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INDUCTION AND REGULATION OF PATHOGENIC Th17 CELL RESPONSES IN SCHISTOSOMIASIS

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Abstract

Schistosomiasis is a major tropical disease caused by trematode helminths in which the host mounts a pathogenic immune response against tissue-trapped parasite eggs. The immunopathology consists of egg antigen-specific CD4 T cell-mediated granulomatous inflammation that varies greatly in magnitude in humans and among mouse strains in an experimental model. New evidence, covered in this review, intimately ties the development of severe pathology to CD4 T helper 17 cells, a finding that adds a new dimension to the traditional CD4 Th1 vs. Th2 cell paradigm. Most examined mouse strains, in fact, develop severe immunopathology with substantial Th17 as well as Th1 and Th2 cell responses; a Th2 polarized response is an exception that is only observed in low-pathology strains such as the C57BL/6. The ability to mount pathogenic Th17 cell responses is genetically determined and depends on the ability of antigen presenting cells to produce IL-23 and IL-1 β following recognition of egg antigens; analyses of several F2 progenies of (high $\times$ low)-pathology strain crosses demonstrated that quantitative trait loci governing IL-17 levels and disease severity vary substantially from cross to cross. Low pathology is dominant, which may explain the low incidence of severe disease in humans; however, coinfection with nematodes can also dampen pathogenic Th17 responses by promoting regulatory mechanisms such as those afforded by alternatively activated macrophages and T regulatory cells. A better understanding of the pathways conducive to severe forms of schistosomiasis and their regulation should lead to interventions similar to those presently used to manage other immune-mediated diseases.

Introduction to schistosomiasis

Schistosomiasis is an ancient parasitic disease caused by trematode helminths that continues to afflict over 200 million people throughout tropical regions of the world. It is contracted by humans (and other vertebrates) when wading in bodies of fresh water contaminated with free-swimming cercariae, the larval and infective form of the schistosomes released from aquatic vector snails. Cercariae penetrate the skin, and over a period of a few weeks mature into adult worms that home to a specific anatomic site within the host's venous vasculature, where males and females mate and lay eggs. The eggs exit the vascular compartment to access an emunctory organ that will set them free in search of snails for the purpose of propagating the life cycle. However, many eggs are trapped locally in the process of exiting or embolize in regional organs where they precipitate a fierce immune response, which, if undisturbed, sets off lifelong immunopathology and disease that can result in death.

Of the many existing schistosome species in nature, *Schistosoma mansoni*, *S. japonicum* and *S. hematobium* are the main human pathogens. In the first two cases, the adult worms colonize the mesenteric vascular plexus and consequently their eggs mostly affect the liver

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and intestine, whereas in the latter the worms home to the perivesicular veins and produce pathology predominantly in the bladder. In this review we focus mainly on *S. mansoni*, which is the most extensively studied species and also serves as an excellent and remarkably faithful model of experimental schistosomiasis in the mouse.

Human schistosomiasis mansoni occurs in two forms: the now less common acute form contracted mostly by visitors to endemic areas, and the chronic form affecting vast segments of the local populations in areas of Africa and northeastern South America. It has been known for a long time that most chronic patients develop relatively mild “intestinal” schistosomiasis, whereas only 5–10% suffer the severe “hepatosplenic” form of the disease, in which there is progressive liver fibrosis, portal hypertension, splenomegaly, ascites, gastrointestinal hemorrhage and death [1]. While schistosomiasis is currently susceptible to curative treatment with drugs such as praziquantel, swift reinfection unfortunately remains a serious drawback to eliminating the disease. No vaccine is available to this date despite vast efforts, the description of which is outside the scope of this article. Greater general details about schistosomiasis can be found in a number of informative review articles [1–4].

In all cases of schistosomiasis the key pathology is a granulomatous inflammatory reaction against tissue-trapped parasite eggs, and it is the nature of the host immune response, rather than the parasite itself, that shapes the magnitude of immunopathology in each case. Of great significance is the fact that much about the mechanisms governing the development and regulation of the pathogenic immune response in schistosomiasis has been learned from observations in the mouse.

The murine model

Murine susceptibility to patent infection with the hepatotropic species *S. mansoni* and *S. japonicum* has allowed for extensive investigation of this parasitic disease in a host in which a well-understood immune system has been the target of vast genetic manipulations. Since the 1970's it has been known that the outcome of murine infection with *S. mansoni* varies greatly according to the host's genetic background. Nevertheless, despite the awareness of considerable differences in infection intensity and pathology among mouse strains [5], most laboratories reported their findings and drew their conclusions based on observations made in only one strain, usually the C57BL/6 (BL/6) strain.

We performed studies on mouse strains of contrasting pathology to examine the underlying immune response to infection and to determine the genetic basis of dissimilar pathology. Prototypic strains are the CBA and C3H mice, which develop severe [5, 6], or extremely severe pathology [7], and BL/6 mice, in which the pathology is milder (Table 1) (Figure 1). Severe liver pathology after 7–8 weeks of infection with 80–85 cercariae is typically characterized by large, poorly-circumscribed perioval granulomatous lesions composed of a mixture of mono- and polynuclear leukocytes, additional interstitial parenchymal inflammation with variable hepatocyte necrosis, and increasing fibrous scarring. The inflammatory cells include lymphocytes, macrophages and eosinophils; more recently, neutrophils have also been recognized as important cellular constituents, especially in severe pathology [8]. Unlike in humans, murine neutrophils are more difficult to distinguish from eosinophils by routine histopathological technique and may have been underestimated in the past; assessment by cell marker expression is a more reliable method for proper identification [9]. By comparison, in mice that develop mild pathology, granulomatous and interstitial inflammation in the liver are greatly reduced, and there is little if any hepatocyte necrosis. However, prolonged survival allows for increasing hepatic fibrous scarring during chronicity.

Naturally high-pathology mouse strains, such as the CBA, seem ideal to study the mechanisms conducive to severe disease, yet these strains do not easily lend themselves to gene deletion or transgenesis. On the other hand, mutant low-pathology BL/6 mice are available for a vast array of immunologically relevant molecules. To adapt to this situation, we developed a model of *induced* severe pathology in BL/6 mice, which consists of subcutaneous immunization with a soluble schistosome egg antigen (SEA) preparation in CFA (SEA/CFA) prior to and once during infection with schistosomes [10]. This treatment results in a proinflammatory shift in immune response with pronounced exacerbation of egg-induced hepatic immunopathology which bears substantial similarity to the natural high-pathology disease (see Table 1 and below).

Our own experience with various mouse strains in terms of immune response and pathology during schistosome infection is summarized in Table 1 and further detailed in subsequent sections of this review. In essence, the mouse model affords a methodical analysis of the host immune response leading to the granulomatous inflammatory process, which, for obvious reasons, cannot be performed in humans.

Parasites and parasite antigens

Schistosomes are parasitic flatworms (platyhelminthes) belonging to the class of trematodes (blood flukes). They are dioecious, that is, there are separate males and females, and they are digeneic, in that they require a permanent and an intermediate host: a vertebrate and a fresh water snail unique to each schistosome species. Upon penetrating the skin of the permanent host, cercariae transform into schistosomula which mature for 3–4 weeks in the lung before arriving at their final intravascular location, where each pair of adults lays hundreds of eggs per day. The molecular cues that allow permissiveness only in certain hosts (permanent or intermediate), or that determine the characteristic final anatomic homing location are not known; as such, for unknown reasons, the mouse is not suitable for patent infection with *S. hematobium*. The schistosomes typically survive for many years in the permanent host effectively evading immune recognition and expulsion, yet their eggs are highly immunogenic. The capacity of the eggs to engender inflammation was likely selected for during evolution to debilitate tissues and facilitate migration through vascular, mesenchymal and musculo-epithelial barriers for the purpose of exiting the permanent host and completing the life cycle. Of similar interest are the observations that, for proper development and maturation, schistosomes are dependent on host signals such as those delivered by TGF- β and TNF- α [11, 12].

