress from the bone marrow into the circulation, adhesion and transmigration through blood vessel walls into the tissue and recruitment to the site of inflammation in the presence of molecular gradients formed by chemokines. Selective differentiation of eosinophils and their release from the bone marrow into the peripheral circulation is governed by IL-5 [42]. This cytokine is also strongly implicated in eosinophil migration to the site of inflammation in which process it cooperates with a chemokine, eotaxin [43]. Many publications to date have shown that the number of

eosinophils found in the lungs of mice with experimentally-induced asthma could be significantly down-regulated by active worm infection, injection with worm-derived excretory-secretory products, and injection with purified or recombinant single worm molecules. Among helminths that upon infection of the host can attenuate the number of eosinophils are *Trichinella spiralis* [44], *Nippostrongylus brasiliensis* [45], *Schistosoma mansoni* [46, 47], *Litomosoides sigmodontis* [48], *Strongyloides stercoralis* [49] and *Heligmosomoides polygyrus* [50-53]. In most of the cases down-regulation of eosinophil number correlated with diminished levels of IL-5 and eotaxin in the lung or impaired production of IL-5 from T lymphocytes in the draining lymph nodes upon OVA recall. This confirms that helminths can reduce the number of eosinophils by diminishing levels of immunological mediators directly involved in proliferation and migration of eosinophils towards the lungs under inflammatory conditions. Interestingly, a down-regulated level of IL-5 was, in some cases, accompanied by diminished production of additional Th2 cytokines e.g. IL-4 and/or IL-13 whereas in other reports no modulation of IL-4 and/or IL-13 concomitant to decreased IL-5 production was noted. It is therefore uncertain at the moment whether worms attenuate eosinophil responses in asthma by specifically blocking IL-5 production by T lymphocytes or suppress the Th2 cell-mediated allergic responses in a more general fashion.

Involvement of IL-10 and TGF- β Derived from T and B Regulatory Cells in the Helminth-Induced Down-Regulation of Eosinophilia

Several mechanisms, revolving mostly around activation by helminths of T and B regulatory cells producing IL-10 and/or TGF- β , have been proposed to

Table 2. Role of Eosinophils and Neutrophils in Health and Disease

Characteristic	Eosinophils	Neutrophils
Basic biology	- originate from bone marrow - released into blood stream as mature cells - mature cells recruited into tissue - proliferative ability reported - very short lifespan	- originate from bone marrow - released into blood stream as mature cells - mature cells recruited into tissue - proliferative ability not reported - very short lifespan
Effector functions	- cytotoxic granule proteins release upon activation: major basic protein, eosinophil cationic protein, eosinophil peroxidase, eosinophil-derived neurotoxin, and b-glucuronidase - release of lipid mediators – leukotriene C4 and platelet activating factor	- release of lytic enzymes, and production of reactive oxygen species with anti-microbial potential - release of lipid mediator – leukotriene B4
Immunoregulatory role	- ability to internalize, process, and present antigenic peptides to CD4⁺ T cells - production of various cytokines, chemokines and growth factors: IL-3, GM-CSF, IL-4, TNF-α, TGF-α, TGF-β1, IL-6, IL-10, APRIL, RANTES, MIP-1α	- neutrophils isolated from patients with chronic inflammatory conditions acquire the ability to act as antigen presenting cells and induce T cell activation - production of various cytokines, chemokines and growth factors: IL-8, MIP-1α, CXCL2, VEGF, IL-1β
Role in disease	- beneficial: mediate protective immunity against helminths, particularly their tissue-migrating stages - detrimental: pathogenesis of asthma, numerous gastrointestinal disorders, hypereosinophilic syndrome	- beneficial: combating bacterial and fungal infections - detrimental: pathogenesis of asthma, cystic fibrosis, chronic obstructive pulmonary disease, and rheumatoid arthritis

be responsible for the above-described effect of the active worm infections on IL-5/eotaxin production and eosinophil numbers. It is well established that the lymphoid regulatory cell subsets, especially T regulatory cells, are crucial in maintaining immunological tolerance to allergens in healthy individuals. In agreement with this is the observation that in asthma patients, a reduction and/or defect in regulatory T cells has been noted and that the adoptive transfer of T regulatory cells effectively attenuated allergic airway inflammation in mice [54, 55]. It is also known that promotion of regulatory T cells and IL-10/TGF- β production are key mechanisms in the allergen-specific immunotherapy that induces peripheral T cell tolerance to allergens and potentially cures allergic diseases [56].

