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

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Chapter 8

SUMMARIZING DISCUSSION

WHAT WAS ALREADY KNOWN

Helminths, being well-known inducers of type 2 immune responses, also promote immune regulatory networks. The resulting immune modulation conveys 'spill-over' suppression to hyper-inflammatory conditions such as allergy [1].

Schistosomes, which establish chronic infection in both humans and mice, have been widely used to dissect the mechanisms underlying the development and activation of regulatory B (Breg) cells, a prominent member of the immune regulatory network. It is widely recognized that Breg cells are induced by schistosomes in both humans and mice [2, 3]. Breg cells comprise a heterogeneous group of cells of different cellular origin as well as phenotypical and functional characteristics. Multiple stimuli are known to contribute to Breg cell activation, of which most-well described are signalling via the B cell receptor (BCR), CD40 and Toll-like receptors (TLRs). Schistosome antigens have been described to induce Breg cells [4-6], but the molecular identity of the stimulus and the signalling pathways engaged remain to be characterized.

Hyper-inflammatory conditions such as allergy are less common in helminth-endemic areas. This relationship has both been studied in humans and in animal models. Human studies have yielded heterogeneous results over the years [7], suggesting that many factors including helminth species, time, location, intensity and chronicity of infection as well as host genetics influence helminth-mediated immunomodulation. Advances in understanding helminth-mediated protection from allergy have been made in animal models, where antigen mixtures and single molecules have been shown to protect from experimental allergic airway inflammation (AAI) in the absence of infection. A range of mechanisms mediating protection have been described, including the induction of various components of the immune regulatory network and suppression of pro-inflammatory responses [8]. The description of single, schistosome-derived molecules that mediate protection against AAI are however limited.

HOW DID OUR STUDIES ADVANCE THE FIELD

Molecular signals for schistosome-induced Breg cell development

Although Breg cells have been most well-characterized in the murine spleen, B cells with regulatory properties can also be found at other sites. We show that B cells with regulatory properties, protecting mice from AAI, can be found in the lung of chronically *S. mansoni*-infected mice (**chapter 2**). While we and others have previously shown that *S. mansoni*-induced splenic Breg cells exert their suppressive function through interleukin (IL)-10 production and induction of regulatory T (Treg) cells [2, 4], we found these schistosome-induced pulmonary B cells to be phenotypically different from splenic Breg cells and to suppress in an IL-10- and Treg cell-independent manner, rather displaying a reduced T helper 2 (Th2)-driving function. This study suggests that B cells with inhibitory properties exist at the site of inflammation, which are phenotypically and functionally different from 'classical' Breg cell subsets described in the spleen. Our study contributes to the body of evidence that Breg cells reside not only in lymphoid organs, but can also be found at other sites including human adipose tissue [9] and nasal polyps [10].

While it is well-established that helminths induce Breg cells, no single helminth-derived molecule had been described that mediates this effect. We show that the egg glycoprotein IPSE/alpha-1 induces splenic Breg cells *in vitro*, as assessed by IL-10 production and Treg cell induction, following direct interaction (**chapter 3**). Importantly, SEA depleted of IPSE/alpha-1 is also capable of Breg cell induction, highlighting that there are other, yet unidentified factors in SEA that induce Breg cells. We found IPSE/alpha-1 to also induce IL-10 production in human $CD1d^+$ B cells, thus identifying IPSE/alpha-1 as the first helminth molecule with direct Breg cell-inducing capacity in both mice and humans. That the Breg cell-inducing effect can be replicated by a recombinant, plant-expressed version of

the molecule, as previously described for the *S. mansoni* egg glycoprotein omega-1 (ω-1) [11], is an important point of departure for further investigations into the therapeutic potential of this molecule.

We could identify IPSE/alpha-1, previously mainly recognized for its capacity to activate basophils [12, 13], as one Breg cell-inducing factor within SEA *in vitro*, but *in vivo* multiple signals likely synergize to induce Breg cells. To study the molecular signals required for Breg cell induction in schistosomiasis more globally, we analysed the transcriptome of splenic MZ and FO B cells from chronically *S. mansoni* infected mice (**chapter 4**). We found marginal zone (MZ) and follicular (FO) B cells to display clearly distinct transcriptional profiles, both at steady-state and after infection. Both cell types moreover clearly responded to infection. We analysed genes, pathways and upstream regulators that are more unique to one B cell subset or the other, and identified interesting leads for further investigation. Amongst those, the cytokines IL-1β and IL-6, as well as members of the type I interferon (IFN-I) family, seem to preferentially activate MZ B cells, which is interesting as initial observations in the literature suggest their involvement in Breg cell induction [14, 15]. Furthermore, our data suggest TLR7 and TLR9 as upstream regulators that are more active in MZ than FO B cells, which also aligns with the literature [16, 17] and overall suggests the involvement of several innate signals in MZ B cell activation.

As IFN-I have been suggested as one upstream regulator of the transcriptional changes that MZ B cells undergo in *S. mansoni* infection, we further investigated whether IFN-I also provide signals for Breg cell induction in the context of *S. mansoni* (**chapter 5**). Schistosomes [18, 19] and other helminths [20, 21] induce IFN-I, and a recent report shows that IFN-I provide signals for the induction of human Breg cells [15]. We found Breg cell IL-10 production in response to *S. mansoni* antigens to be enhanced by IFN-I *in vitro*, but not *in vivo*. Stimulation with *S. mansoni*-derived antigens might provide a pre-activation signal *in vitro*, but the identification of optimal conditions for Breg cell expansion *in vivo* warrants further investigation.

