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

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Chapter 1

GENERAL INTRODUCTION

The human immune system has evolved to fulfil a myriad of functions. It has to confer protection from potentially harmful infectious agents including bacteria, viruses and parasites and fight malignant cells, but tolerate self-tissue and foreign, but harmless substances introduced via e.g. the lungs or the digestive system. This is reflected in the complexity of the immune system, with various innate and adaptive, often highly specialized effector functions at interplay. Helminth (greek: 'intestinal worm') infections are likely as old as humanity itself. The co-evolution of helminths and humans has not only resulted in specified immune responses against helminths, but also in the development of immune evasion mechanisms by these parasites and even a selective pressure to alter the host's genetic composition and thereby shape the immune system [1-3].

1. AN INTRODUCTION TO *SCHISTOSOMA*

Schistosoma (S.) spp. are parasitic flatworms of the genus trematode. In humans, they cause an acute and chronic disease known as schistosomiasis/bilharzia. The species causing most human schistosomiasis cases are *S. mansoni*, *S. japonicum* and *S. haematobium* [3]. In 2016, an estimated 206.4 million people, mainly in poor and rural communities of tropical and subtropical areas, required preventative treatment [4]. *Schistosoma spp.* parasites can cause severe disease, but can also survive in the human host up to 20 years, meaning both mortality and morbidity are major concerns.

1.1. Immunity to *Schistosoma spp.*

Many features of schistosomiasis and the underlying immunological processes can be replicated well in animal models, most commonly infected with *S. mansoni* or *S. japonicum*. The immunological response to *Schistosoma* can be divided into an acute and a chronic phase. The acute phase, in response to premature life cycle stages that penetrate the host skin and subsequently migrate through several tissues including the lung, is initially characterized by a type 1 immune response. A much stronger type 2 immune response is however induced in response to eggs released by mating adult worm pairs starting 5-6 weeks post infection. *S. mansoni* and *S. japonicum* adult worm pairs reside in the mesenteric vein, and part of the released eggs penetrate through the tissue into the intestinal lumen where they are released into the environment with the faeces. Importantly, many eggs are transported with the blood flow into the liver and other organs. Tissue-entrapped eggs and the resulting inflammatory granulomatous response are the main drivers of pathology [5]. The egg-specific type 2 response predominates the earlier type 1 response [6]. In the chronic phase of infection, regulatory responses are induced which contribute to limiting immunopathology despite the continuous release of eggs, and allow long-term parasite survival in the host.

Type 2 immune responses have a major role in both the host defence against helminths or venoms and in allergic disorders. The central features of a type 2 response shared by immune responses to helminth and allergens include the induction of $CD4^+$ T helper (Th) 2 cells and their innate counterpart, type-2 innate lymphoid cells (ILC2s), which both produce the type 2 cytokines interleukin-4 (IL-4), IL-5, IL-9 and IL-13, the induction of B cell IgE production and the recruitment and activation of granulocytes and alternatively activated macrophages (AAMs) [7]. The type 2 immune response to helminth is diverse and adapted to the type of helminth, with the classical 'weep and sweep' response including increased smooth-muscle-cell contractility, intestinal permeability and goblet-cell mucus production that has developed to expel intestinal helminths [8]. The type 2 response to *Schistosoma spp.*, resident in the vasculature, is induced in response to antigens released by the eggs [6, 9]. Characteristic for this type 2 response is the formation of granulomas around tissue-entrapped eggs, which mainly consist of $CD4^+$ Th2 cells, ILC2s as well as eosinophils and macrophages. The central, and dichotomous, role of type 2 immunity in schistosomiasis becomes apparent in transgenic mouse models. Mice deficient in IL-4 display intestinal and hepatic pathology and increased mortality around the time of egg production

onset [10, 11], highlighting the modulatory and host-protective role of Th2 responses especially during the acute stage of disease. Mice deficient in IL-13 or IL4Rα, the shared receptor for both IL-4 and IL-13, do not develop severe hepatic fibrosis as wild-type (WT) animals do, resulting in prolonged survival [10, 12]. This in turn demonstrates that prolonged type 2 responses during chronic infection contribute to morbidity.