All *T. spiralis*, *H. polygyrus* and *L. sigmodontis* infections in allergen-sensitized mice coincided with up-regulated levels of IL-10 and TGF- β as well as increased recruitment of T regulatory cells into the draining lymph nodes [44, 48, 50]. However, transfer of local lymph node cells from IL-10-deficient animals infected chronically with *H. polygyrus* into Der p 1-sensitized hosts resulted in the same degree of protection against asthma as when wild type cells from C57BL/6 mice were used indicating that IL-10 is not the primary modulator of the allergic response in this model [50]. In the same paper, the authors confirmed the lack of role for IL-10 in BALB/c mice by blocking IL-10R signaling with the use of specific antibodies administered to the mice one day before airway challenge. Contrary to these data, were findings reported by Kitagaki *et al.* [51], which showed a protective role of IL-10 in *H. polygyrus*-mediated effects on asthma in BALB/c mice lacking the IL-10 gene. Similarly, *S. mansoni*- and *N. brasiliensis*-mediated protection against allergic airway inflammation was shown to be dependent on IL-10 [45, 46]. The lack of conclusive data defining the role of IL-10 in the helminth-induced asthma suppression might reflect differential involvement of this cytokine depending on the model used.

Fewer publications have addressed the functional role of TGF- β even though this cytokine represents a very strong candidate for being responsible for asthma modulation by helminths. Indeed, depletion experiments showed that induction of TGF- β by *L. sigmodontis* was implicated in the reduction of airway hyperreactivity, however airway inflammation seemed not to depend on this cytokine [48]. IL-10 and TGF- β have been associated with activation and function of regulatory T and B lymphocytes. Both subpopulations of lymphocyte have been shown to contribute to the suppression of allergic asthma by helminths [46, 50].

Even though it is relatively well established that regulatory T and B cells producing IL-10 and/or TGF- β may play an important role in the helminth-induced suppression of allergic airway inflammation little is known about their mechanism of action with regard to this particular model. It could only be hypothesized, as it has been shown in other models, that they act by impairing the inflammatory properties of dendritic cells

while promoting development of tolerogenic DC phenotypes, inhibiting the activation of allergen-specific Th2 cells, and directly suppressing effector cells such as mast cells, basophils and eosinophils [57].

Characterization of Defined Worm Products with Anti-Allergic Properties

From the perspective of creating new drugs to combat asthma, it seems vital to define single active molecules that can bypass the need for employing living worms as this might be linked to some adverse health effects and cause ethical and logistic problems. A few research teams have undertaken an intermediate step on this path and used excretory-secretory products, a subset albeit uncharacterized, of worm products to block allergic reaction in mice.

Infections with *N. brasiliensis* and *H. polygyrus* had previously been shown to have therapeutic effects in experimental asthma, therefore their products NES and HES respectively were tested next and, as expected, showed strong inhibition of eosinophil recruitment into the airways when co-administered together with the OVA allergen at the sensitization stage [58, 59]. The suppression of pulmonary eosinophilia in both models correlated with diminished IL-5 production in the worm products-injected mice. In addition, airway eosinophilia could be suppressed to a significant extent even when HES was administered at airway challenge to mice previously sensitised to allergen in the absence of the helminth products [59].

In terms of the mechanism proposed to explain the action of NES it was shown that even though active infection with the helminth from which NES was derived suppressed asthma *via* IL-10, NES-mediated inhibition of OVA-specific Th2 responses was IL-10-independent [58]. In addition, the same authors have shown that the NES protective effects were independent from toll like receptor 2 and 4 innate immunity signaling and IFN- γ presence. Similar to NES, excretory-secretory products of *H. polygyrus* suppressed asthma *via* a mechanism not relying on IL-10 as the frequency of IL-10-producing CD4⁺ T cells was diminished in a similar way to the frequencies of allergic IL-4 and IL-13 CD4⁺ T cells [59]. HES was shown to contain a TGF- β mimic which was capable of inducing regulatory T cells *in vitro*, therefore the authors hypothesized that HES could suppress allergic immune responses through this pathway. To test their hypothesis, they treated asthmatic mice with an equivalent amount of recombinant mammalian TGF- β but found that such a treatment could not suppress allergies at sensitization or at challenge [59].

So far only a few single defined nematode-derived molecules have been described and tested for their potential therapeutic effect on eosinophils in allergic airway inflammation. Amongst them, PAS-1 a 200 kDa protein isolated from *A. suum* extract, was shown to dampen down eosinophilic lung inflammation induced either by APAS-3, an allergic component of the same worm extract or following immunization with OVA [60-63]. Elevated levels of IL-10 and TGF- β , activation of

regulatory T cells, and increased IFN- γ production were amongst the proposed mechanisms considered responsible for the PAS-1-mediated effects in asthma.

