Immune modulation by schistosomes: mechanisms of regulatory B cell induction and inhibition of allergic asthma

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Chapter 6

MICROORGANISM-INDUCED SUPPRESSION OF ALLERGIC AIRWAY DISEASE: NOVEL THERAPIES ON THE HORIZON?

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Expert Rev Respir Med. 2014 Dec;8(6):717-30
DOI: 10.1586/17476348.2014.949244

ABSTRACT

Allergic airway diseases are a major global health burden, and novel treatment options are urgently needed. Numerous epidemiological and experimental studies suggest that certain helminths and bacteria protect against respiratory allergies. These microorganisms are strong regulators of the immune system, and a variety of potential regulatory mechanisms by which they protect against allergic airway inflammation have been proposed. Whereas early studies addressed the beneficial effect of natural infections, the focus now shifts toward identifying the dominant protective molecules and exploring their efficacy in models of allergic airway diseases. In this article we will review the evidence for microbe-mediated protection of allergic airway diseases, the potential modes of action involved, and discuss advances as well as limitations in the translation of this knowledge into novel treatment strategies against allergic airway disease.

INTRODUCTION

Asthma is an atopic, chronic inflammatory disorder that is estimated to affect 315 million people worldwide [1]. First episodes of atopic disease usually occur during childhood following sensitization to inhaled allergens. Infants that suffer from atopic dermatitis (AD) and/or allergic rhinitis (AR) often develop allergic asthma (AA) later in life. Different risk factors for the development of asthma have been identified in children including infection with respiratory viruses (respiratory-syncytial virus, rhinovirus) and impaired lung development in children born prematurely [2].

AA, the most common form of asthma, is characterized by bronchial hyperresponsiveness and airway obstruction, resulting in episodes of wheezing and breathlessness. These symptoms are fueled by chronic inflammatory responses against allergens, in which T helper (Th) 2 cells, eosinophils and elevated immunoglobulin (Ig) E levels play a central role [3]. Dendritic cells (DCs) are important in the initiation phase when they take up allergens, migrate to the draining lymph nodes and induce the differentiation of allergen-specific Th2 cells (**Figure 1**). Renewed exposure to the allergens induces a cascade of Th2-cytokine production (interleukin (IL)-4, IL-5, IL-13), which promotes class-switching of B cells to IgE production, the development of eosinophilic airway inflammation, goblet cell hyperplasia and airway hyperresponsiveness. Notably, the recently discovered innate lymphoid cells type 2 (ILC2s), activated by innate cytokines from damaged epithelial cells, have been described as a significant early source of Th2-cytokines after local allergen exposure [4].

The prevalence of childhood asthma has increased dramatically in westernized countries in the second half of the 20th century. Recent studies suggest that the incidence of asthma has now reached a plateau in high prevalence countries [5], the global burden of asthma however continues to rise as incidence rates in Africa, South America, and parts of Asia still rapidly increase [6]. Asthma is a multivariable disease in which genetic predispositions are certainly important risk factors. However, the rapid increase in incidence rates over the last few decades points toward a strong contribution of environmental factors to disease development. One explanation is provided by the 'hygiene hypothesis' suggesting that a decrease in childhood exposure to infectious agents as a result of increasing sanitation standards, improved health care, and life style changes attributes to the increase in incidence of allergies and asthma [7].

Microbial organisms such as commensal bacteria and helminths are part of human evolutionary history. These microorganisms have developed various strategies to modulate the host immune system, often by amplifying the natural immune regulatory network of the host and simultaneously reducing immunopathology. Many of these organisms establish chronic infections, which may also reflect the assumption that the host immune system tolerates these non-lethal organisms rather than inducing potent inflammatory responses at the cost of severe tissue damage and immunopathology [8]. As such, the immune system categorizes the degree of danger associated with invading pathogens and only reacts strongly to the most threatening organisms such as certain viruses, bacteria and protozoan parasites. In contrast, mainly repair and tissue integrity responses are activated following exposure to less dangerous microorganisms. More recently, the concept has been advocated that certain helminths and bacteria, which are tolerated by the host, may actually imply benefits. Bacteria of the intestinal microbiome for example ferment food, produce essential vitamins and prevent colonization by other, more harmful bacteria [9]. Furthermore, these helminths and microbiota prevent exaggerated immune responses to ubiquitous antigens, as they are strong inducers of the regulatory arm of the immune system. In line with that, the 'hygiene hypothesis' implies that a reduced infectious pressure leads to an immune imbalance, which promotes allergic disorders in genetically susceptible people [10]. In addition, potential benefits of certain microorganisms for the human body are being lost as the diversity of the microbiome decreases [9].