1.2. *Schistosoma spp.* eggs & egg antigens

In the context of experimental intraperitoneal (i.p.) administration, eggs can be considered as 'antigen reservoirs'. After intravenous (i.v.) injections, eggs transported with the blood stream get trapped in the lung [13], which serves as a model to study lung inflammation in response to *Schistosoma spp.* In contrast, eggs likely remain in the peritoneal cavity after i.p. injection. *S. mansoni* soluble egg antigens (SEA), which is the soluble fraction of homogenized eggs, is a complex mixture of hundreds of antigens, many of which are glycoproteins [14, 15]. The *S. mansoni* egg excretory-secretory product (ES), which represents the entirety of molecules released by cultured eggs, has been described to still contain 188 proteins [16]. Some of the most abundant proteins, including IPSE/alpha-1 [17-21] and omega-1 [22-27], have been well-characterized and described to fulfil distinct functions. Others, such as kappa-5 [28, 29], lyso-PS [30] or SmCKBP [31] are less well characterized, and for many the function is completely unknown. Some immunomodulatory molecules of *Schistosoma spp.* life cycle stages other than eggs have been described, including Smteg, Sm22.6, PIII and Sm29, all of which have been described to prevent allergic airway inflammation (AAI) [32, 33], and cyclophilin A [34]. The only description of a *Schistosoma spp.* egg-derived molecule inhibiting AAI is the *S. japonicum* molecule SjP40 [35]. *Schistosoma spp.* single molecules and their described functions are summarized in **Table 1.**

1.3. The regulatory network

The immune regulatory network, induced during the chronic phase of infection, suppresses the host immune system to allow parasite survival while preventing excessive tissue damage. Immunomodulation is thus advantageous for both the parasite and the host. The induction of regulatory T (Treg) cells, the most prominent component of the regulatory network, by *Schistosoma spp.* has been described in both human and murine studies [36-38]. Treg cells suppress effector T cell responses in murine schistosomiasis [38, 39], and depletion of Treg cells during the acute or chronic phase of infection results in increased granulomatous immunopathology [40, 41]. An adoptive transfer of *S. mansoni*-primed Treg cells or a retroviral transfer of the *Foxp3* gene suppresses granuloma formation and ameliorates immunopathology [40-42]. More recently, additional members of the regulatory network, such as regulatory B (Breg) cells (see section 2) and AAMs [43], have been described. Importantly, cells of the regulatory network are interrelated and synergize, e.g. Breg cells induce Treg cell generation. The aforementioned regulatory immune cells possess various effector functions. One of the most prominent ones is immune modulation through the regulatory cytokine IL-10 [44, 45]. As a result of the induced regulatory network, both antigen-specific and non-specific, more generalized immune modulation occurs. Importantly, the regulatory network also results in 'spill-over suppression' and diminished responses to allergens (see section 3), vaccinations and co-infections [46]. Another member of the regulatory network are AAMs, which play a central role in tissue remodelling [47]. This rapid wound healing response is a central component of type 2 immunity, as it prevents excessive host tissue damage and pathology caused by large, multicellular parasites such as helminths [48].

2. REGULATORY B CELLS

The first description of B cells with suppressive capacities stems from 1974, when it was observed that B cells suppress delayed-type hypersensitivity (DTH; [49, 50]). More than 20 years later, the first descriptions

TABLE 1. Literature on *Schistosoma spp.* single molecules and described functions