ES-62 is another protein employed in native form, which was purified from excretory-secretory products of a filarial nematode *Acanthocheilonema viteae*. This tetrameric protein with a unique helminth post-translational modification where phosphorylcholine (PC) is attached to an N-type glycan was shown to suppress eosinophil recruitment in mice with experimentally induced asthma [64]. Work is currently underway to mechanistically explain how ES-62 exerts its beneficial effects on eosinophil counts during allergic lung inflammation.

A few total proteins with immunomodulatory functions were characterized and their recombinant versions were tested for inhibition of eosinophil recruitment into the lungs of mice with experimentally induced asthma. Amongst them, there were proteins from *S. mansoni* Sm22-6 and Sm29 [65], a cystatin from *A. viteae* [66] and macrophage migration inhibitory factor (MIF)-like protein from third-stage larvae of the nematode parasite of marine mammals, *Anisakis simplex* [67]. There were some variations in terms of the mechanisms of action among the molecules. *S. mansoni* antigens and *A. simplex* MIF-like protein, for example, were able to down-modulate allergic inflammatory mediators through expansion of CD4⁺Foxp3⁺ T cells [65, 67]. *A. viteae* cystatin, on the other hand, did not act *via* induction of regulatory T lymphocytes, rather IL-10-producing macrophages were implicated [66].

Other Ways Developed by Helminths to Suppress Eosinophil Responses in Asthma

As mentioned earlier, eotaxin, together with IL-5, plays a crucial role in navigating migration of eosinophils towards inflamed tissue. It is not surprising therefore that in mice with experimentally-induced asthma that were treated with helminths or their products the reduction in the number of lung eosinophils correlates with diminished levels of eotaxin. It is uncertain however at the moment how helminths regulate production of this chemokine. In the lung, eotaxin is produced by resident cells (epithelial, endothelial, and smooth-muscle cells) as well as by inflammatory cells (macrophages, lymphocytes, and eosinophils themselves) [68]. As the non-immune cells and innate immunity cells seem to be the major source of eotaxin it is plausible to think that helminths and their products might directly target those cell populations to inhibit eotaxin release and, as a consequence, eosinophil flux to the lung. Indeed, McSorley *et al.* [59] reported that administration of HES at the OVA challenge abrogated eosinophil infiltration (along with eotaxin and IL-5) but the adaptive Th2 responses were unaffected.

Eotaxin specifically binds to the CCR3 receptor [69] that is found on the cell surface of both human and murine eosinophils. Ligation of CCR3 by eotaxin results in chemotaxis and adhesion of eosinophils to

endothelial cells *in vitro* and *in vivo*. It was shown that infection with *H. polygyrus* suppressed not only the concentration of local eotaxin in the OVA/Alum treated mice but also significantly down-regulated expression of CCR3 on bronchoalveolar eosinophils [52]. Such down-regulation of CCR3 decreased the migratory potential of eosinophils toward eotaxin in an *in vitro* assay.

Interestingly, certain component(s) with metalloprotease activity contained within excretory-secretory products of *N. americanus* was shown to specifically cleave eotaxin which resulted in inhibition of eosinophil recruitment *in vivo* [70]. A few publications have also reported that helminths and their products may directly act on eosinophil populations and induce eosinophil cell death by caspase-dependent apoptosis [71, 72] or attenuate degranulation of eosinophils stimulated with IgG antibody [73] and their antibody-mediated attachment to larvae [70]. None of these processes, however, have been investigated during allergic airway inflammation.

REGULATION OF NEUTROPHIL FUNCTION BY HELMINTHS

Neutrophils are recruited early in helminth infections, usually at the stage when infective larvae penetrate the tissue forcing their way to the anatomical locations where adult stages may develop and reside. This early influx of neutrophils can facilitate parasite establishment by increasing tissue permeability that results in faster migration of larvae through the tissue. Not surprisingly therefore helminths have been shown to directly attract neutrophils *via* numerous soluble molecules that facilitate migration and establishment of the parasite within its host [74, 75]. Many larval stages of different helminth species can cause significant damage to host tissue during their migration. Larvae of *Schistosoma* and *Strongyloides* species, as well as hookworms penetrate the skin, and migrate into the lungs where they cause inflammation. An elegant study using *N. brasiliensis* as a model system, investigated the origin of such early inflammatory responses induced by migrating stages of helminths [76]. The authors found that the early lung damage was due to IL-17 dependent recruitment of neutrophils. Indeed, IL-17 has previously been shown to induce the recruitment of neutrophils *via* lung expression of CXC chemokines [77]. Neutrophil-rich inflammation has to be subject to tight control mechanisms as neutrophils can be toxic to host tissue (Table 2). Chen *et al.* [76] therefore also studied if and how the inflammation was controlled by helminths. Indeed, they assigned an essential role for the Th2-type response triggered by the worm in controlling this acute tissue damage in lungs. The study found that IL-4 receptor signaling reduced elevations in IL-17 mRNA levels, enhanced the expression of insulin-like growth factor 1 and IL-10 and stimulated the development of wound healing macrophages, all of which contributed to the rapid resolution of tissue damage [76].