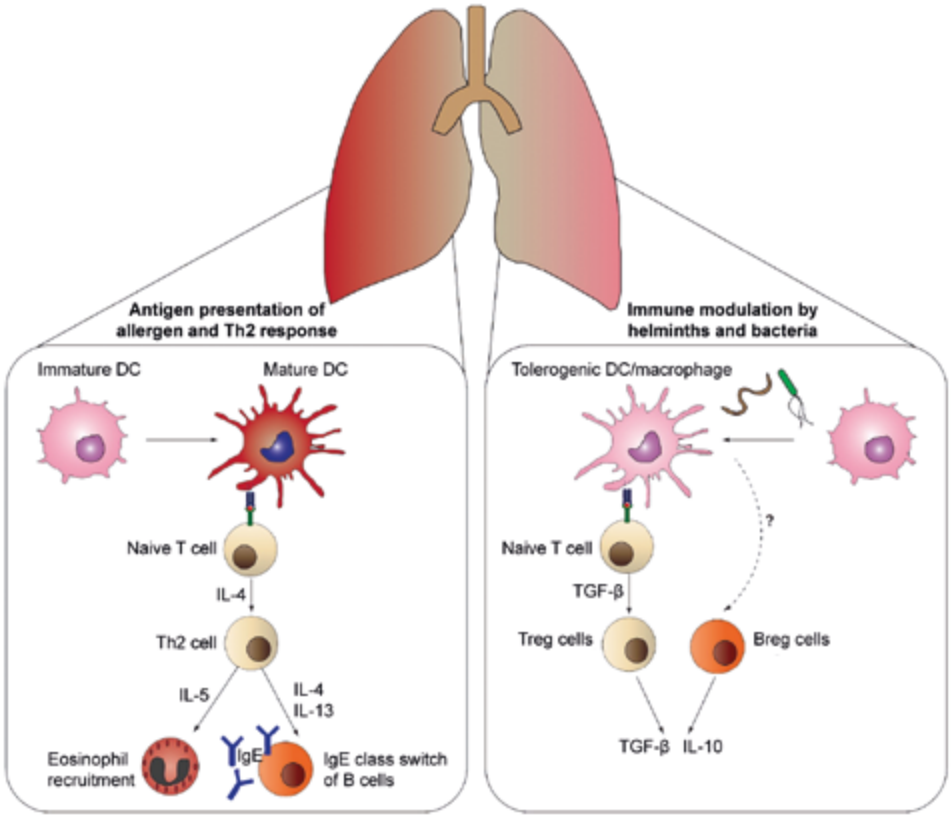

Figure 1. Bacteria and helminths induce T regulatory and B regulatory cell responses that dampen allergic airway inflammation. Left panel: Allergic airway inflammation is most often characterized by a predominant Th2 response. This is initiated by mature dendritic cells that after allergen recognition and presentation, skew T cells towards a Th2 phenotype. Th2 cells initiate effector responses by in particular recruitment and activation of eosinophils and by class switching B-cells to produce IgE antibodies.
Right panel: Bacteria and helminths can intervene in this response at various levels. An example is given in which they induce T regulatory and B regulatory cells. These cells can dampen ongoing inflammation or prevent induction of Th2 mediated inflammation.

Despite the increase in asthma prevalence worldwide in the last decades not many novel therapeutic options have been developed. Current therapies for asthma patients still consist of inhaled steroids and bronchodilators. These medications only alleviate symptoms, which will reoccur after medication has stopped. The treatment of severe asthma proves to be difficult and available therapeutic options are limited to systemic steroids and anti-IgE treatment. Thus, new therapeutic approaches which alter the immune response and promote long-lasting tolerance against allergens are needed. Exploiting the modulatory capacities of evolutionarily-conserved 'old friends' [11], like bacteria and helminths, is a promising strategy to design novel treatments for airway allergies such as asthma.

In this review, we will highlight the current evidence for the protective effect of helminths and certain bacteria in allergic disorders from both epidemiological studies and animal models. We will address both life infections and antigens derived from these organisms, and summarize the proposed mechanisms of protection. Finally, we will discuss the recent advances in the field and give recommendations for future microbial-based treatments against allergic diseases.

Evidence for inhibition of allergic airway inflammation (AAI) by microorganisms

The association between microbial infections and the reduced incidence of allergic disorders has been addressed in numerous epidemiological studies. Meta-analyses consistently show that several helminth species, e.g., *Acaris, Trichuris*, hookworm and Schistsoma can protect against allergic sensitization [12-14]. Hookworm infection is most clearly associated with a reduced risk of developing asthma [14], while other species give either no significant protective effect or have not been extensively studied. Nevertheless, individual studies have suggested that infection with *Schistosoma mansoni* reduces the severity of asthma [15] and allergic skin reactions [16;17]. In addition, a range of other factors have been suggested to determine the protective effect of helminth infection on atopic disease, such as time, intensity and chronicity of infection and host genetics [18]. Of note, worm infections have also been linked to protection against other inflammatory non-communicable diseases, such as inflammatory bowel disease (IBD), multiple sclerosis (MS) and type-1 diabetes [19].