In conclusion, our studies contribute to the understanding of how Breg cells develop and are activated in response to *S. mansoni*. *S. mansoni* actively secretes molecules to target host immunity, including molecules that directly bind and induce Breg cells such as IPSE/alpha-1. *In vivo*, multiple signals synergize leading to optimal Breg cell induction and activation, which seem to be a combination of both helminth-derived and host-derived factors. The further identification of such factors, their synergistic effects and the role of tissue-specific niches warrants further investigation.

Identification of single, schistosome-derived molecules for the inhibition of AAI

As discussed in the 2014 review (**chapter 6**), various helminths and helminth-derived molecules have been described to modulate allergic disease, and a range of different mechanisms of suppression have been suggested. However, the role of schistosome eggs in AAI protection was not well defined and hardly any protective, single molecules had been described. We subsequently showed that isolated *S. mansoni* eggs, in the absence of infection, protect from AAI when administered during the allergic sensitization phase (**chapter 7**). In contrast to several other studies that previously reported helminth- and, more specifically, *Schistosoma* spp.-mediated protection to be dependent on Treg and/or Breg cells, we still observed protection in the absence of Treg and B cells, respectively. Interestingly, protection from the allergic type 2 response occurred despite a strong antigen-specific type 2 response to the eggs. We could reproduce the protective effect of eggs on AAI by treatment with a plant-derived, recombinant version of the single, egg-derived glycoprotein ω-1, for which no such role had been described before. As for *S. mansoni* eggs, protection by ω-1 also seemed independent of regulatory lymphocytes and occured despite a strong antigen-specific Th2 response. Protein mutants lacking T2 RNase activity and protein variants with modified glycosylation pattern will help to further assess e.g. the importance of the ribonuclease activity of ω-1 and consequently the (partial) breakdown of mRNA [22] in mediating protection. Moreover, tracking experiments as well as mouse lines with mutation in ω-1-

binding receptors mannose receptor (MR) and SIGN-R1 will help to understand which tissues and cells take up ω-1, potentially allowing further insight into the mechanism of protection.

Collectively, our work on the protection from AAI by schistosome-derived eggs or single molecules has contributed to revealing the diversity in mechanisms involved in mediating bystander suppression in hyper-inflammatory settings such as allergic asthma. This adds to the expanding realization that helminths have developed various mechanisms of modulating host immunity which go beyond the development of regulatory lymphocytes.

DIRECTION FOR FUTURE RESEARCH

Molecular signals for schistosome-induced Breg cell development

It is currently unclear whether Breg cells develop from a committed precursor, or whether any B cell can acquire suppressive capacity in response to environmental stimuli. It becomes more and more clear that B cells at different stages of maturation and differentiation (from transitional B cells [23, 24] to plasma cells [25-27]), and B cells at different sites (from secondary lymphoid organs [28, 29]) to the peritoneal cavity [30] and the lung [31] in mice, and in human adipose tissue [9] and nasal polyps [10]) possess suppressive capacities, which argues against a committed precursor. Most recently, natural regulatory plasma cells have been identified that develop at steady-state and respond by IL-10 production within hours of stimulation [27].

Breg cells comprise a heterogeneous group of cells that lack a specific marker. The markers currently used to identify Breg cells are likely not specific enough to selectively target them in cellular immunotherapy. Novel technologies such as mass cytometry will allow a better characterization of Breg cell heterogeneity in both animal models and humans, similarly to studies already performed for Treg cells [32].

Breg cell activation i*n vivo* is more complex than the *in vitro* Breg cell-activating stimuli which have been extensively studied. A variety of receptors including BCR, CD40 and TLRs have been described to be involved in Breg cell activation in different models both *in vitro* and *in vivo*. While an inflammatory environment seems a shared feature between all models and central to Breg cell induction, it has been suggested that different Breg cell subsets require different additional stimuli for their development and activation. Innate-like Breg cells may require the ligation of TLRs, while other Bregs especially in autoimmunity seem to require BCR and CD40 ligation [33]. Work from us and others suggests that Breg cells induced during infection - and in particular during schistosome infections - might be of the innate-like type that develop in response to inflammatory cytokines and TLR ligation.

With respect to stimuli that might contribute to Breg cell activation *in vivo*, the gut microbiome is of particular interest. A recent study shows changes in the gut microbiome following *S. mansoni* infection [34]. Some studies moreover suggest that gut microbiota can support Breg cell induction [14, 35, 36], whereas others describe the development of regulatory plasma cells to be microbiota-independent [27]. Although changes in the composition of the lung microbiome have both been shown to promote airway disease [37] and to induce Treg cells that can suppress AAI [38], the influence of the lung microbiota on local B cells has so far not been addressed. It will be of special interest to further define whether and how changes in microbiome composition, and the subsequent interaction with the host immune system, contribute to Breg cell induction.

The importance of Breg cell antigen-specificity for maximal and focussed suppressive function, or for minimizing side effects due to non-specific immune suppression, needs to be addressed in more detail. It is clear from animal models that BCR signalling is one of the critical stimuli for Breg cell development at least in models of autoimmunity, as mice with a BCR fixed for a non-relevant antigen or with impaired BCR signalling harbour less Breg cells and cope less well with EAE [39, 40]. However, stimulation with mitogenic anti-IgM antibody alone does not induce Breg cells [41], suggesting that low

affinity BCR ligation induces regulatory properties rather than the strong signal provided by anti-IgM. Whereas the importance of B cell specificity in autoimmune models has been acknowledged, the nature of the antigen(s) remains to be defined. Interestingly, the frequency of IL-10- and IgG4-producing Breg cells specific for the bee venom allergen phospholipase A2 (PLA) was increased in patients allergic to bee venom after successful allergen immunotherapy, and reached levels comparable to tolerant beekeepers, whereas B cells not specific for PLA produced only little IL-10 and IgG4 and did not respond to allergen immunotherapy [42]. In this study, B cells specific for a defined antigen are the ones to acquire regulatory properties. In helminth infection, the importance of antigen-specificity for Breg cell development is less defined.