Owing to an impressive multinational effort, the genome of the schistosomes was fully sequenced and in the case of *S. mansoni* shown to encode at least 11809 genes, including those critical for parasite assembly, adaptation, survival and function [13]. Relevant to the potent immunopathogenicity of the schistosome eggs, 188 of the encoded proteins were found in secretions from live eggs [14]. Among the most abundant of these potentially host-sensitizing egg components that have been the subject of more detailed study are IPSE/alpha 1 (SmEP25) [15, 16], the omega 1 ribonuclease [17, 18], the major egg antigen (Ag) Sm-p40 [19–21], glutathione S-transferases 26 and 28 kD [22], phosphoenolpyruvate carboxykinase [23], thioredoxin [24], thioredoxin peroxidase [25] and the secretory glycoprotein kappa 5 (k5) [26]. A salient feature of the schistosome proteins is their rich glycosylation with a complex array of characteristic terminal glycan structures, such as GalNAc α 1–4GlcNAc (LacdiNAc, LDN), fucosylated LDN (F-LDN) and Lewis X (Le^x), all of which are composed of relatively few monosaccharide units typically including fucose, N-acetyl glucosamine, N-acetyl galactosamine and galactose [27–29]. Some of the better characterized egg proteins, such as IPSE/ alpha 1, omega 1 and k5, have been shown to carry these glycan motifs [27, 30].

The major egg Ag Sm-p40 is the most abundant component of the schistosome egg and thus in SEA. It is a 40 kD protein of 354 amino acids with homology to α -crystallins and small heat shock proteins. Sm-p40 is a dominant egg immunogen in high-pathology (H-2^k) CBA and C3H mice, in which it elicits a proinflammatory Th1/Th17 cell response [6, 31]. To date, the glycosylation status of Sm-p40 has not been determined, and it is conceivable that it may not be glycosylated (C. H. Hokke, personal communication). In the CBA and C3H mice a vast proportion of the anti-Sm-p40 T cell repertoire is directed against immunodominant epitope peptide Sm-p40_{234–246}[32], utilizing a T cell receptor (TCR) composed of V α 11.3V β 8 [33]. This striking immunodominance prompted the development of CBA mice expressing a transgenic (Tg) TCR specific for Sm-p40_{234–246}[31] (Box 1). Interestingly no single dominant Sm-p40 epitope was found in BL/6 mice and none is predicted in this strain for this Ag by epitope software.

The pathogenic host immune response in schistosomiasis

Schistosome products typically induce robust immune responses in the permanent host. The strongest and most consequential responses are directed against the eggs and egg products. Nevertheless, the immune system targets all of the parasite's life cycle forms although it is still poorly understood how the adult worms so successfully evade and/or resist immune attack. In general, the parasite proteins elicit cellular (T cell) responses, whereas the anti-glycan responses are humoral [34]. Such Ab responses, particularly those of the IgE isotype, have been shown to play a protective role in schistosomiasis [35, 36]. Although Ab and B cells and their interactions with and dependence on Th2 [37] and T follicular helper cells [38] represent an important element of the host's anti-schistosome immune response, their further discussion is beyond the scope of this review.

Egg-Ag-specific MHC class II-restricted CD4 T cells bearing clonally rearranged $\alpha\alpha$ chain receptors play an essential role in mediating the egg-induced granulomatous pathology; mice lacking these cells exhibit significantly reduced immunopathology [39–41]. Recognition of peptides from processed egg Ags on different Ag presenting cell (APC) types leads to CD4 T cell activation and cytokine production essential for shaping the subsequent inflammatory response. The absence of eggs in the stools of HIV+ patients with schistosomiasis mansoni is an indirect but elegant proof that CD4 T cells are critical in humans for granulomatous inflammation facilitating the transmigration of eggs into the intestinal lumen [42].

Earlier discoveries revealed that egg Ag-specific CD4 T cell responses result in the production of IL-2 [43] and that parasite egg deposition is a decisive factor in switching an initial Th1-type (IL-2 and IFN- γ) to a Th2-type (IL-4 and IL-5) dominant cytokine environment during schistosome infection [44]. However, appropriate function of the CD80/86-CD28, CD40-CD154 and B7RP1-inducible costimulator (ICOS) costimulatory systems is necessary to enable the switch [45–47]. The roles of Th1 vs. Th2 cytokines in schistosome immunopathology were further defined following the demonstration that in the absence of IL-4 signaling there is exacerbated inflammation and death, implying an overall host-protective function for the Th2 cell subset [48, 49]. However, during the chronic phase of schistosomiasis, the Th2 cell-derived cytokine IL-13 contributes to hepatic fibrosis [50, 51]; this pathogenic attribute of Th2 cells is covered more extensively in the review article by K. Fairfax et. al. in this series. Nonetheless, in the majority of human patients a fully Th2 polarized cytokine environment is associated with the mild intestinal form of the disease, in which hepatic fibrosis is not a determining factor [52, 53]. TNF- α has also been shown to positively contribute to immunopathology [53], whereas IL-10 clearly plays a potent immunoregulatory and anti-inflammatory role [54–56].

Cytokine kinetics, shifts and functions during the schistosome infection have been largely inferred from studies performed in the low-pathology BL/6 mouse. However, these conclusions are not necessarily applicable to other mouse strains; in fact, mice with different genetic backgrounds mount strikingly heterogeneous immunopathological reactions to the schistosome infection (see Table 1 and below). Most significantly, in CBA, C3H, and other high-pathology mice, overall cytokine production is more robust than in BL/6, and the initial proinflammatory Th1 response does not taper off, but persists alongside the rising Th2 response [57].

Worth recapitulating at this point is the long-known gradual reduction of overall cytokine secretion and consequent granulomatous inflammation in response to tissue-lodging eggs produced after the acute phase of the disease, called “immunomodulation”, which to a greater or lesser extent occurs in all mice, regardless of strain. Over time, numerous causes of immunomodulation have been proposed including a role for regulatory T cell (Treg) populations and alternatively activated macrophages (AAM) (see below), T cell anergy and IL-2 exhaustion [58, 59], idiotypic networks [60] and CD4 T cell hyporesponsiveness due to upregulation of *grail* in response to persistent antigenic stimulation [61]. Of interest is the fact that some regulatory mechanisms underlying immunomodulation may be similar to, or overlap with, those responsible for limiting inflammation in naturally low-pathology strains. They will be discussed in another section below.

The advent of Th17 cells

Until 2005, the heterogeneous outcome of immunopathology and clinical course in a vast number of inflammatory and infectious conditions, including human and experimental schistosomiasis, was considered to be a consequence of the interplay between pro- and anti-inflammatory forces driven largely by Th1 vs. Th2 cells [62]. At this time, however, the field of T cell immunology was revolutionized with the discovery that the immunopathology in several autoimmune diseases was mainly driven by CD4 T cells producing IL-17, rather than by Th1 cells producing IFN- γ [63–68]. As a consequence, the long-lasting, classical Th1/Th2 paradigm was no longer tenable.