Helminth species	Name	Molecule	Source	Suggested immunological function	Reference
S. mansoni	Cyclophilin A	Peptidyl-prolyl cis-trans isomerase	adult worms, secretory	immunomodulatory; regulation of APC activity	(34)
	IPSE/alpha-1	glycoprotein	eggs (sub-shell area), secretory	binding of IgE and DNA; induction of basophil IL-4 production; alters transcription profile of DCs	(17-21)
	Kappa-5	glycoprotein	eggs, secretory	interaction with CLRs	(28, 29)
	Lyso-PS	lyso-phosphatidylserine	tegument	induction of Treg cells via TLR2; Th2 polarization	(30, 125)
	Omega-1	T2 RNase, glycoprotein	eggs, secretory	Th2 polarization of DCs; induction of Treg cells; inflammasome activation and IL-1β induction in MFs; IL-33/ILC2-mediated improvement in metabolic homeostasis	(22-27)
	PIII	fraction of soluble adult worm antigen	adult worm antigen	immunomodulatory; inhibition of AAI	(32)
	Sm22.6 (=SmTAL-1)	tegument-allergen-like (TAL)	tegument of all life cycle stages except eggs, soluble	immunomodulatory; inhibition of AAI	(32)
	Sm29	glycoprotein	tegument of adult worms during lung stage, membrane-bound	immunomodulatory; inhibition of AAI	(32)
	SmCKBP	chemokine-binding protein	eggs, secretory	inhibition of CXCL8 and suppression of neutrophil recruitment	(31)
	Smteg	soluble fraction of schistosomula tegument	tegument	induction of IL-10 in lung monocytes & inhibition of AAI	(33)
S. japonicum	SjP40		eggs	induction of Th1 cytokines & inhibition of AAI	(35)

of suppressive B cells in murine models of autoimmune diseases were published [51-53] and the term 'regulatory B cell' introduced.

2.1. Phenotype & origin

Breg cells is an umbrella term for a heterogeneous group of B cells that comprise immunomodulatory capacity. Various Breg cell subsets have been described both in humans and mice, depending on their origin, the tissue they reside in, their phenotype and mode of action. To date, it is unknown whether Breg cells develop from a committed precursor or whether any B cell can acquire suppressive capacity in response to certain environmental stimuli, and different models are discussed in the literature [54, 55]. Extensive overviews over different Breg cell subsets and the range of described suppressor mechanisms has also been provided in the literature [44, 55, 56]. In brief, both murine B cell lineages, innate-like B1 B cells abundant in peritoneal and pleural cavity [57] and B2 B cells that populate secondary lymphoid organs, can give rise to Breg cells. Of B2 B cells, especially marginal zone (MZ) B cells [58, 59] and their precursors (transitional type MZ precursors, T2-MZP) [60, 61] have been described to acquire regulatory functions. More recently, plasma blasts and plasma cells have also been acknowledged to produce regulatory cytokines and act suppressive [62, 63]. In humans, Breg cells are mainly characterized within the $CD24^{hi}CD38^{hi}$ immature B cell and the $CD24^{hi}CD27^{+}$ B cell compartment [64, 65]. Suppressive functions of Breg cells other than IL-10 production, include e.g. the release of other regulatory cytokines including IL-35 and transforming growth factor beta (TGF-β), the induction of Treg cells and suppression of effector T cells through expression of ligands such as FasL, ICAM-1/LFA-1, GITRL or PD-L1 [66].

2.2. Signals for the induction and expansion of Breg cells

Breg cells are present in naïve individuals and mice, but often expand and display increased suppressive activity in the context of autoimmunity and infection [44, 67, 68]. Various signals have been described to play a role in Breg cell development and activation, of which stimulation through the B cell receptor (BCR) [53, 69, 70], CD40 [53, 60, 71, 72], and Toll-like receptors (TLRs; especially TLR4 [73-75], TLR7 [76] and TLR9 [58, 73]) are considered most central. Moreover, different cytokines including IL-15 [77], IL-21 [78], IL-35 [62, 79], BAFF [80], APRIL [81] and type I interferons [63] have been described to support Breg cell development and activation.

Recently, it has also been acknowledged that microbiota-derived signals contribute to Breg cell activation. The microbiome induces Breg cells in the spleen and the mesenteric lymph node (LN) in an IL-1β- and IL-6-dependent manner. Mice with a disrupted microbiome have an impaired Breg cell compartment and develop exacerbated autoimmunity [82]. Other studies confirmed that alterations of the gut microbiome, either by increased estrogen levels or housing animals under specific-pathogen-fee conditions, induce IL-10-producing Breg cells in the spleen and the mesenteric LN [83, 84].

It is likely that more than one stimuli and converging signalling pathways are needed to achieve maximal Breg cell development and activation. A two-step model has been proposed for the acquisition of regulatory properties by B cells, with the exposure to innate stimuli (e.g. TLR ligands) as first step initiating IL-10 production, and BCR or CD40 ligation as a second step promoting B cell survival and activation and thus amplifying suppression [61, 73]. Other studies suggest that TLR and CD40 ligation may enable B cell IL-10 expression by promoting differentiation into IL-10-competent plasmablasts or plasma cells [62, 63].