The standard immunization protocol used to study the effect of helminths in experimental asthma relies on sensitization of mice with OVA followed by multiple intranasal challenges with the same antigen. This method of immunization leads to predominant infiltration of the lungs with eosinophils. In some cases however, the increased numbers of eosinophils are accompanied by significantly elevated numbers of neutrophils. The majority of the publications studying effects of helminths in asthma concentrated primarily on the effect of the worms on eosinophil numbers. Only a few publications so far have reported the effect of worms on neutrophils numbers in the lungs of asthmatic mice. It was shown in these studies that infection with *H. polygyrus*, and immunization with *H. polygyrus* and *N. brasiliensis* excretory-secretory products at the sensitisation stage all significantly down-regulated the number of neutrophils in the lungs [50, 58, 59]. In the case where HES was used, diminished number of neutrophils correlated with decreased frequencies of CD4+ T cells expressing IL-17A.

In contrast to the above-described results, Park *et al.* [44] found that the level of neutrophils in mice with experimentally-induced asthma was elevated by *T. spiralis* infection however no IL-17 data were available to corroborate this finding.

In another model, neutrophil infiltration of the lung tissue was induced by intranasal treatment with IL-

8/CXCL8. Intravenous injection of recombinant smCKBP—a protein secreted by *S. mansoni* eggs, completely ablated the CXCL8-induced pulmonary inflammation and changes in lung function [78].

CONCLUDING REMARKS

Modern asthma management involves application of inhaled corticosteroids (anti-inflammatory drugs) and β_2 -adrenoreceptor agonists (bronchodilators), which provide a majority of patients with effective control of the disease symptoms. In many countries however the number of asthma sufferers continues to increase and, in addition to this, disease cases tend to be more severe [79]. Thus, there is a strong argument for developing novel therapies that not only allow patients to better manage their symptoms but also can ideally cure and prevent the disease. Unfortunately, many promising targets identified through *in vitro* and *in vivo* studies have not translated into clinical practice [80]. This might be due to the poorly, so far recognized problem of different phenotypes of asthma characterized by different causal pathways. Successful treatment of patients with asthma would firstly require correct assignment of individual patients to their respective phenotype using appropriate biomarkers and then their treatment with specifically designed biologics. This complex diagnostic/therapeutic process

Table 3. Modulation of Different Types of Lung Inflammation by Active Helminth Infections

Granulocytes in BAL	Helminth	Effect	Mechanism	Reference
Exclusively eosinophilic; OVA/Alum model, LPS - free	<i>Schistosoma mansoni</i> infection	Eosinophils ↓ IL-5 ↓ Eotaxin ↓	B cell and IL-10-dependent suppression	Mangan <i>et al.</i> , 2006
Predominantly eosinophilic (or neutrophil number not specified); OVA/Alum model, LPS - not specified	<i>Nippostrongylus brasiliensis</i> infection	Eosinophils ↓ Eotaxin ↓	IL-10-dependent	Wohleben <i>et al.</i> , 2004
	<i>Litosomoides sigmodontis</i> infection	Eosinophils ↓ IL-5 ↓	Treg and TGF- β -dependent	Dittrich <i>et al.</i> , 2005
	<i>Heligmosomoides polygyrus</i> infection	Eosinophils ↓ IL-5 ↔	Elevated Tregs in lung lymph nodes; IL-10-dependent	Hartmann <i>et al.</i> , 2009
	<i>Heligmosomoides polygyrus</i> infection	Eosinophils ↓ IL-5 ↓ Neutrophils ↓	Increased Tregs in lung lymph nodes	Kitagaki <i>et al.</i> , 2005
	<i>Heligmosomoides polygyrus</i> infection	Eosinophils ↓ IL-5 ↓ Eotaxin ↓ CCR3 ↓	Decrease in chemotactic activity of eosinophils	Rzepecka <i>et al.</i> , 2007
	<i>Strongyloides stercoralis</i> infection	Eosinophils ↔ IL-5 ↑ Eotaxin ↓	No mechanism proposed	Wang <i>et al.</i> , 2000
	<i>Trichinella spiralis</i> infection	Eosinophils ↓ IL-5 ↓ Neutrophils ↑	Up-regulated IL-10 and TGF- β , recruitment of Tregs into lung lymph nodes	Park <i>et al.</i> , 2011
Mixed granulocytic; OVA/Alum model, LPS - not specified	<i>Heligmosomoides polygyrus</i> infection	Eosinophils ↓ IL-5 ↓ Neutrophils ↓	Tregs-dependent	Wilson <i>et al.</i> , 2005