IL-17 is the collective designation of a family of cytokines among which IL-17A, and to a lesser extent IL-17F, are endowed with a powerful pro-inflammatory function [66]. IL-17A, henceforth called IL-17 and the main focus of the studies described in this review, is a 21kD homodimeric glycoprotein mainly produced by activated CD4 T cells [66, 69]. These IL-17-producing CD4 T cells were subsequently recognized as a separate lineage of proinflammatory cells unrelated to Th1 or Th2 cells, and were designated Th17 cells after their signature cytokine [70, 71].

The first notion of the role of Th17 cells in the chronic inflammation associated with experimental autoimmune encephalomyelitis (EAE) came from experiments showing that mice lacking IL-12p40 and IL-23p19 failed to develop EAE, whereas in those lacking IL-12p35 the disease was similar to that observed in wild-type controls. These results demonstrated that the immunopathology was dependent on IL-23, a member of the IL-12 superfamily of heterodimeric cytokines which is composed of subunits IL-12p40 and IL-23p19 and is involved in the generation of Th17 cells, rather than on IL-12 itself, which is composed of subunits IL-12p40 and IL-12p35 and is necessary for the development of Th1 cells [72]. A pathogenic role of Th17 cells, similar to that in EAE, was also demonstrated in other human and experimental autoimmune disorders including collagen-induced arthritis (CIA) [73, 74], myocarditis [75] and colitis [76]. However, Th17 cells were also shown to be involved in immunity to some pathogens including the bacteria *Klebsiella pneumoniae* [77, 78] and *Citrobacter rodentium* [79], as well as the fungus *Candida*

albicans [80, 81]. Ironically, Th17 cells were first discovered for their harmful role in T cell autoimmunity, and only later were they shown to be effective in the control of certain intracellular microbial infections.

Of great interest were reports from a number of laboratories describing the requirement for several cytokines in the generation of Th17 cells, suggesting that there is considerably greater complexity in, and controversy about, the development of cells of this lineage than is the case for Th1 or Th2 cells. Such cytokines, derived from dendritic cells (DC) and other APC, which include IL-1 α and IL-21 in addition to IL-23, IL-6, and TGF- β , were shown to play overlapping and complementary roles in the differentiation and expansion of Th17 cells [82–85]. This process requires the expression of the corresponding receptors for these cytokines on naïve target T cells and activation of signaling pathways including the signal transducer and activator of transcription (STAT)-3 and the master lineage-specific transcription factor retinoic acid-related orphan receptor (ROR)- γ [86]. Th17 cells exert their potent proinflammatory function by secreting IL-17A and IL-17F, as well as IL-22, IL-21 and granulocyte colony-stimulating factor (G-CSF), which stimulate the production of the chemokines CXCL1, CXCL2, (and CXCL8 in humans) by fibroblasts, lymphoid and epithelial cells expressing the corresponding receptors such as the IL-17 receptor (IL-17RA), ultimately resulting in neutrophil-rich inflammatory cell infiltrates.

The role of Th17 cells in schistosomiasis

Since the immunobiology of the schistosome infection has so much in common with T cell-mediated autoimmune diseases, we began exploring the role of IL-12 superfamily cytokines using subunit-deficient BL/6 mice, which were immunized with SEA/CFA to boost their otherwise natural low pathology [10]. In BL/6 mice, such an immunization causes marked exacerbation of egg-induced hepatic inflammation together with a significant increase in both IFN- α and IL-17 production by SEA-stimulated T cells (Table 1). Remarkably, the immunization completely failed to elicit pathology exacerbation and IL-17 production in IL-12p40 $^{-/-}$ (IL-12, IL-23 deficient) mice, while in IL-12p35 $^{-/-}$ (IL-12 deficient, IL-23 sufficient) mice IL-17 production and immunopathology were similar to that seen in wild type controls [87]. These findings suggested that IL-17 driven by IL-23, but not IFN- α , was at the root of the severe disease, a fact further supported by the inhibition of pathology following treatment with anti-IL-17 Ab [87]. A close direct look at IL-23, afforded by mice deficient in IL-23p19 (IL-23p19 $^{-/-}$), revealed a significantly impaired immunopathology together with a marked reduction in IL-17 in the hepatic granulomas but not in the mesenteric lymph nodes [8]. In the IL-23p19 $^{-/-}$ mice there were also low levels of IFN- α regulated by high IL-10, and there was evidence that macrophages in the granulomas were in a state of alternative activation (see later). Taken together, these results not only demonstrated that IL-17 is critically involved in the immunopathology but also that IL-23 is not the only requirement for IL-17 production. Interestingly, a third cytokine of the IL-12 superfamily, IL-27, did not exert significant influence on the granulomatous inflammation [88].

Severe egg-induced immunopathology, whether naturally occurring in several mouse strains (Table 1) or in this case induced by immunization of BL/6 mice with SEA/CFA, is invariably linked to high IFN- α and IL-17 levels produced by SEA-stimulated mesenteric lymph node cells (MLNC). However, these cytokines play entirely disparate roles in granuloma pathogenesis as demonstrated in BL/6 mice deficient in the T-box transcription factor T-bet, which is required for IFN- α production and Th1 cell development and function [89]. Schistosome-infected T-bet $^{-/-}$ mice unexpectedly displayed significantly more severe neutrophil-rich hepatic granulomatous inflammation and greater T cell activation with increased production of IL-17, IL-21, IL-22 and the neutrophil chemokines CXCL1 and

CXCL2 together with lower levels of IL-4, IL-5 and IL-10 and a decrease in markers associated with AAM and Treg cells. Mice deficient in IFN- γ (IFN- γ -/-) similarly exhibited increased immunopathology with elevated IL-17 levels, whereas in mice lacking IL-17 (IL-17-/-) the pathology was markedly reduced despite IFN- γ levels that were even higher than in wild-type controls [90]. Interestingly, novel mice double-deficient in IL-17 and IFN- γ (IL-17/IFN- γ -/-) displayed the smallest granulomas, similar to those seen in unimmunized wild type BL/6 mice, along with higher levels of IL-5, IL-10 and the AAM marker resistin-like molecule α (Relm α , also known as FIZZ1), as well as reduced expression of IL-23p19, IL-1 α and CXCL1. Taken together, these findings demonstrate a) that Th17 cells play a critical role in the development of severe egg-induced inflammation, which is normally regulated by Th1 cells, b) that in the absence of IL-17, Th1 cells can mediate limited pathology, c) that IL-17 and IFN- γ are cross-regulatory during schistosome infection, and d) that in the absence of both IL-17 and IFN- γ , default background low pathology is mediated by Th2 cells with a likely contribution from innate immune mechanisms.