It is worth noting that the signals required for the development and activation of Breg cells likely differ *in vivo* and *in vitro*. During an inflammatory response *in vivo*, multiple processes occur in parallel at any given time, while *in vitro* systems allow to dissect the role of individual signals and pathways, but often fail to mimic the complexity *in vivo*.

2.3. Breg cells in helminth infection

The first indication that B cells with regulatory properties are induced during helminth infection has been made 20 years ago in μMT mice lacking mature B cells, which develop augmented pathology following *S. mansoni* infection [85]. Subsequently, different helminth species have been shown to induce Breg cells [86-89]. These helminth-elicited Breg cells have immunomodulatory capacities in a variety of autoimmune and inflammatory conditions, including anaphylaxis [90], experimental autoimmune encephalomyelitis (EAE) [87, 91] and AAI [86, 87, 92]. In humans, Breg cells have also been identified in helminth-infected individuals [88, 93].

Options of how helminth or their products induce Breg cells are a) the direct ligation of above mentioned receptors, e.g. the BCR or TLRs on B cells by helminth molecules, and b) signals derived from accessory cells (e.g. via the ligation of CD40) or the general inflammatory environment (e.g. cytokines) induced by helminth infection. That Breg cells can be directly induced by *Schistosoma spp.* products in both mice and humans has been shown by *in vitro* co-culture of murine, purified splenic B cells with living *S. mansoni* worms [86], and by *in vitro* stimulation of PBMC-derived B cells with SEA [94], respectively. *S. mansoni* infection has been described to induce the expression of *Tlr7* in Breg cells. TLR7 ligation was moreover found to facilitate IL-10 production, it remains unclear however whether this is the consequence of direct TLR7 ligation by a *S. mansoni* molecule [76]. Despite these studies suggesting a direct Breg cell induction by helminths and their products is possible, it is plausible that *in vivo*, Breg cells also receive signals from accessory cells and their environment.

The description of helminth-specific signals that induce Breg cells is limited. In the context of *Schistosoma spp.*, *in vitro* exposure of murine splenocytes to live worms [86, 90] and of human peripheral blood B cells from helminth-infected individuals to SEA [94] has been reported to induce IL-10-producing Breg cells. In all these cases the molecular identity of the stimulus has however not been identified. The milk oligosaccharide lacto-*N*-fucopentaose III (LNFP-III), which carries a glycosylation pattern that also occurs on *Schistosoma spp.* products and is therefore cross-reactive, has been described to induce B cell IL-10 production [95]. Except for the stimulation of isolated human B cells from helminth-infected individuals with SEA [94], which may be a recall reaction of memory B cells, it remains unclear whether the observed Breg cell induction is the result of a direct interaction of with receptors on B cells. For helminth other than *Schistosoma spp.*, the glycoprotein ES-62 from *Acanthocheilonema viteae* and an extract from *Brugia malayi*, both filarial nematodes, also induce IL-10 production by B cells [96, 97].

2.4. Breg cell transcriptomics

Transcriptomics allows to study the complete set of genes transcribed at a certain time point and potentially allows insight into the whole breadth of cellular processes at interplay. To date, only few studies have utilized transcriptomics approaches to gain insight into the signals required for the development and activation of Breg cells. Khan et al. reported that *Tlr7* is significantly upregulated on *S. mansoni*-induced $CD19^{+}CD1d^{hi}$ B cells compared to naïve control cells and $CD19^{+}CD1d^{lo}$ B cells from infected animals, and that TLR7 ligation increases their IL-10 production [76]. Another study by Sun et al. identifies CD9 as a marker of murine IL-10-competent, $CD19^{+}CD1d^{hi}CD5^{+}$ Breg cells induced by *in vitro* polyclonal stimulation. Although the exact role of CD9 remains unclear, the study also suggests that CD9 might play a role in the immunosuppressive function of Breg cells, as blocking CD9 *in vitro* impaired their ability to suppress T cell proliferation [98]. $CD9^{+}$ B cells have subsequently been described to suppress house dust mite-induced AAI [99].