Helminths release a multitude of antigens, which can potentially activate B cells through the BCR and innate receptors like TLRs simultaneously. While signalling through TLRs has been repeatedly reported to induce Breg cells in helminth infection, the relative contribution of BCR signalling should be further defined by using e.g. mouse models with fixed BCRs as done in the field of autoimmunity. In addition, studies on Breg cell antigen-specificity are possibly hampered by technical challenges, such as low abundance of these cells and the co-staining of other, non-specific B cells with labelled antigens. This could potentially be overcome in the future through advancements in the field of single cell analysis.

It is furthermore not well understood whether Breg cells acquire suppressive capacity in a tissue-dependent manner, and what their migration pattern is. Many studies assess splenic Breg cells after intraperitoneal injection of a stimulus, which does not resemble the physiological situation well. Studying Breg cells at the site of inflammation and/or tracking B cell residency and migration would help to understand the role of the tissue micro-environment in Breg cell development and activation. Interestingly, it has been shown that tumour-infiltrating B cells acquire suppressive capacity upon exposure to metabolites of the 5-lipoxygenase pathway and growth factors in the tumour micro-environment [43, 44], suggesting an important role of local signals for Breg cells. Lipoxygenase derivatives were found in the lipidome of several *S. mansoni* life-cycle stages [45], and it would be interesting to further investigate those in the context of Breg cell induction during schistosome infections.

In view of potential therapeutic applications of Breg cells in conditions such as allergy and autoimmunity, it is important to identify conditions for optimal Breg cell activation and to study if suppression by Breg cells is a lasting phenotype, or whether these cells require frequent (re)stimulation to exert their function. To this end, the study of Breg cells should always include the characterization of their suppressive capacity e.g. by adoptive transfer into animal models of allergy or autoimmunity. This might pose challenges as active suppression via IL-10 is likely a transient feature, with recent cell activation necessary for active cytokine secretion [46]. In addition, proof of suppressive capacity might be more challenging to confirm in humans as it largely relies on *in vitro* assays [47, 48] that only yield limited information about *in vivo* suppressive capacity.

B cells are important therapeutic targets in many diseases with an immune component, including autoimmunity and cancer. Most prominently, B-cell depletion by anti-CD20 monoclonal antibody (rituximab) has been approved for the treatment of certain forms of leukaemia and rheumatoid arthritis (RA), and is under investigation for e.g. treatment of multiple sclerosis (MS) [49]. Treatment with anti-CD20 affects the balance of CD20-expressing and non-expressing B cells. It preferentially leaves immature, transitional B cells - a population that contains progenitor Breg cells [50, 51] - intact, thus targeting the Breg cell compartment indirectly. Another monoclonal antibody that, in addition to inhibiting pro-inflammatory B cells, might affect the Breg cell compartment is an IL-6R antagonist (tocilizumab), which has been shown to increase the TGF-β expression of $CD25^+$ Breg cells when used in RA patients [52]. Finally, IFNβ therapy commonly applied in patients with certain types of MS increases IL-10 production not only by monocytes and T cells [53, 54], but also by B cells and plasmablasts [55, 56].

None of these treatments have been developed to directly target Breg cells and the results are merely off-target effects. Proof-of-concept studies in mice have shown that suppressive Breg cells can be expanded, either *in vivo* by agonistic anti-CD40 antibody [23], or *ex vivo* by stimulation with CD40L, IL-4 and IL-21 [57]. More research is however necessary to understand the conditions that lead to optimal Breg cell activation and targeted suppressive function especially *in vivo* before novel therapies based on Breg cells can be developed.

With respect to applying treatment that targets the Breg cell compartment in allergy or asthma, any such treatment would have to be advantageous, meaning be of higher efficacy or lower side effects, compared to the currently applied options including anti-IgE therapy and allergen-specific immunotherapy. Anti-IgE treatment targets IgE produced by pro-allergic B cells as one of the central players in allergy and asthma, but is only short-lived [58]. Allergen-specific immunotherapy (AIT) aims to restore tolerance to the allergen by shifting the Th2 and IgE-dominated response to a more balanced one including IgG4 isotype switch and regulatory cells like Treg and Breg cells, achieving more long-lasting effects [59, 60]. Drawbacks of AIT are that it takes 3-5 year before AIT treatment reaches its full beneficial effect, and that this curative effect gradually declines over time [61]. Furthermore, the question remains how much of the symptom suppression is dependent on the induction of a long-lasting pool of (memory) Breg cells. Nevertheless, an interesting strategy could be to develop a new generation of allergen immunotherapeutics including an (helminth-derived) adjuvant component that specifically boosts the Breg cell compartment to perhaps shorten treatment time and increase the timespan of the curative effect.

Identification of single, schistosome-derived molecules for the inhibition of AAI

Although some of the clinical trials using *Trichuris suis* eggs (TSO) to treat patients with allergic and autoimmune conditions initially showed promising results, the progress in the field has slowed down in recent years. Larger trials have failed to confirm the early results, and efforts to use live infections as treatment strategies have diminished. Single, well-defined helminth-derived molecules circumvent the risks that full infections bear and are therefore advantageous. The identification of such immunomodulatory candidate molecules benefits from systemic approaches using computational tools and screening in high-throughput assays, such as already applied for the excretory-secretory products of *H. polygyrus* and *S. mansoni* adult worms [62-64]. The search for candidate molecules can by guided by the immunological activity of parasite-derived antigen mixtures such as excretory-secretory products, followed by molecule identification using e.g. fractionation, screening assays or proteomics approaches. Additionally, molecules of interest can also be identified in the absence of data from antigen mixtures, by e.g. identifying cytokine and chemokine homologues or family member of proteins already known to be of interest, such as proteases and protease inhibitors, members of the venom-allergen-like (VAL) family and lectins [65-68]. Notably, potential immunomodulatory molecules do not only include (glyco)proteins, but also lipids [69, 70], short chain fatty acids [71], and exosomes containing proteins, lipids and nucleic acids [72-74].