To more carefully dissect interactions between immune cells and the parasite, we examined cytokine levels in an in vitro co-culture system involving bone marrow-derived DC stimulated with live schistosome eggs in the presence of naïve syngeneic CD4 T cells non-specifically stimulated with anti-CD3/CD28 coated beads [91]. In CBA-derived co-cultures T cells produced high levels of IL-17. In contrast, BL/6 T cells, while capable of producing IL-17 in response to a combination of exogenous IL-6, TGF- β and IL-23, failed to do so in response to DC plus eggs, indicating that enhanced IL-17 production in CBA co-cultures is due to stimulation with the schistosome eggs. The CBA cells also expressed significantly higher levels of transcripts for IL-12p40, IL-23p19, IL-1 α , IL-6, CXCL1 and CXCL2, whereas IL-4, IL-10 and Foxp3 were higher in BL/6. Notably, blocking antibodies specific for IL-12p40 and IL-23, but surprisingly not for IL-6 and IL-21, significantly inhibited IL-17 production in the CBA cultures [91]. In related experiments IL-17 production by novel Sm-p40-specific Tg T cells (Box 1) in response to stimulation with DC plus eggs, was found to be sequentially dependent on IL-23 and IL-1 α , but again not on IL-6. Consequently, there was significant inhibition of immunopathology and IL-17 production in schistosome-infected IL-12p40-/- CBA mice and in mice treated with IL-1R antagonist [31]. These results indicated that both the naïve and Tg T cells were dependent on IL-23 and IL-1 α for differentiation into Th17 cells, while IL-6 was dispensable. This pattern differs with studies demonstrating a requirement for IL-6 and TGF- β [79, 81, 92, 93]. However, it is well established that the cytokine requirements for Th17 cell differentiation and pathogenicity, including IL-6, TGF- β , IL-23, IL-1 α and IL-21, vary significantly depending on the system examined, the nature of the T cells, and the species (human vs. mouse) [94–96]. For example, IL-6 and TGF- β were shown to be necessary to drive Th17 differentiation in sorted CD4⁺ CD44^{lo} CD62L^{hi} naïve T cells [97], whereas our studies were performed with unselected purified naïve CD4 T cells obtained from normal mice, which were exposed to IL-6 in vivo and are more representative of cells naturally confronting a new Ag or pathogen in vivo [31].

An important notion from the above findings is the remarkable heterogeneity of the host immune response to schistosome infection involving different APC, T cell subsets and cytokines, resulting in contrasting levels of immunopathology (Figure 2). Such differences are well documented in other parasitic diseases, notably leishmaniasis [98, 99], but have not received equal attention in schistosomiasis. The disparity in strain-specific responses to schistosome Ags begs the investigation of innate immune recognition mechanisms that may be responsible for stimulating different cytokine secretion programs and inducing different T cell subsets. Initial gene profiling of bone marrow-derived DC populations from naïve CBA and BL/6 mice revealed profound differences in innate pattern recognition receptor expression between these strains. Of particular interest were the C-type lectin receptors,

specifically the murine homologues of human DC-specific ICAM-3-grabbing non-integrin (DC-SIGN, CD209) which was shown to bind schistosome-derived glycans [100, 101]. Current efforts focus on elucidating potential innate receptors that recognize egg components and precipitate signaling cascades resulting in proinflammatory cytokine production and severe disease.

Recent data from other groups have significantly broadened the scope of Th17 cell involvement in schistosomiasis. In BL/6 mice infected with *S. japonicum* the natural hepatic egg-induced granulomatous inflammation is considerably more pronounced than in *S. mansoni* infection and correlates with increased expression of IL-17, IL-6, TGF- α , IL-23p19, IL-1 β , TNF- α , CXCL1 and CXCL2 [102]. Moreover, neutralization of IL-17 in vivo resulted in significant amelioration of pathology, diminished lesional neutrophil recruitment, and a reduction in proinflammatory cytokines [102]. Most significantly, flow cytometry analysis has shown that IL-17 and ROR γ t expression by CD4⁺ T cells, as well as IL-17/IL-10 and ROR γ t/Foxp3 ratios, are significantly increased in peripheral blood mononuclear cells (PBMC) from *S. hematobium*-infected Senegalese patients with bladder pathology in comparison with uninfected individuals or infected patients without detectable bladder pathology. Interestingly, when comparing *S. mansoni*-infected CBA and BL/6 mice, the characteristic disparity in the magnitude of immunopathology directly correlates with flow cytometric analysis of IL-17 expression in CD4 T cells isolated from PBMC, spleen and hepatic granuloma cells, suggesting that the PBMC profile seen in humans is representative of that prevailing in lymphoid and target organs [103].

Regulation of the immune response and immunopathology in schistosomiasis

Studies aimed at elucidating the complex immunopathogenic host response in schistosomiasis run parallel to, and are intimately interwoven with, those examining the regulatory mechanisms that prevent or oppose the development of excessive disease. Over the past three to four decades, many immunoregulatory pathways put forward in general immunology have been studied in the context of schistosomiasis. However, since these pathways were described before the discovery of Th17 cells, their direct impact on this T cell subset has not been formally documented and can only be inferred.

Treg cells and AAM are cell types that can exert a powerful regulatory function in numerous biological systems [104–108]. Together with Th2 cells, Treg cells and AAM represent key players in the regulation of excessive schistosome-induced pathology [109–111], and it is likely that these cells make use of some common mechanisms to dampen inflammation in the chronic phase of infection (immunomodulation) as well as in mouse strains that naturally develop mild immunopathology. In particular, IL-4-dependent AAM are indispensable for the development of a Th2 response and for averting severe disease and death [49]. We have observed the expression of AAM markers, including the chitinase-related molecule Ym1, Relm α , and arginase, and the Treg lineage-specific transcription factor Foxp3 to inversely correlate with hepatic egg-induced immunopathology and IL-17 levels [112]. Similarly, these cell markers were decreased in infected SEA/CFA immunized T-bet^{−/−} mice displaying exacerbated immunopathology and high levels of IL-17 [89]. Whether in conjunction with or independent of Treg cells and AAM, IL-10 represents a powerful regulatory force during schistosome infection as demonstrated by its capacity to curtail immunopathology when administered in vivo, as well as by the marked exacerbation of disease that results from its absence [54–56]. Interestingly, co-production of IL-10 by Th17 cells induced with IL-6 and TGF- α completely abrogated their pathogenicity in a model of central nervous system inflammation [113]. Formerly considered a Th2 cytokine, IL-10 is now known to be produced by all CD4 T cell subsets and by other lymphoid and non-

lymphoid cells [114, 115]. One caveat is that the inhibitory role of IL-10 in schistosomiasis has been assessed and well documented in the BL/6 strain and that in CBA mice its ample presence does not necessarily result in reduced pathology. It is possible that genetic polymorphisms might affect IL-10 or IL-10R structure and signaling, and thus appropriate function; however, the failure to regulate high pathology in some strains likely extends beyond the biology of IL-10.

Of increasing importance among the regulatory mechanisms of the immune response are coinhibitory systems mediating inhibition of T cell activation and proinflammatory cytokine production. Programmed death 1 (PD-1), largely expressed on T cells, and its ligands PD-L1, widely expressed on lymphoid and non-lymphoid cells, and PD-L2, expressed mainly on DC and macrophages, represent one such coinhibitory system that has been demonstrated to regulate autoimmune and pathogen-induced inflammation [116]. In schistosomiasis, increased PD-L2 expression on CD11c+ DC was associated with decreased morbidity [117]. Additional studies are necessary to more thoroughly explore the role of the PD-1/PD-L and other coinhibitory systems on the outcome and possible control of immunopathology in schistosomiasis.