3. ALLERGY AND ASTHMA

Asthma is an atopic, chronic inflammatory disorder that is estimated to affect 315 million people worldwide [100]. Asthma is characterized by chronic lung inflammation, bronchial hyper-responsiveness

and airway obstruction, resulting in episodes of wheezing and breathlessness. First episodes of atopic disease often occur during childhood following sensitization to inhaled allergen, and infants who suffer from atopic disease often develop allergic asthma later in life. Recent studies suggest that the incidence of asthma has now reached a plateau in high prevalence countries [101]. The global burden of asthma however continues to rise as incidence rates in Africa, South America, and parts of Asia still rapidly increase [102]. Asthma is a multivariable disease in which genetic predispositions are certainly important risk factors. Genome-wide association studies (GWAS) have identified different susceptibility loci, most prominently the chromosome 17q21 region and various genes of the type 2/IgE cluster, including e.g. *Il33* and *Tslp* (thymic stromal lymphopoeitin), which are genes encoding type 2 innate cytokines produced by bronchial epithelial cells [103, 104]. However, the rapid increase in incidence rates over the last few decades points toward a strong contribution of environmental factors to disease development.

3.1. Hygiene & Old Friends hypothesis, epidemiological evidence

The 'hygiene hypothesis' already postulated in 1989 [105] suggests that a decrease in childhood exposure to infectious agents as a result of increasing sanitation standards, improved health care, and life style changes contributes to the increase in incidence of allergies and asthma. This conceptual framework has been developed further into the 'old friends hypothesis', suggesting an evolutionary adaptation of our immune system to the continuous encounter with microbes and infectious agents, and a tendency towards an over-reactive immune system in their absence. Both concepts suggest that a reduction in infectious pressure over time leads to an immune system imbalance promoting allergic and other immune-mediated disorders such as inflammatory bowel disease (IBD) or multiple sclerosis (MS) [106].

The increase in asthma and other immune-mediated diseases correlates with urbanization and economic development, but a causal link has not been demonstrated so far. As one possible factor, changes in the exposure to pathogenic microbes, including helminths, have been suggested. The incidence of helminth infections is inversely correlated with increasing asthma rates in westernized countries. The ability of helminths to establish chronic infections and induce regulatory immunity has generated strong interest in the possibility that helminths or their products suppress hyper-inflammation and could be used as new anti-inflammatory treatment strategy [107].

The association between helminth infections and the reduced incidence of allergic disorders has been addressed in numerous epidemiological studies, yielding heterogeneous results. Meta-analyses show that there is no overall effect of helminth infections on asthma, but that hookworm infections do protect against allergic sensitization [108-110]. Individual studies have also suggested that infections with *S. mansoni* reduce the severity of asthma [111] and allergic skin reactions [112, 113]. While early clinical trials using eggs of the pig whipworm *Trichuris suis* (TSO) or experimental hookworm infections, both of which are intestinal helminths, resulted in promising safety and efficacy data in patients with intestinal inflammatory diseases, efficacy could not be demonstrated in larger studies or studies with allergic or asthmatic subjects [107]. Potential reasons for the heterogeneity in results from epidemiological studies and clinical trials, apart from the helminth species, include a range of other factors such as time, location, intensity and chronicity of infection and host genetics [114].

3.2. Immunity in asthma

The asthmatic immune response is complex, with a multitude of innate and adaptive, cellular and humoral processes at interplay. During allergic sensitization, epithelial cells produce cytokines including TSLP, IL-25 and IL-33, which activate DCs and ILC2s to induce Th2 cell polarization. Upon renewed allergen exposure, Th2 cells and ILC2s quickly produce large amounts of type 2 cytokines including IL-4, IL-5 and IL-13, which in turn cause class-switching of B cells to IgE and the recruitment and activation of eosinophils, mast cells and basophils [115, 116]. Mast cells and basophils release inflammatory mediators

such as histamines following cross-linking of allergen-specific IgE bound to surface IgE Fc-receptors, amplifying the allergic response and resulting in an acute reaction including mucus production and bronchoconstriction [115, 117].