A growing body of literature shows that helminths including *S. mansoni* modulate allergic asthma via various mechanisms that are not limited to the induction of regulatory lymphocytes [75]. Bioinformatics-guided approaches such as dual transcriptomics and genomics databases such as WormBase ParaSite will not only aid the identification of immunomodulatory candidate molecules, but can also support the prediction of functional characteristics, interactions with host immunity and ultimately the putative mechanisms of action based on the presence of known homologues, predicted folding structures and functional domains [76-80].

Helminth parasites do not only directly interact with host immune cells to modulate immune responses, but also do so indirectly by their effect on the intestinal microbiome. Rodent intestinal nematodes have been shown to change the microbiome composition and thereby reduce AAI severity, or confer protection to respiratory syncytial virus (RSV) infection [20, 81, 82]. Whether helminths, and in particular *Schistosoma spp.* residing in the vasculature, alter the intestinal microbiome in humans, e.g. by the release of eggs in the intestinal lumen, is currently less well understood as studies come to conflicting conclusions [34, 83-86]. Future studies should therefore address the role of the microbiome in Breg cell induction in more detail.

Studies investigating the role of single, helminth-derived molecules are often limited by the amount and purity of the natural, isolated protein, and heterologous expression systems often lead to unintended alterations in the glycan composition. The development of a plant-derived expression system for schistosome molecules allows to produce large amounts of recombinant, helminth-glycosylated proteins [11], and the generation of protein mutants and variants with altered glycosylation will help to understand the role of protein activity and glycosylation pattern in protection.

There are multiple factors that might explain the mixed results obtained in clinical trials so far, and that thus are crucial points of consideration for the development of helminth-based therapies in the future, such as the timing, intensity/dose and localization of helminth infection or exposure to helminth-derived products. The body of evidence from studies in both animal models and humans suggest that, while exposure to helminths is able to prevent disease onset, treatment of established allergic disease is much harder to achieve. The importance of early-life exposure to microbial agents is supported by studies showing that growing up in a traditional farming environment with exposure to certain bacteria and fungi [87, 88] as well as unprocessed farm milk [89] protects against allergies and asthma. These studies suggest that stimuli shaping the immature immune system are critical for the protection against allergy and asthma later in life, and that in consequence also helminth-based therapies might have the most impact at a stage where the immune system can still be shaped. Supporting evidence also comes from studies on the impact of maternal helminth exposure on the offspring's susceptibility to allergy and asthma [90, 91]. As a prophylactic application of helminth-based therapies to the general public is likely not feasible and bears risks of side effects, such approach would require the identification of people at risk of developing allergy or asthma later in life, e.g. because of their genetic pre-disposition or aberrant immune reactions to allergens.

Most clinical trials so far have assessed helminth infections or their products that are restricted to the intestine, such as *T. suis* OVA. While intestinal exposure might be sufficient to alleviate local inflammation such as observed in inflammatory bowel disease, it might prove insufficient to induce systemic immunomodulation and suppress inflammation at distal sites such as the lung. With respect to treatment based on helminth-derived molecules, the choice of administration route could potentially overcome questions of local versus systemic exposure. Regarding the dose of treatment, animal studies naturally use higher infection or molecule doses than found in humans, where strict ethical regulations require dose escalation studies to determine that maximum tolerated dose and to minimize side effects [92]. The intraperitoneal administration of large doses of compound as employed in the majority of animal studies, in which the principles guiding human clinical trials are rarely applied, therefore provide only very limited guidance for translation into the human situation. Furthermore, once immunomodulatory candidate molecules are identified, it has to be assessed whether they can directly be applied in humans or whether alternative strategies are needed. For example, reproduction by small molecules or modified molecules that contain only the active motifs needed for interaction with host cells [93, 94] might be warranted to minimize side effects due to high immunogenicity of foreign, parasite-derived proteins in the human host. It however remains to be established whether

immunogenicity is a concern, as there are examples of pathogen-derived molecules where this is not the case. The hookworm molecule AIP-2 is an example of a helminth product that is minimally immunogenic in mice [95], and the bacteria-derived product streptokinase has been successfully used in humans despite its immunogenicity [96].

Additional efforts to fully understand the multiple variables at interplay that determine the effect of helminth infections and helminth-derived products on hyper-inflammatory conditions such as allergy may provide valuable leads for the development of novel pharmaceutical agents for the treatment of allergic disorders.

CONCLUDING REMARKS

The human immune system successfully distinguishes between benign and harmful agents, and mounts the appropriate response, in the majority of cases. It is however not without error, as apparent from e.g. allergic and autoimmune disorders. The rapid pace at which our living environments change, faster than any evolutionary adaptation can take place, poses challenges for the immune system. A deeper understanding of fundamental immunological processes, including immunomodulation such as induced by parasitic agents, might open up opportunities to develop novel treatment options based on targeted intervention in immune processes.