The low prevalence of autoimmune diseases in populations inhabiting tropical regions of the world has been attributed to the coexistence of helminthic infections and has led to the formulation of the hygiene hypothesis [118–120]. Indeed, down-modulation of disease severity by helminth coinfection in various models of experimental autoimmunity has lent abundant credence to this hypothesis [121–125]. Given the many mechanistic similarities between the immunopathology of T cell-mediated autoimmune diseases and schistosomiasis, we assessed the impact of a coinfection with the murine gastrointestinal parasitic nematode helminth *Heligmosomoides polygyrus* on the severe schistosome-induced pathology characteristic of CBA mice and SEA/CFA-immunized BL/6 mice. In both instances, there was significantly reduced immunopathology in the co-infected mice, which was accompanied by decreased MLNC and granuloma cell production of IL-17, IFN- γ and TNF- α and increased production of IL-4, IL-5, IL-10, and TGF- β [112]. How *H. polygyrus* manages to switch schistosome-infected mice from a Th17/Th1-polarized response with severe pathology to a predominantly Th2 response with mild pathology still remains to be fully understood, but may depend on its ability to stimulate Foxp3 expression and regulatory capabilities in naïve CD4 T cells by way of excretory/secretory products [126] or to induce AAM differentiation [49]. In support of the latter possibility, expression of the AAM markers Ym1 and Relm α was significantly increased in the livers of *H. polygyrus* and *S. mansoni* co-infected mice compared to mice infected with *S. mansoni* alone [112]. Yet another significant way that intestinal nematodes such as *H. polygyrus* could influence the immunopathology of schistosomiasis is by virtue of their ability to alter the composition of the gut microbiota [127]. Recent studies have demonstrated a connection between specific microbial communities and the development of immune responses and immunopathology [128–130] both locally in the intestine and systemically, including models of Th17-dependent autoimmune disease such as EAE [131, 132]. Further evidence linking microbes with the outcome of T cell subset development and pathology in schistosomiasis comes from two observations. The first is the previously mentioned stimulation of Th17 cell development and exacerbation of egg-induced inflammation in infected BL/6 mice immunized with an emulsion of SEA in CFA (which contains killed mycobacteria) and the second is the ability of BL/6 DC co-pulsed with SEA and heat-killed *Propionibacterium acnes* to switch induction of SEA-specific T cell cytokine production from a Th2 (IL-5/IL-13) to a Th17 (IL-17) phenotype [133]. In both cases engagement of, and subsequent signaling by, innate pattern recognition receptors by microbial products likely accounts for the altered responses. Certainly, in both instances the results were obtained from experimental setups but the findings likely reflect real-life situations such as

the perforation of the intestine by schistosome eggs, potentially facilitating the entry of bacteria into the circulation. The important point to be made is that gut microbial communities, which are determined by a multiplicity of factors including habitat, diet, host genetic background and co-existence of parasitic nematodes, can significantly influence pathogenic immune responses and thus can up- or down-regulate the severity of the schistosome infection both in experimental systems as well as naturally in humans.

Genetic control of murine schistosomiasis

The marked heterogeneity of immune response and immunopathology in humans and among inbred mouse strains following infection with schistosomes has prompted considerable efforts to map the genetic intervals responsible for governing such disparate traits. Despite formidable obstacles afforded by genetic polymorphisms of humans and schistosomes alike, by uneven parasitic loads, and by intercurrent infections and/or other medical and social factors, quantitative trait loci (QTL) analysis succeeded in identifying genomic intervals associated with disease susceptibility or pathology. Work by Dessein and colleagues has identified two major loci associated with human disease: the first, designated *SM1*, was identified in a Brazilian population and linked to intensity of infection, while the second, designated *SM2*, was identified in a Sudanese population and linked to severe hepatic fibrosis [134, 135]. These loci were found to be independent of one another, supporting earlier evidence that infection levels do not necessarily correspond to disease severity. Interestingly, these data suggest that anti-infection and anti-disease immunity are under distinct major gene control, which may have a significant clinical impact in the future, particularly as the risk of schistosome resistance to praziquantel increases.

Notwithstanding this early work and subsequent major advances in genome sequencing and analysis, it remains particularly difficult to identify polymorphic genes associated with complex diseases in humans. Fortunately, in *S. mansoni* infection, the murine disease closely mimics that in the human, making it an ideal model for genetic study. We used multiple crosses of genetically diverse mice to identify relevant loci governing differences in immune response and immunopathology (Figure 3). Of great interest was the observation that in all the examined crosses between high and low-pathology strains, low pathology was dominant in the F1 progenies, whereas in the F2 generations it predictably ranged from very low to very high.

An initial F2 analysis between high-pathology CBA and low-pathology BL/6 mice disclosed a linkage between granuloma size and IFN- γ levels in supernatants from SEA-stimulated MLNC and identified several genetic intervals linked to IFN- γ production, including two highly suggestive loci on chromosomes 1 and 5 [136]. These loci were shown to have a significant epistatic interaction and contain several candidate genes, including *Adprt1* and *TLR5*. In addition, a single locus on chromosome 13 had a highly suggestive linkage to granuloma formation; this locus contains several genes of the serpin family of protease inhibitors and has been implicated previously in wound healing and neutrophil infiltration.

To further understand the genetic complexities of the schistosome infection, we performed a second, more comprehensive analysis using a cross between a different strain of mice with particularly severe pathology, SJL, and the low-pathology BL/6 mice (Table 1) [137]. This study provided us with the first opportunity to assess genetic control of the IL-17 response during murine schistosome infection, which had been recently identified as a close correlate and more reliable marker of high pathology [87]. In an (SJL $\times$ BL/6) F2 cohort, there was a highly significant correlation between granuloma size and IL-17, IFN- γ and TNF- α , but not with IL-4, IL-5 and IL-10. QTL analysis identified two loci that were highly significantly linked to granulomatous inflammation, located on chromosomes 4 and 17. The locus on

chromosome 4 contains a number of overlapping QTL for several autoimmune diseases with similar pathogenic mechanisms to schistosomiasis, including EAE and systemic lupus erythematosus (SLE) [138, 139], with several candidate genes implicated in immune functions including the signaling proteins, *lck* and *map3k6*, as well as *IL-22ra1*. The locus on chromosome 17 has also been linked with EAE and SLE and contains additional overlapping QTL with resistance to malaria (*Char3*) and leishmaniasis (*Lmr1*) [140, 141]. The most immunologically relevant candidate genes within this interval are *Tnfa* and *Notch3*, both of which are known to play roles in autoimmune and inflammatory diseases, as well as T cell development in the case of Notch [142]. In addition to granuloma formation, we also identified several loci associated with IL-17 production, among which *D4Mit211* exhibited a highly significant linkage, whereas in 4 additional loci the linkage was suggestive (Figure 3). Interestingly, the loci on chromosomes 3 and 9 contain the genes encoding the Th17-specific transcription factors ROR α and ROR γ , respectively. Potential candidate genes residing within this locus include the runx-like transcription factor *Runx1tl* and *Map3k7* or TAK1.

Since QTLs governing schistosome-induced murine (and human) immunopathology and/or cytokine expression could be directly or indirectly influenced by genes within the MHC, as exemplified by the described locus on chromosome 17 in the SJL $\times$ BL/6 cross [137], we sought to investigate genetic intervals controlling phenotypic differences in a system unbiased by the MHC. C57BL/10 (BL/10) and BL/6 mice share approximately 95% of the genome including the H-2^b haplotype, yet BL/10 mice develop more severe hepatic egg-induced granulomatous inflammation (Table 1). Enhanced pathology seen in parental BL/10 mice, as well as in a fraction of mice from a (BL/10 $\times$ BL/6) F2 cohort, is associated with an increase in IL-17 and IFN- γ produced by SEA-stimulated MLNC. Most interestingly, recent work involving a series of congenic mice containing defined BL/10 segments on a BL/6 background, revealed enhanced pathology to be associated with intervals in chromosome 4 and 13, in locations similar to those previously mapped, respectively, in the above described BL/6 $\times$ SJL and BL/6 $\times$ CBA crosses. Congenic mice with enhanced pathology also expressed higher levels of IL-17, IL-23 and IL-1 β together with lower levels of IL-5 and the AAM markers Relm α and Ym1 (P. M. Smith, T. Sproule, D. Roopenian and M. J. Stadecker, unpublished observation).