3.3. Helminth-mediated protection against AAI

Helminths have developed various strategies to modulate the host immune system. They amplify the natural immune regulatory network of the host and modify pro-inflammatory immunity, thereby preventing exaggerated immune responses to ubiquitous antigens such as allergens.

Early insight into the protective effects of helminth infections stems from research using a variety rodent and human nematodes and *Schistosoma spp*. The latter provides a unique model of helminth infection as it, in contrast to the other models of mainly gastrointestinal nematodes used, allows to study both the role of egg deposition and chronicity of infection on protection against AAI. The literature published thus far on the protective effect of *Schistosoma spp*. infections is summarized in **Table 2**. Building up on this work, several groups have successfully employed excretory-secretory products (ES) or, in the case of *Schistosoma spp*., SEA and eggs instead of natural infections. Ultimately, a range of single molecules that harbour protective effects against AAI have already been identified and their mode of action characterized, including *Ancylostoma caninum* AIP-2, *Anisakis simplex* MIF-like protein, *Ascaris suum* PAS-1, *Acanthocheilonema vitae* cystatin and ES-62 as well as *Heligmosomoides polygyrus* HpARI. For schistosomes, *S. mansoni* Smteg, Sm22.6, Sm29 and PIII as well as *S. japonicum* SjP40 have been described in the literature as single molecules with protective effects against AAI. The literature published thus far on the protective effect of helminth-derived molecules is summarized in **Table 3**.

3.4. Mechanisms of protection

A range of different mechanisms by which helminths and their molecules protect against AAI have been described, including the induction of Treg cells, regulatory macrophages and regulatory cytokines (IL-10, TGF-β), the induction of Th1 responses and IFN-γ which result in a shift in the Th1/ Th2 balance, as well as the inhibition of pro-inflammatory processes such as IL-33 release and mast cell degranulation (also see **Table 2** and **Table 3**).

Different mechanisms of protection have been suggested to be involved in conferring *Schistosoma spp*.-mediated protection from AAI. *Schistosoma spp*. provides an especially interesting model for experimental studies as its life cycle in the host includes the formation of adult worm pairs and continuous deposition of eggs. Infections with either mixed sex worms or only male worms allows to dissect the contribution of eggs to protection. Various studies identified Treg cells and Breg cells, respectively, as mediators of protection [32, 86, 88, 92, 118-120]. Only some of these studies however provide functional experiments to formally prove their role in protection rather than solely showing an association between reduced AAI and elevated numbers or activity of these cells. One recent study links protective effects against AAI to an increased IFN-γ production [35].

The variety of proposed mechanisms most likely reflects the variety of different species, molecules and models studied. It is plausible that helminths have evolved distinct ways of altering the host immune response and promote their own survival.

3.5. Development of novel therapies & outlook

Despite the drastic worldwide increase in asthma prevalence, not many novel therapies have been developed. The current treatment of asthmatic patients still consists of inhaled steroids and bronchodilators, which only alleviates symptoms and is impeded by the development of steroid resistance. New treatment approaches that alter the immune response and promote long-lasting

tolerance are needed. Exploiting the modulatory capacities of evolutionary conserved 'old friends' like helminths might serve as a promising strategy towards the development of novel treatment options. Although early clinical trials investigating the use of natural helminth infections initially showed promising results in intestinal inflammatory diseases [121, 122], follow-up studies have shown little effect in allergy [123, 124] and efforts to use live infections as treatment strategies have since diminished. Treatment with live helminth infections also bears risks, and efforts have therefore intensified to identify single, helminth-derived molecules that mediate protection.

4. SCOPE OF THIS THESIS

The induction of Breg cells by helminth parasites and the implication of helminth-induced immune modulation for providing protection from hyper-inflammatory disorders such as allergies has been extensively studied over the last decades. However, due to the complex immunity to helminth infections, it proved difficult to a) dissect the molecular signals for induction of Breg cells, and b) fully characterize cellular mechanisms of protection in allergic airway inflammation. Experimental *S. mansoni* infection and isolated *S. mansoni* eggs have provided us with tools to further address these research areas.

The first part of this thesis focusses on the role of Breg cells in protection from AAI by chronic *S. mansoni* infections and aims at identifying molecular signals required for schistosome-induced Breg cell development.