Infection with or exposure to certain bacteria can also interfere with the development of allergies and airway disease. For example, there is mounting evidence that environmental factors such as living on a farm protect from developing asthma [20-22] and this protective effect is attributed to the environmental exposure to different bacterial and fungal microorganisms [23]. In contrast to previous belief, the lung is not sterile but colonized by certain bacterial species, and it was suggested that the composition of the lung microbiome was different in patients with asthma compared to healthy individuals [24;25]. Changes in the bacterial composition may have consequences for disease severity as patients with steroid resistance displayed a different composition of the lung microbiome compared to patients responsive to steroids [26]. Whether these changes are an effect of pathophysiological processes in the airways of patients with asthma or a predisposing factor for the development of asthma is not clear. Nevertheless, it is clear that colonization of the airways with certain bacteria increased the risk of developing allergic airway disease in children [27;28]. In contrast, exposure and infection with a range of other microorganisms may protect from the development of asthma. In epidemiological studies the prevalence of asthma was negatively associated with Herpes simplex virus I, Hepatitis A, and *Toxoplasma gondii* infection [29]. Not only exposure to external bacteria but also the composition of the microbiome can affect the development of asthma. The total diversity of the gut microbiota during the first month of life has been associated with the development of AA in infancy and childhood [30] and a higher prevalence of asthma has been found in children treated with antibiotics [31;32]. These associations must however be reviewed carefully as the causal relationship can be overestimated as reverse causation and confounding factors may have impacted the analysis [33]. Further examples are provided by infections with the gut bacterium *Helicobacter pylori* (*H. pylori*), which is reversely linked with asthma prevalence, particularly in children [34-37]. Interestingly, infection with *H. pylori* is also negatively associated with the development of other non-communicable disease such as IBD and MS [38]. The association between the decreasing prevalence of infection with *H. pylori* [39] and the increase in non-communicable diseases such as asthma has been linked to the so called `disappearing microbiota hypothesis` [9]. Experimental studies in models of allergic airway disease have confirmed this relation and furthermore unraveled the underlying mechanisms of this protective effect [40-42].

Protective, microbial-induced mechanisms against respiratory allergies

A number of different mechanisms by which helminths, certain bacteria and their molecules protect against AAI have been described. The variety of proposed mechanisms most likely reflects the variety of different molecules and models studied. Although part of these mechanisms may have overlapping features, it is plausible that helminths and bacteria have evolved distinct ways of altering the immune responses of the host to promote their survival and use different molecules to do so.

In general, helminths are recognized as potent regulators of the host immune system. They strongly induce Th2 cells, IgE class-switching and eosinophil-rich tissue infiltrations, but at the same

time promote regulatory cells, like regulatory T (Treg) and B (Breg) cells, tolerogenic/immature DCs and alternatively activated macrophages (AAMs). The regulatory cytokines IL-10 and transforming growth factor (TGF)-β play a key role in their capacity to regulate the host immune system. Bacteria of the microbiome are associated with various types of pro-inflammatory immune responses. However, as certain bacteria try to maintain their niche, they produce factors which are able to inhibit inflammation. *H. pylori* infection in the stomach for example can induce a strong Th1 response, but also secretes molecules that induce tolerogenic DCs and inhibit T cell proliferation as reviewed by Müller [43]. These factors may simultaneously affect immune responses to bystander antigens such as allergens. Collectively, both helminths and bacteria as well as their products have various regulatory effects on the host immune system. A large number of protective mechanisms have been described by which microbes and/or their products can inhibit experimental airway allergies (see **Table 1** and **Figure 2** for an overview of the discussed literature) The following main mechanisms appear to be important in microbial-induced protection against allergies and airway disease:

1) T cell polarization

AA is a predominantly Th2-mediated disease. Initial observations of protective effects from microbial compounds were thought to be mediated by influencing the Th1/Th2 balance. Indeed, many microorganisms and some microbial molecules that protect against AA induce Th1 cells and/or the Th1 cytokine IFN-γ, and/or reduce Th2 cytokines [44-48]. Interestingly, a similar shift from allergen-specific Th2 to Th1 cytokines was demonstrated *in vitro* in peripheral blood mononuclear cells from patients allergic to timothy grass pollen in response to cystatin from the filarial helminth *Acanthocheilonema viteae* [49]. Other helminth-derived material dampens allergen-specific Th2 immunity while simultaneously inducing a strong helminth antigen-specific Th2 response [50]. CpG oligodeoxynucleotides (CpG ODNs) [51] and *H. pylori* neutrophil-activating protein (HP-NAP) [52] were shown to inhibit AAI and to skewe human allergen specific Th2 cells to Th1 cells *in vitro* [53;54].