The complex genetics of the schistosome infection has made it difficult to discover true candidate genes, despite the number of QTL identified to date. We reasoned this could be due, at least in part, to the limited genetic diversity among classical inbred mouse strains. To overcome this limitation, we analyzed the schistosome infection in wild-derived mouse strains, which diverged from a common ancestor with classically inbred strains over one million years ago and possess a more diverse gene pool, making them more likely to reveal novel mechanisms of immune regulation [143–145]. Interestingly, schistosome-infected wild-derived inbred mice of the MOLF/ei strain develop severe natural perioval granulomatous inflammation together with high levels of IL-17 [146] (Table 1). Using congenic mice on a BL/6 background with a MOLF/ei locus on chromosome 6, we discovered that the high pathology developed by these mice was based on a single gene, IL-1-receptor associated kinase-like 2 (*Irak2*), enhancing T cell-intrinsic sensitivity to IL-1 β -induced activation of the Th17-lineage-specific transcription factors ROR α and basic leucine zipper transcription factor, ATF-like (BATF), and thus of Th17 cell differentiation.

Taken together, we have used different genetic crosses as well as classical and non-classical mouse strains to better understand the complex genetics underlying murine schistosomiasis, and identified a number of loci throughout the genome that are linked to both cytokine production and immunopathology (Figure 3). Some loci were identified repeatedly and had significant epistatic interactions with one or more additional loci, whereas others appear to

function independently and were identified in only one cross, which is indicative of the multiplicity of genes involved in the host immunopathological response to infection with schistosomes. In future work it will be challenging to refine these identified QTL and to discover the underlying genes.

Conclusions

The granulomatous inflammatory pathology in schistosomiasis centers around parasite eggs and is mediated and orchestrated by CD4 T cells specific for egg Ags. Until recently, the markedly dissimilar outcome of disease severity, found both in humans and among mouse strains, was largely attributed to the interplay of pro- vs. anti-inflammatory forces carried out by Th1 vs. Th2 cells. However, the discovery of Th17 cells and their central pathogenic role in T cell-mediated autoimmune diseases prompted a dramatic paradigm shift regarding the role of Th cell subsets in a number of immune and inflammatory responses. Before their discovery, many functions of Th17 cells were attributed to Th1 cells, with which they frequently overlap, but new evidence suggests that in many instances Th1 cells regulate Th17 cells, since pathogenic Th17 cell responses are magnified in their absence. Our investigation of Th17 cells indeed uncovered this subset to be intimately tied to the development of exacerbated egg-induced inflammation in schistosomiasis. Studies in infected gene knock-out mice and in an in vitro system using either normal T cells or Tg T cells bearing a transgenic TCR specific for the major Sm-p40 egg Ag, demonstrated Th17 cell differentiation to be dependent on IL-23 and IL-1 α produced by APC following stimulation with egg-derived products (Figure 2).

Historically schistosome infections have been considered to result in Th2-dominated cytokine polarization. However, in the murine infection, a plain Th2 polarization seems to be the exception applicable only to low-pathology strains such as the BL/6. Rather, most examined strains, including members of the subspecies of wild-derived mice, develop considerably more severe pathology associated with elevated Th17, Th1, as well as Th2 cell responses (Table 1). Genetic analysis disclosed that severe pathology and high IL-17 levels are controlled by several independent or overlapping loci. However, the loci vary among the different crosses between high and low-pathology strains, which is indicative of the complex, multigenic nature of these traits in each case (Figure 3).

Aside from the cognate interaction with egg Ag, a number of factors shape the ensuing schistosome-specific T cell response. Central among these are the innate receptor repertoire and cytokine profile of the APC that activate T cells, as well as the costimulatory and coinhibitory signals affecting APC-T cell interactions. Once established, pathogenic T cell responses must be subjected to regulatory forces, such as those delivered by Treg cells and AAM. Of note also are the effects of coinfection with intestinal helminths in mitigating schistosome egg-induced immunopathology, which in humans may explain the net prevalence of mild clinical disease in areas of coendemicity. Our current research is directed at further elucidating the nature and impact of innate receptors affording the initial recognition of schistosome-derived ligands as well as the mechanisms involved in mediating regulation. In practical terms, these studies are aimed at devising strategies to control damaging inflammation similar to those presently used to manage other immune-mediated diseases.

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References

1. Bica I, Hamer DH, Stadecker MJ. Hepatic schistosomiasis. *Infect Dis Clin North Am.* 2000; 14:583–604. viii. [PubMed: 10987111]
2. Fallon PG. Immunopathology of schistosomiasis: a cautionary tale of mice and men. *Immunol Today.* 2000; 21:29–35. [PubMed: 10637556]
3. Pearce EJ, MacDonald AS. The immunobiology of schistosomiasis. *Nat Rev Immunol.* 2002; 2:499–511. [PubMed: 12094224]
4. Wilson MS, Mentink-Kane MM, Pesce JT, Ramalingam TR, Thompson R, Wynn TA. Immunopathology of schistosomiasis. *Immunol Cell Biol.* 2007; 85:148–54. [PubMed: 17160074]
5. Cheever A, Duvall R, Hallack T Jr, Minker R, Malley J, Malley K. Variation of hepatic fibrosis and granuloma size among mouse strains infected with *Schistosoma mansoni*. *Am J Trop Med Hyg.* 1987; 37:85–97. [PubMed: 3111283]
6. Hernandez HJ, Trzyna WC, Cordingley JS, Brodeur PH, Stadecker MJ. Differential antigen recognition by T cell populations from strains of mice developing polar forms of granulomatous inflammation in response to eggs of *Schistosoma mansoni*. *Eur J Immunol.* 1997; 27:666–70. [PubMed: 9079807]
7. Bosshardt SC, Freeman GL Jr, Secor WE, Colley DG. IL-10 deficit correlates with chronic, hypersplenomegaly syndrome in male CBA/J mice infected with *Schistosoma mansoni*. *Parasite Immunol.* 1997; 19:347–53. [PubMed: 9292893]
8. Rutitzky LI, Bazzone L, Shainheit MG, Joyce-Shaikh B, Cua DJ, Stadecker MJ. IL-23 is required for the development of severe egg-induced immunopathology in schistosomiasis and for lesional expression of IL-17. *J Immunol.* 2008; 180:2486–95. [PubMed: 18250458]
9. Dyer KD, Garcia-Crespo KE, Killoran KE, Rosenberg HF. Antigen profiles for the quantitative assessment of eosinophils in mouse tissues by flow cytometry. *J Immunol Methods.* 2011; 369:91–7. [PubMed: 21565196]
10. Rutitzky LI, Hernandez HJ, Stadecker MJ. Th1-polarizing immunization with egg antigens correlates with severe exacerbation of immunopathology and death in schistosome infection. *Proc Natl Acad Sci USA.* 2001; 98:13243–8. [PubMed: 11606762]
11. Amiri P, Locksley RM, Parslow TG, Sadick M, Rector E, Ritter D, McKerrow JH. Tumour necrosis factor alpha restores granulomas and induces parasite egg-laying in schistosome-infected SCID mice. *Nature.* 1992; 356:604–7. [PubMed: 1560843]
12. Freitas TC, Jung E, Pearce EJ. TGF-beta signaling controls embryo development in the parasitic flatworm *Schistosoma mansoni*. *PLoS Pathog.* 2007; 3:e52. [PubMed: 17411340]
13. Berriman M, Haas BJ, LoVerde PT, Wilson RA, Dillon GP, Cerqueira GC, Mashiyama ST, Al-Lazikani B, Andrade LF, Ashton PD, Aslett MA, Bartholomeu DC, Blandin G, Caffrey CR, Coghlan A, Coulson R, Day TA, Delcher A, DeMarco R, Djikeng A, Eyre T, Gamble JA, Ghedin E, Gu Y, Hertz-Fowler C, Hirai H, Hirai Y, Houston R, Ivens A, Johnston DA, Lacerda D, Macedo CD, McVeigh P, Ning Z, Oliveira G, Overington JP, Parkhill J, Pertea M, Pierce RJ, Protasio AV, Quail MA, Rajandream MA, Rogers J, Sajid M, Salzberg SL, Stanke M, Tivey AR, White O, Williams DL, Wortman J, Wu W, Zamanian M, Zerlotini A, Fraser-Liggett CM, Barrell BG, El-Sayed NM. The genome of the blood fluke *Schistosoma mansoni*. *Nature.* 2009; 460:352–8. [PubMed: 19606141]
14. Cass CL, Johnson JR, Califf LL, Xu T, Hernandez HJ, Stadecker MJ, Yates JR 3rd, Williams DL. Proteomic analysis of *Schistosoma mansoni* egg secretions. *Mol Biochem Parasitol.* 2007; 155:84–93. [PubMed: 17644200]
15. Williams DL, Asahi H, Oke TT, Lopes da Rosa J, Stadecker MJ. Murine immune responses to a novel schistosome egg antigen, SmEP25. *Int J Parasitol.* 2005; 35:875–82. [PubMed: 15970293]
16. Schramm G, Falcone FH, Gronow A, Haisch K, Mamat U, Doenhoff MJ, Oliveira G, Galle J, Dahinden CA, Haas H. Molecular characterization of an interleukin-4-inducing factor from *Schistosoma mansoni* eggs. *J Biol Chem.* 2003; 278:18384–92. [PubMed: 12624091]
17. Steinfelder S, Andersen JF, Cannons JL, Feng CG, Joshi M, Dwyer D, Caspar P, Schwartzberg PL, Sher A, Jankovic D. The major component in schistosome eggs responsible for conditioning