REFERENCES

1. Maizels RM, Yazdanbakhsh M. Immune regulation by helminth parasites: cellular and molecular mechanisms. Nat Rev Immunol. 2003;3(9):733-44.
2. van der Vlugt LE, Labuda LA, Ozir-Fazalalikhan A, Lievers E, Gloudemans AK, Liu KY, et al. Schistosomes induce regulatory features in human and mouse CD1d(hi) B cells: inhibition of allergic inflammation by IL-10 and regulatory T cells. PLoS One. 2012;7(2):e30883.
3. Correale J, Equiza TR. Regulatory B cells, helminths, and multiple sclerosis. Methods Mol Biol. 2014;1190:257-69.
4. Amu S, Saunders SP, Kronenberg M, Mangan NE, Atzberger A, Fallon PG. Regulatory B cells prevent and reverse allergic airway inflammation via FoxP3-positive T regulatory cells in a murine model. J Allergy Clin Immunol. 2010;125(5):1114-24 e8.
5. Mangan NE, Fallon RE, Smith P, van Rooijen N, McKenzie AN, Fallon PG. Helminth infection protects mice from anaphylaxis via IL-10-producing B cells. J Immunol. 2004;173(10):6346-56.
6. Correale J, Farez M. Helminth antigens modulate immune responses in cells from multiple sclerosis patients through TLR2-dependent mechanisms. J Immunol. 2009;183(9):5999-6012.
7. Helmby H. Human helminth therapy to treat inflammatory disorders - where do we stand? BMC Immunol. 2015;16:12.
8. Danilowicz-Luebert E, O'Regan NL, Steinfelder S, Hartmann S. Modulation of specific and allergy-related immune responses by helminths. J Biomed Biotechnol. 2011;2011:821578.
9. Nishimura S, Manabe I, Takaki S, Nagasaki M, Otsu M, Yamashita H, et al. Adipose Natural Regulatory B Cells Negatively Control Adipose Tissue Inflammation. Cell Metab. 2013.
10. Ickrath P, Kleinsasser N, Ding X, Ginzkey C, Beyersdorf N, Kerkau T, et al. Impact and Modulations of Peripheral and Edaphic B Cell Subpopulations in Chronic Rhinosinusitis With Nasal Polyposis. Clin Exp Otorhinolaryngol. 2018;11(2):133-40.
11. Wilbers RH, Westerhof LB, van Noort K, Obieglo K, Driessen NN, Everts B, et al. Production and glyco-engineering of immunomodulatory helminth glycoproteins in plants. Sci Rep. 2017;7:45910.
12. Schramm G, Falcone FH, Gronow A, Haisch K, Mamat U, Doenhoff MJ, et al. Molecular characterization of an interleukin-4-inducing factor from Schistosoma mansoni eggs. J Biol Chem. 2003;278(20):18384-92.
13. Schramm G, Gronow A, Knobloch J, Wippersteg V, Grevelding CG, Galle J, et al. IPSE/alpha-1: a major immunogenic component secreted from Schistosoma mansoni eggs. Mol Biochem Parasitol. 2006;147(1):9-19.

14. Rosser EC, Oleinika K, Tonon S, Doyle R, Bosma A, Carter NA, et al. Regulatory B cells are induced by gut microbiota-driven interleukin-1beta and interleukin-6 production. Nat Med. 2014;20(11):1334-9.

15. Menon M, Blair PA, Isenberg DA, Mauri C. A Regulatory Feedback between Plasmacytoid Dendritic Cells and Regulatory B Cells Is Aberrant in Systemic Lupus Erythematosus. Immunity. 2016;44(3):683-97.

16. Miles K, Heaney J, Sibinska Z, Salter D, Savill J, Gray D, et al. A tolerogenic role for Toll-like receptor 9 is revealed by B-cell interaction with DNA complexes expressed on apoptotic cells. Proc Natl Acad Sci U S A. 2012;109(3):887-92.

17. Khan AR, Amu S, Saunders SP, Hams E, Blackshields G, Leonard MO, et al. Ligation of TLR7 on CD19(+) CD1d(hi) B cells suppresses allergic lung inflammation via regulatory T cells. Eur J Immunol. 2015;45(6):1842-54.

18. Trottein F, Pavelka N, Vizzardelli C, Angeli V, Zouain CS, Pelizzola M, et al. A type I IFN-dependent pathway induced by Schistosoma mansoni eggs in mouse myeloid dendritic cells generates an inflammatory signature. J Immunol. 2004;172(5):3011-7.

19. Webb LM, Lundie RJ, Borger JG, Brown SL, Connor LM, Cartwright AN, et al. Type I interferon is required for T helper (Th) 2 induction by dendritic cells. EMBO J. 2017;36(16):2404-18.

20. McFarlane AJ, McSorley HJ, Davidson DJ, Fitch PM, Errington C, Mackenzie KJ, et al. Enteric helminth-induced type I interferon signaling protects against pulmonary virus infection through interaction with the microbiota. J Allergy Clin Immunol. 2017;140(4):1068-78 e6.

21. Connor LM, Tang SC, Cognard E, Ochiai S, Hilligan KL, Old SI, et al. Th2 responses are primed by skin dendritic cells with distinct transcriptional profiles. J Exp Med. 2017;214(1):125-42.

22. Everts B, Hussaarts L, Driessen NN, Meevissen MH, Schramm G, van der Ham AJ, et al. Schistosome-derived omega-1 drives Th2 polarization by suppressing protein synthesis following internalization by the mannose receptor. J Exp Med. 2012;209(10):1753-67, S1.

23. Blair PA, Chavez-Rueda KA, Evans JG, Shlomchik MJ, Eddaoudi A, Isenberg DA, et al. Selective targeting of B cells with agonistic anti-CD40 is an efficacious strategy for the generation of induced regulatory T2-like B cells and for the suppression of lupus in MRL/lpr mice. J Immunol. 2009;182(6):3492-502.

24. Evans JG, Chavez-Rueda KA, Eddaoudi A, Meyer-Bahlburg A, Rawlings DJ, Ehrenstein MR, et al. Novel suppressive function of transitional 2 B cells in experimental arthritis. J Immunol. 2007;178(12):7868-78.

25. Shen P, Roch T, Lampropoulou V, O'Connor RA, Stervbo U, Hilgenberg E, et al. IL-35-producing B cells are critical regulators of immunity during autoimmune and infectious diseases. Nature. 2014;507(7492):366-70.