2) Induction of Treg/Breg cells

Anti-microbial pro-inflammatory responses are often accompanied by compensatory regulatory responses to prevent excessive damage as a result of inflammatory responses. Different regulatory cells are involved in protection against AAI induced by helminth parasites and bacteria. Treg cells are currently the most well-studied regulatory cell type and include the thymus derived, peripheral derived, and *in vitro* induced regulatory T cells (tTreg, pTreg, and iTreg respectively) [55;56]. They suppress other effector T cells via the regulatory cytokines IL-10 and TGF-β, as well as through cytokine-independent or cell-cell contact-mediated mechanisms. A role for Treg cells induced by natural helminth [57-64] or microbial infection [40;41;46;65;66] in the suppression of AAI has been demonstrated by different groups (**Figure 1**). In addition to live infection, a number of helminth- [67-69] and bacteria-derived molecules also mediate protection in association with the induction of Treg cells [42;70-74]. Infection with many helminths and certain microbiota is associated with the induction of IL-10 and TGF-β [66;75], and the protective effects of these microorganisms or their molecules on AA are often, but not always, dependent on these cytokines. One study with schistosome eggs for example described a Treg cell-mediated protection against AAI which appeared to be independent of these cytokines [76].

Another suppressive cell type, which has been increasingly studied is the Breg cell. Various model systems suggest that Breg cells can mediate protection against AA. Both IL-10-dependent and -independent effects have been described depending on the tissue source of these Breg cells: e.g., spleen IL-10-dependent; [77] versus gut/lungs IL-10-independent; [78;79]. Moreover, Breg cells enforce their protective effects not only by inhibiting effector T cell responses, but also by inducing Treg cells and inhibiting the effector function of antigen-presenting cells (APCs) [80]. Importantly, both helminths and

a number of bacteria have shown to be strong inducers of Breg cells [78;79;81-84]. For some of these species, it has been demonstrated in experimental models that their protective effect against allergic airway disease critically depends on Breg cells (**Figure 1**) [78;79;81]

Parasites or bacteria and their products may not only work via the direct induction of regulatory cells but may also influence the availability of other molecules that exert this effect. For example, certain strains of the human gut bacterium *Clostridia* can protect mice from developing colitis and allergic diarrhoea by the production of short chain fatty acids (SCFAs) [85]. These SCFAs stimulate epithelial cells to produce TGF-β, which is likely to contribute to the induction of IL-10-producing Treg cells. Especially the SCFA butyrate was able to induce Treg cells [86]. Interestingly, the gut microbiome and the production of SCFAs is dependent on diet. A high-fiber diet induces more SCFAs compared to one low-in-fiber via alterations of the microbiota with a direct inhibitory effect on the development of AAI [87].

In summary, many studies report a microbial-mediated induction of Treg/Breg cells, and show that either blocking their effector functions inhibits the protective effect or that the adoptive transfer of

Table 1. An overview of mechanisms by which bacteria and helminths and their products may modulate allergic inflammatory responses. Various cell types seem to be modulated by microorganisms or their compounds during their inhibition of an allergic response. Both results obtained in animal models and from *in vitro* experiments on human cells are summarized in this table.

Affected cell	Mechanism	Microorganism	Composition or molecule	Reference
T cell	Skewing to Th1 rather than Th2 cells	*Ascaris suum*	PAS-1	[44]
		Acanthocheilonema viteae	Cystatin	[49]
			ES-62	[47]
		Caenorhabditis elegans	Crude extract	[45]
		Nippostrongylus brasiliensis	ES	[50]
		Helicobacter pylori	HP-NAP	[52;53]
		Lactobacillus paracasei with *Lactobacillus rhamnosus*	Live infection	[46]
		Salmonella typhimurium SL7207	Attenuated	[48]
		N/A	CpG ODNs	[51;54]
	Induction Treg cells	*Anisakis simplex*	MIF-like protein	[69]
		Ascaris suum	PAS-1	[68]
		Heligmosomoides polygyrus	Live infection	[59;60;63]
		Litomosoides sigmodontis	Live infection	[58]
		Nippostrongylus brasiliensis	Live infection	[64]
		Schistosoma mansoni	Live infection	[61]
			Sm22.6/Sm29/PIII	[67]
			eggs	[76]
		Trichinella spiralis	Live infection	[57;62]
		Alcaligenes faecalis	β-glucan curdlan	[70]
		Clostridium leptum	Live infection	[66]
		Helicobacter pylori	Live infection	[40;41]
			VacA and GGT	[42]
		Lactobacillus rhamnosus GG or *Bifidobacterium lactis*	Live infection	[65]
		Lactobacillus paracasei with *Lactobacillus rhamnosus*	Live infection	[46]

Table 1. (continued)