- dendritic cells for Th2 polarization is a T2 ribonuclease (omega-1). *J Exp Med.* 2009; 206:1681–90. [PubMed: 19635859]
18. Everts B, Perona-Wright G, Smits HH, Hokke CH, van der Ham AJ, Fitzsimmons CM, Doenhoff MJ, van der Bosch J, Mohrs K, Haas H, Mohrs M, Yazdanbakhsh M, Schramm G. Omega-1, a glycoprotein secreted by *Schistosoma mansoni* eggs, drives Th2 responses. *J Exp Med.* 2009; 206:1673–80. [PubMed: 19635864]
 19. Cai Y, Langley J, Smith D, Boros D. A cloned major *Schistosoma mansoni* egg antigen with homologies to small heat shock proteins elicits Th1 responsiveness. *Infect Immun.* 1996; 64:1750–5. [PubMed: 8613387]
 20. Nene V, Dunne D, Johnson K, Taylor D, Cordingley J. Sequence and expression of a major egg antigen from *Schistosoma mansoni*. Homologies to heat shock proteins and alpha-crystallins. *Mol Biochem Parasitol.* 1986; 21:179–88. [PubMed: 3097539]
 21. Cao M, Chao H, Doughty B. Cloning of a cDNA encoding an egg antigen homologue from *Schistosoma mansoni*. *Mol Biochem Parasitol.* 1993; 58:169–71. [PubMed: 8459829]
 22. Tiu WU, Davern KM, Wright MD, Board PG, Mitchell GF. Molecular and serological characteristics of the glutathione S-transferases of *Schistosoma japonicum* and *Schistosoma mansoni*. *Parasite Immunol.* 1988; 10:693–706. [PubMed: 3146049]
 23. Asahi H, Osman A, Cook RM, LoVerde PT, Stadecker MJ. *Schistosoma mansoni* phosphoenolpyruvate carboxykinase, a novel egg antigen: immunological properties of the recombinant protein and identification of a T-cell epitope. *Infect Immun.* 2000; 68:3385–93. [PubMed: 10816489]
 24. Alger HM, Sayed AA, Stadecker MJ, Williams DL. Molecular and enzymatic characterisation of *Schistosoma mansoni* thioredoxin. *Int J Parasitol.* 2002; 32:1285–92. [PubMed: 12204228]
 25. Williams DL, Asahi H, Botkin DJ, Stadecker MJ. Schistosome infection stimulates host CD4(+) T helper cell and B-cell responses against a novel egg antigen, thioredoxin peroxidase. *Infect Immun.* 2001; 69:1134–41. [PubMed: 11160011]
 26. Schramm G, Hamilton JV, Balog CI, Wuhler M, Gronow A, Beckmann S, Wippersteg V, Grevelding CG, Goldmann T, Weber E, Brattig NW, Deelder AM, Dunne DW, Hokke CH, Haas H, Doenhoff MJ. Molecular characterisation of kappa-5, a major antigenic glycoprotein from *Schistosoma mansoni* eggs. *Mol Biochem Parasitol.* 2009; 166:4–14. [PubMed: 19428667]
 27. Hokke CH, Deelder AM. Schistosome glycoconjugates in host-parasite interplay. *Glycoconj J.* 2001; 18:573–87. [PubMed: 12376724]
 28. Hokke CH, Yazdanbakhsh M. Schistosome glycans and innate immunity. *Parasite Immunol.* 2005; 27:257–64. [PubMed: 16138846]
 29. Cummings RD, Nyame AK. Schistosome glycoconjugates. *Biochim Biophys Acta.* 1999; 1455:363–74. [PubMed: 10571025]
 30. Wuhler M, Balog CI, Catalina MI, Jones FM, Schramm G, Haas H, Doenhoff MJ, Dunne DW, Deelder AM, Hokke CH. IPSE/alpha-1, a major secretory glycoprotein antigen from schistosome eggs, expresses the Lewis X motif on core-difucosylated N-glycans. *FEBS J.* 2006; 273:2276–92. [PubMed: 16650003]
 31. Shainheit MG, Lasocki KW, Finger E, Larkin BM, Smith PM, Sharpe AH, Dinarello CA, Rutitzky LI, Stadecker MJ. The pathogenic Th17 cell response to major schistosome egg antigen is sequentially dependent on IL-23 and IL-1beta. *J Immunol.* 2011; 187:5328–35. [PubMed: 22003203]
 32. Hernandez HJ, Stadecker MJ. Elucidation and role of critical residues of immunodominant peptide associated with T cell-mediated parasitic disease. *J Immunol.* 1999; 163:3877–82. [PubMed: 10490987]
 33. Finger E, Brodeur PH, Hernandez HJ, Stadecker MJ. Expansion of CD4 T cells expressing a highly restricted TCR structure specific for a single parasite epitope correlates with high pathology in murine schistosomiasis. *Eur J Immunol.* 2005; 35:2659–69. [PubMed: 16106372]
 34. Eberl M, Langermans JA, Vervenne RA, Nyame AK, Cummings RD, Thomas AW, Coulson PS, Wilson RA. Antibodies to glycans dominate the host response to schistosome larvae and eggs: is their role protective or subversive? *J Infect Dis.* 2001; 183:1238–47. [PubMed: 11262206]