26. Matsumoto M, Baba A, Yokota T, Nishikawa H, Ohkawa Y, Kayama H, et al. Interleukin-10-producing plasmablasts exert regulatory function in autoimmune inflammation. Immunity. 2014;41(6):1040-51.

27. Lino AC, Dang VD, Lampropoulou V, Welle A, Joedicke J, Pohar J, et al. LAG-3 Inhibitory Receptor Expression Identifies Immunosuppressive Natural Regulatory Plasma Cells. Immunity. 2018;49(1):120-33 e9.

28. Wilson MS, Taylor MD, O'Gorman MT, Balic A, Barr TA, Filbey K, et al. Helminth-induced CD19+CD23hi B cells modulate experimental allergic and autoimmune inflammation. Eur J Immunol. 2010;40(6):1682-96.

29. Mauri C, Menon M. The expanding family of regulatory B cells. Int Immunol. 2015;27(10):479-86.

30. O'Garra A, Chang R, Go N, Hastings R, Haughton G, Howard M. Ly-1 B (B-1) cells are the main source of B cell-derived interleukin 10. Eur J Immunol. 1992;22(3):711-7.

31. van der Vlugt L, Obieglo K, Ozir-Fazalalikhan A, Sparwasser T, Haeberlein S, Smits HH. Schistosome-induced pulmonary B cells inhibit allergic airway inflammation and display a reduced Th2-driving function. Int J Parasitol. 2017;47(9):545-54.

32. Mason GM, Lowe K, Melchiotti R, Ellis R, de Rinaldis E, Peakman M, et al. Phenotypic Complexity of the Human Regulatory T Cell Compartment Revealed by Mass Cytometry. J Immunol. 2015;195(5):2030-7.

33. Mizoguchi A, Bhan AK. A case for regulatory B cells. J Immunol. 2006;176(2):705-10.

34. Jenkins TP, Peachey LE, Ajami NJ, MacDonald AS, Hsieh MH, Brindley PJ, et al. Schistosoma mansoni infection is associated with quantitative and qualitative modifications of the mammalian intestinal microbiota. Sci Rep. 2018;8(1):12072.

35. Benedek G, Zhang J, Nguyen H, Kent G, Seifert HA, Davin S, et al. Estrogen protection against EAE modulates the microbiota and mucosal-associated regulatory cells. J Neuroimmunol. 2017;310:51-9.

36. Alhabbab R, Blair P, Elgueta R, Stolarczyk E, Marks E, Becker PD, et al. Diversity of gut microflora is required for the generation of B cell with regulatory properties in a skin graft model. Sci Rep. 2015;5:11554.

37. Huang YJ, Marsland BJ, Bunyavanich S, O'Mahony L, Leung DY, Muraro A, et al. The microbiome in allergic disease: Current understanding and future opportunities-2017 PRACTALL document of the American Academy of Allergy, Asthma & Immunology and the European Academy of Allergy and Clinical Immunology. J Allergy Clin Immunol. 2017;139(4):1099-110.

38. Gollwitzer ES, Saglani S, Trompette A, Yadava K, Sherburn R, McCoy KD, et al. Lung microbiota promotes tolerance to allergens in neonates via PD-L1. Nat Med. 2014;20(6):642-7.

39. Fillatreau S, Sweenie CH, McGeachy MJ, Gray D, Anderton SM. B cells regulate autoimmunity by provision of IL-10. Nat Immunol. 2002;3(10):944-50.

40. Matsumoto M, Fujii Y, Baba A, Hikida M, Kurosaki T, Baba Y. The calcium sensors STIM1 and STIM2 control B cell regulatory function through interleukin-10 production. Immunity. 2011;34(5):703-14.

41. Yanaba K, Bouaziz JD, Matsushita T, Tsubata T, Tedder TF. The development and function of regulatory B cells expressing IL-10 (B10 cells) requires antigen receptor diversity and TLR signals. J Immunol. 2009;182(12):7459-72.

42. van de Veen W, Stanic B, Yaman G, Wawrzyniak M, Sollner S, Akdis DG, et al. IgG4 production is confined to human IL-10-producing regulatory B cells that suppress antigen-specific immune responses. J Allergy Clin Immunol. 2013;131(4):1204-12.

43. Wejksza K, Lee-Chang C, Bodogai M, Bonzo J, Gonzalez FJ, Lehrmann E, et al. Cancer-produced metabolites of 5-lipoxygenase induce tumor-evoked regulatory B cells via peroxisome proliferator-activated receptor alpha. J Immunol. 2013;190(6):2575-84.

44. Han S, Feng S, Ren M, Ma E, Wang X, Xu L, et al. Glioma cell-derived placental growth factor induces regulatory B cells. Int J Biochem Cell Biol. 2014;57:63-8.

45. Kaisar MMM. Dissertation: Tracking helminths - from molecular diagnostics to mechanisms behind immune polarization. (ISBN: 978-94-6182-826-2). 2017.

46. Maseda D, Smith SH, DiLillo DJ, Bryant JM, Candando KM, Weaver CT, et al. Regulatory B10 cells differentiate into antibody-secreting cells after transient IL-10 production in vivo. J Immunol. 2012;188(3):1036-48.

47. Iwata Y, Matsushita T, Horikawa M, Dilillo DJ, Yanaba K, Venturi GM, et al. Characterization of a rare IL-10-competent B-cell subset in humans that parallels mouse regulatory B10 cells. Blood. 2011;117(2):530-41.

48. Blair PA, Norena LY, Flores-Borja F, Rawlings DJ, Isenberg DA, Ehrenstein MR, et al. CD19(+) CD24(hi)CD38(hi) B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic Lupus Erythematosus patients. Immunity. 2010;32(1):129-40.