Affected cell	Mechanism	Microorganism	Composition or molecule	Reference
		Mycobacterium bovis BCG	Freeze-dried preparation	[71;72]
		Multiple bacteria	BV, a lysate	[73]
		Streptococcus pneumonia	Type-3 polysacharide and pneumolysoid	[74]
	Induction γδTCR^{+} T cells	*Ascaris suum*	PAS-1	[68]
		Escherichia coli	Live infection	[106]
B cell	Induction Breg cells	*Heligmosomoides polygyrus*	Live infection	[79]
		Schistosoma mansoni	Live infection	[78;81]
	Induction IgE cross-reactivity	*Schistosoma haematobium*	Live infection	[105]
DC or macrophage	Alteration activation status	*Acanthocheilonema viteae*	Cystatin	[95]
		Clonorchis sinensis	Total protein	[90]
		commensals in general	Live infection	[100]
		Bacillus cereus	Synthetic lipopeptide	[96]
	Induction DC IL-18 secretion	*Helicobacter pylori*	Live infection	[41]
Mast cell	Blockage mast cell degranulation	*Acanthocheilonema viteae*	ES-62	[104]
Epithelial cell	Activation epithelial cells	*Pseudomonas aeruginosa* transfected with type 1 fimbiae	Inactivated	[107]
Various	Blockage IL-33 release and reduction ILC2 cells	*Heligmosomoides polygyrus*	ES	[103]
	Induction regulatory cytokines (IL-10, TGF-β)	*Clostridium leptum*	Live infection	[66]
		Lactobacillus paracasei NCC	Live infection	[75]
	Reduction chemotaxis eosinophils	*Heligmosomoides polygyrus*	Live infection	[63]
		Nippostrongylus brasiliensis	Live infection	[64]
		Strongyloides stercoralis,	Live infection	[97]
		Escherichia coli	LPS	[99]
	Reduction chemotaxis iNKT	commensals in general	Live infection	[101]
	Reduction development basophil precursors	commensals in general	Live infection	[102]
	Alteration microbiota by diet induced SFAs	Effect on multiple bacteria	Live infection	[87]

these regulatory cells is sufficient to exert protection. Despite the clear notion that Treg or Breg cells are important mediators of suppressive effects in AAI, the precise mechanisms by which these cells function is not yet well-described. Treg and Breg cells inhibit other effector T cells and APCs but may also have a range of other immunomodulatory functions in AAI [3;88].

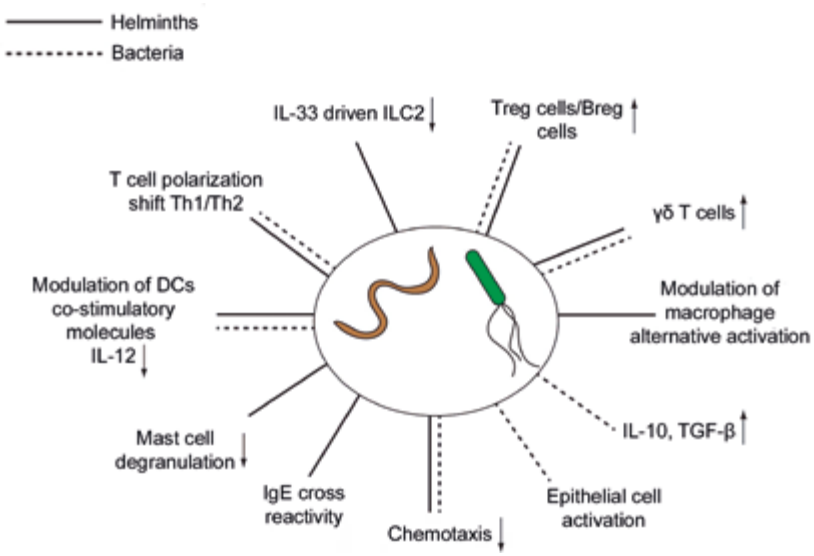

Figure 2. Mechanisms by which bacteria and helminths may modulate allergic inflammatory responses. The proposed mechanisms in which bacteria and helminths can reduce allergic airway inflammation are various in their origin and involve multiple immune cell types. In some studies that are discussed in this review, a combination of multiple mechanisms is proposed.

3) Modulation of DC/macrophage function

While both DCs and macrophages have been primarily implicated in the priming of pro-inflammatory Th1 responses, they are also important contributors to the initiation of regulatory (and Th2) immune responses. Antigen processing in the absence of danger signals retains DCs in an immature state and preferentially induces Treg cells. Several microorganisms have evolved this feature to their benefit. A molecule from Schistosoma directly acts on DCs via the toll-like receptor 2 to promote the polarization of Treg cells [89], while other helminth molecules downregulate co-stimulatory molecules thereby possibly influencing subsequent T cell polarization [90]. AAMs, induced by IL-4 and IL-13, are elicited during polarized type 2 immune responses. They are important innate effectors in anti-parasite immunity and wound healing, especially during helminth infections [91]. Interestingly, AAMs have also been described to possess immunosuppressive activities in models of schistosomiasis and filariasis [92;93]. Lung-stage helminth infections strongly induce alternative activation of alveolar macrophages [94]. A protein from filarial helminths has been reported to protect against experimental AAI in a macrophage- and IL-10-dependent manner [95], further pointing toward the importance of innate immune cells with regulatory properties in protection against allergic inflammation (**Figure 1**). *H. pylori* infection strongly reduces allergic airway disease in experimental models [40], and this protective effect was associated with the production of the cytokine IL-18 by tolerogenic DCs which skew the T cell phenotype toward Treg cells rather than Th17 cells [41]. This effect could be further pinned down to two specific virulence factors of *H. pylori*, the vacuolating cytotoxin (VacA) and the γ-glytamyl transpeptidase (GGT) [42]. A bacterial synthetic lipopeptide was also able to inhibit the disease by keeping the DCs in a tolerant state [96].