Table 4. Modulation of Lung Inflammation by Helminth Derived Molecules

Granulocytes in BAL	Helminth Product(s)	Effect	Mechanism	Reference
Predominantly eosinophilic (or neutrophil number not specified); OVA/Alum model, LPS - not specified	Excretory-secretory products from <i>Nippostrongylus brasiliensis</i>	Eosinophils ↓ IL-5 ↓ Neutrophils ↓	Independent from the presence of TLR2, TLR4, IFN- γ and IL-10	Trujillo-Vargas <i>et al.</i> , 2006
	<i>Schistosoma mansoni</i> antigens Sm22-6, PIII and Sm29	Eosinophils ↓ IL-5 ↓ (only for PIII and Sm22-6)	CD4+Foxp3+ T cells, even in the absence of IL-10 expression, might play an important role	Cardoso <i>et al.</i> , 2010
	<i>Schistosoma mansoni</i> eggs	Eosinophils ↓ IL-5 ↓	Role for CD4+CD25+ Foxp3+ T cells independent of IL-10	Pacifico <i>et al.</i> , 2008
	<i>Schistosoma japonicum</i> egg antigens	Eosinophils ↓ IL-5 ↓ Neutrophils ↔	Stimulation of CD4+CD25+ T cells	Yang <i>et al.</i> , 2006
	ES-62, a molecule secreted by <i>Acanthocheilonema viteae</i>	Eosinophils ↓	Not specified	Melendez <i>et al.</i> , 2007
	Recombinant <i>Acanthocheilonema viteae</i> cystatin	Eosinophils ↓ IL-5 ↔	IL-10-producing macrophages	Schnoeller <i>et al.</i> , 2008
Predominantly eosinophilic (neutrophil number not specified); heat-coagulated egg white implanted subcutaneously, OVA challenge, LPS - not specified	<i>Ascaris suum</i> extract	Eosinophils ↓ IL-5 ↓ Eotaxin ↓	Not specified	Lima <i>et al.</i> , 2002
Predominantly eosinophilic (neutrophil number not specified); OVA/Alum model, LPS - free	PAS-1, a protein from <i>Ascaris suum</i>	Eosinophils ↓ IL-5 ↓ Eotaxin ↓	IFN- γ and IL-10, but not IL-12, play an important role in the PAS-1 modulatory effect	Araujo <i>et al.</i> , 2008
Mixed granulocytic; OVA model, LPS - not specified	Excretory-secretory products from <i>Heligmosomoides polygyrus</i> at sensitisation	Eosinophils ↓ IL-5 and eotaxin ↓ Neutrophils ↓ IL-17A ↓	Not dependent on TGF- β	McSorley <i>et al.</i> , 2012
	Excretory-secretory products from <i>Heligmosomoides polygyrus</i> at challenge	Eosinophils ↓ IL-5 and eotaxin ↓ Neutrophils ↔	No mechanism proposed	McSorley <i>et al.</i> , 2012
	Macrophage migration inhibitory factor homologs of <i>Anisakis simplex</i>	Eosinophils ↓ IL-5 ↓ Neutrophils ↓	CD4+CD25+Foxp3+ T cell recruitment	Park <i>et al.</i> , 2009
Exclusively neutrophilic; CXCL8 administered intranasally	<i>Schistosoma mansoni</i> recombinant protein smCKBP	Neutrophils ↓	Chemokine blocking activity	Smith <i>et al.</i> , 2005

in asthma treatment might prove to be extremely expensive.

As it has been shown in this review, helminths are evolutionary equipped to effectively suppress both eosinophilic and neutrophilic types of lung inflammation (summarized in Tables 3 and 4). Thus, their products seem to offer a broad spectrum of anti-inflammatory activity that can potentially target different asthma phenotypes in humans. It is therefore compelling to continue work on developing novel drugs to treat

allergic asthma using well-characterized single helminth molecules as templates.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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