49. Mulero P, Midaglia L, Montalban X. Ocrelizumab: a new milestone in multiple sclerosis therapy. Ther Adv Neurol Disord. 2018;11:1756286418773025.

50. Horikawa M, Minard-Colin V, Matsushita T, Tedder TF. Regulatory B cell production of IL-10 inhibits lymphoma depletion during CD20 immunotherapy in mice. J Clin Invest. 2011;121(11):4268-80.

51. Bodogai M, Lee Chang C, Wejksza K, Lai J, Merino M, Wersto RP, et al. Anti-CD20 antibody promotes cancer escape via enrichment of tumor-evoked regulatory B cells expressing low levels of CD20 and CD137L. Cancer Res. 2013;73(7):2127-38.

52. Snir A, Kessel A, Haj T, Rosner I, Slobodin G, Toubi E. Anti-IL-6 receptor antibody (tocilizumab): a B cell targeting therapy. Clin Exp Rheumatol. 2011;29(4):697-700.

53. Kozovska ME, Hong J, Zang YC, Li S, Rivera VM, Killian JM, et al. Interferon beta induces T-helper 2 immune deviation in MS. Neurology. 1999;53(8):1692-7.

54. Liu H, MacKenzie-Graham AJ, Kim S, Voskuhl RR. Mice resistant to experimental autoimmune encephalomyelitis have increased thymic expression of myelin basic protein and increased MBP specific T cell tolerance. J Neuroimmunol. 2001;115(1-2):118-26.

55. Schubert RD, Hu Y, Kumar G, Szeto S, Abraham P, Winderl J, et al. IFN-beta treatment requires B cells for efficacy in neuroautoimmunity. J Immunol. 2015;194(5):2110-6.

56. Ramgolam VS, Sha Y, Marcus KL, Choudhary N, Troiani L, Chopra M, et al. B cells as a therapeutic target for IFN-beta in relapsing-remitting multiple sclerosis. J Immunol. 2011;186(7):4518-26.

57. Yoshizaki A, Miyagaki T, DiLillo DJ, Matsushita T, Horikawa M, Kountikov EI, et al. Regulatory B cells control T-cell autoimmunity through IL-21-dependent cognate interactions. Nature. 2012;491(7423):264-8.

58. Segal M, Stokes JR, Casale TB. Anti-immunoglobulin e therapy. World Allergy Organ J. 2008;1(10):174-83.

59. Palomares O, Akdis M, Martin-Fontecha M, Akdis CA. Mechanisms of immune regulation in allergic diseases: the role of regulatory T and B cells. Immunol Rev. 2017;278(1):219-36.

60. van de Veen W. The role of regulatory B cells in allergen immunotherapy. Curr Opin Allergy Clin Immunol. 2017;17(6):447-52.

61. Frew AJ. Allergen immunotherapy. J Allergy Clin Immunol. 2010;125(2 Suppl 2):S306-13.

62. Osbourn M, Soares DC, Vacca F, Cohen ES, Scott IC, Gregory WF, et al. HpARI Protein Secreted by a Helminth Parasite Suppresses Interleukin-33. Immunity. 2017;47(4):739-51 e5.

63. Johnston CJC, Smyth DJ, Kodali RB, White MPJ, Harcus Y, Filbey KJ, et al. A structurally distinct TGF-beta mimic from an intestinal helminth parasite potently induces regulatory T cells. Nat Commun. 2017;8(1):1741.

64. Floudas A, Cluxton CD, Fahel J, Khan AR, Saunders SP, Amu S, et al. Composition of the Schistosoma mansoni worm secretome: Identification of immune modulatory Cyclophilin A. PLoS Negl Trop Dis. 2017;11(10):e0006012.

65. Hewitson JP, Harcus Y, Murray J, van Agtmaal M, Filbey KJ, Grainger JR, et al. Proteomic analysis of secretory products from the model gastrointestinal nematode Heligmosomoides polygyrus reveals dominance of venom allergen-like (VAL) proteins. J Proteomics. 2011;74(9):1573-94.

66. Molehin AJ, Gobert GN, McManus DP. Serine protease inhibitors of parasitic helminths. Parasitology. 2012;139(6):681-95.

67. Yang Y, Wen Y, Cai YN, Vallee I, Boireau P, Liu MY, et al. Serine proteases of parasitic helminths. Korean J Parasitol. 2015;53(1):1-11.

68. Loukas A, Maizels RM. Helminth C-type lectins and host-parasite interactions. Parasitol Today. 2000;16(8):333-9.

69. van der Kleij D, Latz E, Brouwers JF, Kruize YC, Schmitz M, Kurt-Jones EA, et al. A novel host-parasite lipid cross-talk. Schistosomal lyso-phosphatidylserine activates toll-like receptor 2 and affects immune polarization. J Biol Chem. 2002;277(50):48122-9.

70. Angeli V, Faveeuw C, Roye O, Fontaine J, Teissier E, Capron A, et al. Role of the parasite-derived prostaglandin D2 in the inhibition of epidermal Langerhans cell migration during schistosomiasis infection. J Exp Med. 2001;193(10):1135-47.

71. Kaisar MMM, Pelgrom LR, van der Ham AJ, Yazdanbakhsh M, Everts B. Butyrate Conditions Human Dendritic Cells to Prime Type 1 Regulatory T Cells via both Histone Deacetylase Inhibition and G Protein-Coupled Receptor 109A Signaling. Front Immunol. 2017;8:1429.

72. Coakley G, McCaskill JL, Borger JG, Simbari F, Robertson E, Millar M, et al. Extracellular Vesicles from a Helminth Parasite Suppress Macrophage Activation and Constitute an Effective Vaccine for Protective Immunity. Cell Rep. 2017;19(8):1545-57.