4) Other mechanisms

Inhibition of cell migration to the lungs is another immunosuppressive effect of microbial exposure. AAI is characterized by the influx of eosinophils, which is mediated by specific chemokines such as

eotaxin (**Figure 1**). Different studies have described that helminth infection reduces the levels of eotaxin in the bronchoalveolar lavage fluid [63;64;97] and the expression of its receptor CCR3 on eosinophils [98]. Prenatal microbial exposure can modulate AAI in the offspring by inhibition of eosinophil infiltration [99]. Interestingly, mice receiving antibiotics or totally lack gut commensal bacteria show a stronger AAI compared to mice with normal gut flora. This phenomenon has been associated with e.g. a dysregulation in DC recruitment and maturation [100] and an accumulation of invariant natural killer T (iNKT) cells in the lung [101]. Furthermore these mice have an increased frequency of circulating basophils, which was explained by the finding that the basophil precursors of these mice have a higher expression of the IL-3 receptor which regulates their development [102]. Interestingly, the blockage of IL-33 release and subsequent suppression of ILC2 induction by secreted helminth molecules [103] has recently been described in mediating protection against AAI, adding ILCs to the list of effector cells modulated by helminth molecules. Other mechanisms of protection include the blockage of FcεRI-induced degranulation of human mast cells by the helminth-derived glycoprotein ES-62 [104] as well as the induction of carbohydrate cross-reactive, allergen-specific IgE during helminth infections with a reduced capacity to induce basophil degranulation [105]. Furthermore, the induction of γδTCR+ T cells by both helminth and bacteria has been reported [68;106]. A bacterial compound able to inhibit AAI has also been shown to e.g. induce proliferation and wound repair of bronchial epithelial cells [107].

Clinical trials utilizing helminths and microbiota for treatment of respiratory allergies

Modulation of the host immune response against helminths or certain bacteria allows the establishment of chronic infection and a mutual beneficial co-existence in the host. As a bystander effect, immunoregulation often results in suppression of immunity to other antigens such as allergens. Although it is conceivable that part of the activity from life microbes on the immune system can be condensed to the activity of single or multiple molecules, it is elaborate to identify them and it will be a long process before they can be applied in the clinic. Therefore, several researchers have taken the approach of applying clinically controlled full infections in clinical trials, bypassing the process of identifying the responsible molecules.

These controlled infections have been applied now in a number of inflammatory diseases with mixed results. At present, the eggs of the pig whipworm *Trichuris suis* are approved for therapy of IBD, the first helminth-based therapy being used [108-110]. Randomized, controlled trials have shown promising results in terms of safety and efficacy in MS patients [111]. However, no beneficial effect of *T. suis* eggs was observed in patients with AR after 3 weekly doses of 2500 *T. suis* eggs over a 6 month period [112;113]. It remains to be established whether a longer treatment, more or higher dosages, different worms and/or treatment of a younger study population would be more effective. Probiotics and various inactivated bacterial strains have also been widely examined for their health-promoting activities and treatment of various inflammatory disorders. However, probiotics are also not routinely used as a treatment in allergic patients. A meta-analysis of application of probiotics to pregnant women or babies concluded that probiotics do not prevent the development of asthma [114], although it is suggested that probiotics might be effective in preventing AD [115]. For example, AD could be reduced by oral application of a lysate of heath-killed *Escherichia coli* and *Enterococcus faecalis* in a subgroup of children with at least one parent with AD [116]. Broncho-Vaxom (BV), an extract which contains a mixture of multiple bacteria is already used to treat patients from recurrent respiratory tract infections, but it is not yet applied against asthma [73]. Other bacterial preparations showed some effectiveness in clinical trials. For example, newly diagnosed adult patients with moderate persistent asthma experienced some improvement of lung function and asthma symptoms when treated with inactivated *Mycobacterium phlei* [117]. In conclusion, several trials have applied helminths, bacteria or complete extracts/lysates in clinical studies, of which some showed promising results for the treatment of certain inflammatory

diseases. There is little evidence so far for a therapeutic effect of controlled microbial infections on respiratory allergies. Although this is a relative young field and conceptually very exciting, it needs to be further explored and developed in the near future with help from small-medium enterprises (SMEs) and pharmaceutical companies.