In **chapter 2** we build up on previous work and show that not only splenic B cells, but also pulmonary B cells induced during chronic *S. mansoni* infection can provide protection against airway inflammation. In **chapter 3**, we sought to identify *S. mansoni*-derived antigens that induce Breg cells and describe that the egg glycoprotein IPSE/alpha-1 directly interacts with splenic MZ B cells, induces IL-10 production and promotes Treg cell expansion. In **chapter 4**, we aimed to identify molecular signals contributing to Breg cell induction *in vivo* by performing transcriptomics on splenic B cell subsets from chronically infected mice. In **chapter 5**, we describe that type I interferons enhance Breg cell IL-10 production in response to *S. mansoni* antigens *in vitro*, but are dispensable *in vivo*.

In the second part of this thesis, we build up on earlier work by us and others showing a protective effect of *S. mansoni* on experimental AAI, and aimed to identify protective, single *S. mansoni*-derived molecules.

Chapter 6 summarizes and discusses the evidence for helminth-induced protection from AAI, mechanisms of protection, efforts towards the identification of protective, single helminth-derived molecules and the implications for the development of novel treatment strategies (as of 2014). In **chapter 7**, we describe that isolated *S. mansoni* eggs and the single egg-derived glycoprotein omega-1, in the absence of adult worms, also protect from AAI.

The main findings of this thesis are summarized and discussed in **chapter 8,** including directions for future research towards understanding of the link between *Schistosoma*, Breg cells and allergic asthma.

TABLE 2. Literature on the protection from AAI by *Schistosoma spp.* infection

Helminth species	Sex of cercariae	Time of AAI induction	Mouse strain	Cerc. strain	No. of cerc./ mouse	Suggested mechanism	Reference
S. mansoni	mixed	wk 1 (pre-patent); wk 5 (patent)	Balb/c	Brazilian	90	dependent on egg deposition; Treg cells	(118)
	mixed	wk 14 (chronic)	C57BL/6	Puerto Rican	40	Breg cells	(88)
	male only	wk 8 (acute)	Balb/c	Puerto Rican	?	Breg cells	(86)
	mixed	wk 8 (acute)	Balb/c	LE	30		(119)
	mixed	wk 8 (acute); wk 12 (intermed.); wk 16 (chronic)	C57BL/6	Puerto Rican	15/30/45	dependent on infection intensity and chronicity	(126)
	mixed & male only	wk 7-11 (acute); wk12-16 (chronic)	Balb/c	Puerto Rican	30	protection only in 'male only' group; B cell-/IL-10 dependent	(92)
S. japonicum	mixed & male only	wk 4	Balb/c	?	25	protection both in mixed and 'male only' group	(127)

TABLE 3. Literature on the protection from AAI by helminths-derived molecules

Helminth species	Molecule	Time of immunization	Suggested mechanism	Reference
***Schistosoma spp.* products**				
S. mansoni	Smteg	during sensitization	IL-10	(33)
	Sm22.6,Sm29, PIII	during sensitization + pre-challenge	Treg cells	(32)
	eggs	during sensitization	Treg cells; IL-10 independent	(119)
S. japonicum	SjP40	during sensitization	induction of IFN-γ	(35)
	SEA/eggs		Treg cells	(120)
Products from other helminths				
A. caninum	AIP-2	during challenge	Treg cells	(128)
A. simplex	MIF-like protein	during challenge	Treg cell-/IL-10-/TGF-β-dependent	(129)
A. suum	PAS-1	during sensitization and challenge	IFN- γ- and IL-10-dependent	(130)
		during sensitization and challenge	Treg cells, CD8 γδ T cells	(131)
A. vitae	Cystatin	during sensitization	Shift to IFN-γ/Th1 response	(132)
		during sensitization or pre-challenge	IL-10-producing macrophages	(133)
	ES-62	during sensitization and challenge	Shift to IFN-γ/Th1 response	(134)
		during sensitization and challenge	Inhibition of mast cell degranulation	(135)
H. polygyrus	ES	during sensitization	Inhibition of IL-33 release and ILC2s	(136)
	HpARI	during sensitization	Inhibition of IL-33 release	(137)
N. brasiliensis	ES	during sensitization and challenge	TLR2-, TLR4- and IL-10-independent	(138)

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