73. Nowacki FC, Swain MT, Klychnikov OI, Niazi U, Ivens A, Quintana JF, et al. Protein and small non-coding RNA-enriched extracellular vesicles are released by the pathogenic blood fluke Schistosoma mansoni. J Extracell Vesicles. 2015;4:28665.

74. Eichenberger RM, Ryan S, Jones L, Buitrago G, Polster R, Montes de Oca M, et al. Hookworm Secreted Extracellular Vesicles Interact With Host Cells and Prevent Inducible Colitis in Mice. Front Immunol. 2018;9:850.

75. McSorley HJ, Chaye MAM, Smits HH. Worms: Pernicious parasites or allies against allergies? Parasite Immunol. 2018:e12574.

76. Schulze S, Schleicher J, Guthke R, Linde J. How to Predict Molecular Interactions between Species? Front Microbiol. 2016;7:442.

77. Westermann AJ, Barquist L, Vogel J. Resolving host-pathogen interactions by dual RNA-seq. PLoS Pathog. 2017;13(2):e1006033.

78. Ebner F, Kuhring M, Radonic A, Midha A, Renard BY, Hartmann S. Silent Witness: Dual-Species Transcriptomics Reveals Epithelial Immunological

Quiescence to Helminth Larval Encounter and Fostered Larval Development. Front Immunol. 2018;9:1868.

79. Bolt BJ, Rodgers FH, Shafie M, Kersey PJ, Berriman M, Howe KL. Using WormBase ParaSite: An Integrated Platform for Exploring Helminth Genomic Data. Methods Mol Biol. 2018;1757:471-91.

80. Howe KL, Bolt BJ, Shafie M, Kersey P, Berriman M. WormBase ParaSite - a comprehensive resource for helminth genomics. Mol Biochem Parasitol. 2017;215:2-10.

81. Zaiss MM, Rapin A, Lebon L, Dubey LK, Mosconi I, Sarter K, et al. The Intestinal Microbiota Contributes to the Ability of Helminths to Modulate Allergic Inflammation. Immunity. 2015;43(5):998-1010.

82. Houlden A, Hayes KS, Bancroft AJ, Worthington JJ, Wang P, Grencis RK, et al. Chronic Trichuris muris Infection in C57BL/6 Mice Causes Significant Changes in Host Microbiota and Metabolome: Effects Reversed by Pathogen Clearance. PLoS One. 2015;10(5):e0125945.

83. Kay GL, Millard A, Sergeant MJ, Midzi N, Gwisai R, Mduluza T, et al. Differences in the Faecal Microbiome in Schistosoma haematobium Infected Children vs. Uninfected Children. PLoS Negl Trop Dis. 2015;9(6):e0003861.

84. Schneeberger PHH, Coulibaly JT, Panic G, Daubenberger C, Gueuning M, Frey JE, et al. Investigations on the interplays between Schistosoma mansoni, praziquantel and the gut microbiome. Parasit Vectors. 2018;11(1):168.

85. Lee SC, Tang MS, Lim YA, Choy SH, Kurtz ZD, Cox LM, et al. Helminth colonization is associated with increased diversity of the gut microbiota. PLoS Negl Trop Dis. 2014;8(5):e2880.

86. Cooper P, Walker AW, Reyes J, Chico M, Salter SJ, Vaca M, et al. Patent human infections with the whipworm, Trichuris trichiura, are not associated with alterations in the faecal microbiota. PLoS One. 2013;8(10):e76573.

87. von Mutius E. The microbial environment and its influence on asthma prevention in early life. J Allergy Clin Immunol. 2016;137(3):680-9.

88. Schuijs MJ, Willart MA, Vergote K, Gras D, Deswarte K, Ege MJ, et al. Farm dust and endotoxin protect against allergy through A20 induction in lung epithelial cells. Science. 2015;349(6252):1106-10.

89. Lluis A, Depner M, Gaugler B, Saas P, Casaca VI, Raedler D, et al. Increased regulatory T-cell numbers are associated with farm milk exposure and lower atopic sensitization and asthma in childhood. J Allergy Clin Immunol. 2014;133(2):551-9.

90. Guadalupe I, Mitre E, Benitez S, Chico ME, Nutman TB, Cooper PJ. Evidence for in utero sensitization to Ascaris lumbricoides in newborns of mothers with ascariasis. J Infect Dis. 2009;199(12):1846-50.

91. Elliott AM, Mpairwe H, Quigley MA, Nampijja M, Muhangi L, Oweka-Onyee J, et al. Helminth infection during pregnancy and development of infantile eczema. JAMA. 2005;294(16):2032-4.

92. Le Tourneau C, Lee JJ, Siu LL. Dose escalation methods in phase I cancer clinical trials. J Natl Cancer Inst. 2009;101(10):708-20.

93. Rzepecka J, Coates ML, Saggar M, Al-Riyami L, Coltherd J, Tay HK, et al. Small molecule analogues of the immunomodulatory parasitic helminth product ES-62 have anti-allergy properties. Int J Parasitol. 2014;44(9):669-74.

94. Suckling CJ, Alam S, Olson MA, Saikh KU, Harnett MM, Harnett W. Small Molecule Analogues of the parasitic worm product ES-62 interact with the TIR domain of MyD88 to inhibit pro-inflammatory signalling. Sci Rep. 2018;8(1):2123.

95. Navarro S, Pickering DA, Ferreira IB, Jones L, Ryan S, Troy S, et al. Hookworm recombinant protein promotes regulatory T cell responses that suppress experimental asthma. Sci Transl Med. 2016;8(362):362ra143.

96. Sikri N, Bardia A. A history of streptokinase use in acute myocardial infarction. Tex Heart Inst J. 2007;34(3):318-27.