Helminth- and microbial-derived molecules for the treatment of respiratory allergies

Although some of the clinical trials showed promising results, treatment options based on natural infections bear risks and the first trials for respiratory allergies showed that further testing and optimization is needed before implementation in the clinic is possible. Therefore several groups have instead focused their efforts on studying the effects of (single) helminth- or bacteria-derived molecules as opposed to natural infections. However, as this is a more elaborate path to follow, this field is still in its infancy and the current data available are mainly from animal models.

Molecules derived from helminths that show protective effects in models of AA include whole antigen generated from adult worms [45;90;118] or other live cycle stages such as eggs [119], excretory-secretory products (ES) [50;120;121] and isolated [44;47;68;104;122] as well as recombinantly expressed [49;67;69;95] single antigens. Different strategies are also examined with regard to the protective effects of bacterial derived products. Common components of bacteria such as the cell wall LPS [99;123;124], curdlan [70] and CpG sequences in the DNA [51] have been reported to be protective. Importantly, in many of the applied experimental models exposure to LPS actually induces rather than inhibits AAI. These findings demonstrate that exposure to bacteria is not universally protective but may rather depend on certain bacterial traits. Some groups use live-attenuated or inactivated bacteria [48;107;117;125], a lysate [73] or frieze-dried bacteria [71;72]. In some cases more specific single molecules were tested [42;52], however this field is not yet strongly evolved. The type of preparation of the compound may be of great importance, as it was shown that inhibition of AAI was only effective when using frieze-dried but not heat-killed or alive *Mycobacterium bovis* BCG [71].

From the bench to the clinic

Nearly all of the experimental studies have been conducted in models of AAI based on the model allergen ovalbumin that is isolated from chicken egg white. While this model is well established and allows the use of genetically modified mice to study antigen-specificity, there is a great need to apply models based on natural allergens (house dust mite, grass, tree pollen) as it allows to more easily relate findings from the model to the field and the clinic. Furthermore, these models use a natural route of sensitization. First studies show protective effects of both helminth- [49;126] and bacteria-derived products [73;75] in models based on natural allergens. It is of interest that some cases of asthma in humans are induced by fungal antigens, and some studies have also suggested a protective role of helminth and bacterial compounds in fungus-induced AAI in mice [103;127].

Another aspect of interest when studying the protective effect of microorganisms and their molecules is the timing and duration of administration. Allergen-sensitization often occurs early in life, followed by life-long suffering from asthmatic disease. Any preventative treatment approach would therefore be most beneficially applied during infancy. Of interest, it has been suggested in various studies that treatment of neonatal mice with bacteria or their compounds is more effective in inducing tolerance compared to adult mice [40;41;65]. The treatment of newborns might however be difficult to achieve in practice. Interestingly, a number of papers we have discussed have applied treatment to the pregnant and later lactating mothers, showing protection against features of AAI in the offspring [75;99;128]. The rationale for the success of these early treatment approaches might come from epigenetic changes in immune-associated genes. In regard to this, it was shown that epigenetic changes had occurred in mice following contact with environmental bacteria, leading to a reduced

susceptibility to allergies [128]. Altogether, it is clear that microbial molecules should be best applied as early as possible to prevent the development of allergic diseases, it nevertheless is of great importance to investigate microbial molecules for their potential to dampen already established disease.

Although there are some promising results that indicate treatment of ongoing asthmatic disease is possible [45;69], the majority of the data suggests that this is much more difficult to achieve than the prevention of disease onset. But as patients are repeatedly exposed to allergens, testing potential therapeutic molecules in a setting of repeated challenge is thus important. While some molecules fail to protect upon re-challenge [73] others maintain their protective effect [71;74], making them more suitable as a putative treatment for established disease. Working toward the implementation of a novel treatment for asthmatic patients, not only the timing but also the route of administration needs to be considered. In allergen immunotherapy, alternative routes of local (e.g. sublingual, intranasal) and systemic (e.g. oral) allergen administration to mucosal surfaces show promising results in terms of immunogenicity and safety in clinical trials [129] compared to the standard method of subcutaneous injections. In mouse models the same, but also different and less obvious routes of molecule application are applied (intraperitoneal, often in combination with an adjuvant; intravenous; intranasal/aerosol; intratracheal), when testing the therapeutic potential of microbial-derived molecules. In view of human application, we believe that administration routes that are most likely to induce tolerance should be favored also in the animal models to allow extrapolation of the results to humans.

From a clinical point of view, there are several issues that need to be addressed when working toward the implementation of helminth- and microbial-derived molecules in the clinics. As summarized elsewhere [130;131], potential side effects need to be considered. These include anaphylaxis and atopic reactions to helminth parasite-derived molecules, as well as the risk of general immune suppression in the patient. Trials of the recombinant Na-ASP-2 hookworm vaccine for example have shown important differences in the immune response between individuals not exposed to hookworm and individuals from endemic areas that have previously been infected [132;133]. In order to avoid general immune suppression, it is favorable to be able to induce antigen-specific tolerance, or local tolerance restricted to the site of allergen-induced inflammation (e.g. the lungs). Furthermore, it should be ensured that tolerance induced by microbial-derived molecules does not inhibit immune responses to natural infections with potential pathogenic microorganism. Interestingly, in mice the application of freeze-dried BCG to inhibit AAI did not interfere with the diagnosis of live infection with the bacteria [71]. Finally, the risk of cross-reactivity between antigens of microorganisms and allergens needs to be taken into account.

It is becoming clear that asthma is a term that describes a collection of symptoms. Personalized medicine tries to tailor treatment regimens specifically to a patient condition or characteristic by investigating individual-based treatments, as some patients might benefit from therapies that only show efficacy in a subgroup of the asthmatics. In greatest need for novel treatment options are probably patients that suffer from severe asthma, which are often resistant to treatment with steroids and cannot be subjected to specific immunotherapy. For these patients, it would be of special importance to develop a treatment that cures ongoing disease, which is harder to achieve than preventative approaches as discussed above. This may prove to be difficult as advanced local tissue degeneration has occurred which will probably not be restored even when inflammation has been dampened. However, it is important to realize that the larger group of asthma patients with milder forms of disease, which are now well controlled by steroids, form a realistic target group to apply a 'treatment to cure' therapy with microbial molecules. Altogether, we believe that, rather than applying natural infections, the isolation of modulatory, protective components from bacteria and helminths may enable the development of more sustained and controlled preventive or therapeutic strategies.

EXPERT COMMENTARY AND FIVE YEAR VIEW

Despite the increasing worldwide prevalence of asthma little has changed in the last years in the treatment or prevention of this disease. The main therapy is still based on inhaled steroids and bronchodilators. Despite the development of monoclonal antibodies targeting IgE or Th2 cytokines (IL-5, IL-13, IL-4 receptor alpha chain) no ground-breaking advancement has been made in prevention or treatment of asthma. Epidemiological and experimental research has increased our understanding of mechanisms how microorganisms can evade the immune system and suppress the development of allergy and airway disease. Several mechanistic pathways how these microorganisms actively suppress immune reactions have indeed been discovered. In our opinion, these novel insights are promising and open up a novel avenue for the development of innovative therapeutics. As live infection with bacteria and parasites is not always perceived as a desirable strategy, focus has been shifted to isolating molecules from microorganisms. Several molecules have been identified and are currently being evaluated in experimental models. We expect that in the following years the number of identified suppressive molecules will further increase and that these molecules will be evaluated in preclinical models. The predictive values of these models in regard to the effectiveness in human disease may be limited. Nevertheless, these model data will provide interesting and valuable insights about the underlying mechanisms involved in the suppressive capacity of these molecules, timing of treatment and route of administration. One major issue is if these molecules can be used solely for prevention or also for treatment of people with already developed allergic disease. This will certainly also critically determine the therapeutic windows in which treatment is feasible. Next, crucial steps need to be made toward the clinic in which active involvement of SMEs and pharmaceuticals will help to initiate clinical trials in patients with allergic disease such as AA. These trials should then reveal if a molecular approach can advance our therapeutic options in allergic disease and asthma and if we can effectively modulate immune responses to allergens long-term.

KEY ISSUES

- Atopic diseases (e.g. allergic rhinitis, allergic asthma) are a major global health burden today. Medication options are limited and only alleviate the symptoms, therefore novel treatment options need to be developed urgently.
- Helminths/microbiota modulate the host immune system, suppressing the immune response to themselves and bystander antigens such as allergens. The 'hygiene hypothesis' suggests that the drastic increase in incidence rates for atopic diseases can be ascribed to a reduction in infectious pressure and the resulting imbalance of the immune system.
- A body of epidemiological evidence underpins that helminths (e.g. hookworms, schistosomes) and certain bacteria (e.g. *H. pylori*) protect against allergic asthma in humans.
- *In vivo* studies suggested mechanisms by which microorganisms or their products protect against allergic airway disease, including shifting of the Th1/Th2 immune balance, induction of Treg/Breg cells, modulation of DC/macrophage function and impairment of chemotaxis and effector cell (e.g. mast cell, basophil) function.
- Helminth- and microbial-derived molecules have been identified which efficiently protect against experimental allergic airway inflammation, and which include whole antigen preparations, excretory-secretory products and single molecules (single/recombinant) of helminths as well as live-attenuated and freeze-dried bacteria, bacterial lysates and single bacterial molecules.
- At the moment microbial-derived treatments are not routinely applied in the clinics to treat allergic airway disease. Working toward a novel treatment option for atopic patients based on microbial-

derived molecules, a range of factors such as safety, mode of action (general vs. antigen-specific tolerance), timing and route of administration need to be taken into account.

- We expect that more immune suppressive molecules will be described and that these molecules will further progress into clinical trials.

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