35. Dunne DW, Butterworth AE, Fulford AJC, Curtis Kariuki H, Langley JG, Ouma JH, Capron A, Pierce RJ, Sturrock RF. Immunity after treatment of human schistosomiasis: association between IgE antibodies to adult worm antigens and resistance to reinfection. *European Journal of Immunology*. 1992; 22:1483–94. [PubMed: 1601036]
36. King CL, Xianli J, Malhotra I, Liu S, Mahmoud AA, Oettgen HC. Mice with a targeted deletion of the IgE gene have increased worm burdens and reduced granulomatous inflammation following primary infection with *Schistosoma mansoni*. *The Journal of Immunology*. 1997; 158:294–300. [PubMed: 8977202]
37. Hernandez HJ, Wang Y, Stadecker MJ. In infection with *Schistosoma mansoni*, B cells are required for T helper type 2 cell responses but not for granuloma formation. *J Immunol*. 1997; 158:4832–7. [PubMed: 9144498]
38. Zaretsky AG, Taylor JJ, King IL, Marshall FA, Mohrs M, Pearce EJ. T follicular helper cells differentiate from Th2 cells in response to helminth antigens. *J Exp Med*. 2009; 206:991–9. [PubMed: 19380637]
39. Phillips SM, DiConza JJ, Gold JA, Reid WA. Schistosomiasis in the congenitally athymic (nude) mouse. I. Thymic dependency of eosinophilia, granuloma formation, and host morbidity. *J Immunol*. 1977; 118:594–9. [PubMed: 839071]
40. Iacomini J, Ricklan D, Stadecker M. T cells expressing the $\gamma\delta$ T cell receptor are not required for egg granuloma formation in schistosomiasis. *Eur J Immunol*. 1995; 25:884–8. [PubMed: 7737289]
41. Hernandez HJ, Wang Y, Tzellas N, Stadecker MJ. Expression of class II, but not class I, major histocompatibility complex molecules is required for granuloma formation in infection with *Schistosoma mansoni*. *Eur J Immunol*. 1997; 27:1170–6. [PubMed: 9174607]
42. Karanja DM, Colley DG, Nahlen BL, Ouma JH, Secor WE. Studies on schistosomiasis in western Kenya: I. Evidence for immune-facilitated excretion of schistosome eggs from patients with *Schistosoma mansoni* and human immunodeficiency virus coinfections. *Am J Trop Med Hyg*. 1997; 56:515–21. [PubMed: 9180601]
43. Mathew RC, Boros DL. Anti-L3T4 antibody treatment suppresses hepatic granuloma formation and abrogates antigen-induced interleukin-2 production in *Schistosoma mansoni* infection. *Infect Immun*. 1986; 54:820–6. [PubMed: 3096893]
44. Pearce E, Caspar P, Grzych J, Lewis F, Sher A. Downregulation of Th1 cytokine production accompanies induction of Th2 responses by a parasitic helminth, *Schistosoma mansoni*. *Journal of Experimental Medicine*. 1991; 173:159–66. [PubMed: 1824635]
45. MacDonald AS, Patton EA, La Flamme AC, Araujo MI, Huxtable CR, Bauman B, Pearce EJ. Impaired Th2 development and increased mortality during *Schistosoma mansoni* infection in the absence of CD40/CD154 interaction. *J Immunol*. 2002; 168:4643–9. [PubMed: 11971013]
46. Hernandez HJ, Sharpe AH, Stadecker MJ. Experimental murine schistosomiasis in the absence of B7 costimulatory molecules: reversal of elicited T cell cytokine profile and partial inhibition of egg granuloma formation. *J Immunol*. 1999; 162:2884–9. [PubMed: 10072537]
47. Rutitzky LI, Ozkaynak E, Rottman JB, Stadecker MJ. Disruption of the ICOS-B7RP-1 costimulatory pathway leads to enhanced hepatic immunopathology and increased gamma interferon production by CD4 T cells in murine schistosomiasis. *Infect Immun*. 2003; 71:4040–4. [PubMed: 12819093]
48. Brunet LR, Finkelman FD, Cheever AW, Kopf MA, Pearce EJ. IL-4 protects against TNF-alpha-mediated cachexia and death during acute schistosomiasis. *J Immunol*. 1997; 159:777–85. [PubMed: 9218595]
49. Herbert DR, Holscher C, Mohrs M, Arendse B, Schwegmann A, Radwanska M, Leeto M, Kirsch R, Hall P, Mossman H, Claussen B, Forster I, Brombacher F. Alternative macrophage activation is essential for survival during schistosomiasis and downmodulates T helper 1 responses and immunopathology. *Immunity*. 2004; 20:623–35. [PubMed: 15142530]
50. Chiamonte MG, Donaldson DD, Cheever AW, Wynn TA. An IL-13 inhibitor blocks the development of hepatic fibrosis during a T-helper type 2-dominated inflammatory response. *J Clin Invest*. 1999; 104:777–85. [PubMed: 10491413]

51. Fallon PG, Richardson EJ, McKenzie GJ, McKenzie AN. Schistosome infection of transgenic mice defines distinct and contrasting pathogenic roles for IL-4 and IL-13: IL-13 is a profibrotic agent. *J Immunol.* 2000; 164:2585–91. [PubMed: 10679097]
52. Araujo MI, de Jesus AR, Bacellar O, Sabin E, Pearce E, Carvalho EM. Evidence of a T helper type 2 activation in human schistosomiasis. *Eur J Immunol.* 1996; 26:1399–403. [PubMed: 8647223]
53. Joseph AL, Boros DL. Tumor necrosis factor plays a role in *Schistosoma mansoni* egg-induced granulomatous inflammation. *J Immunol.* 1993; 151:5461–71. [PubMed: 8228238]
54. Flores-Villanueva PO, Zheng XX, Strom TB, Stadecker MJ. Recombinant IL-10 and IL-10/Fc treatment down-regulate egg antigen- specific delayed hypersensitivity reactions and egg granuloma formation in schistosomiasis. *J Immunol.* 1996; 156:3315–20. [PubMed: 8617955]
55. Hoffmann KF, Cheever AW, Wynn TA. IL-10 and the dangers of immune polarization: excessive type 1 and type 2 cytokine responses induce distinct forms of lethal immunopathology in murine schistosomiasis. *J Immunol.* 2000; 164:6406–16. [PubMed: 10843696]
56. Sadler CH, Rutitzky LI, Stadecker MJ, Wilson RA. IL-10 is crucial for the transition from acute to chronic disease state during infection of mice with *Schistosoma mansoni*. *Eur J Immunol.* 2003; 33:880–8. [PubMed: 12672053]
57. Stadecker MJ, Asahi H, Finger E, Hernandez HJ, Rutitzky LI, Sun J. The immunobiology of Th1 polarization in high-pathology schistosomiasis. *Immunol Rev.* 2004; 201:168–79. [PubMed: 15361240]
58. Rutitzky LI, Mirkin GA, Stadecker MJ. Apoptosis by neglect of CD4+ Th cells in granulomas: a novel effector mechanism involved in the control of egg-induced immunopathology in murine schistosomiasis. *J Immunol.* 2003; 171:1859–67. [PubMed: 12902487]
59. Stadecker MJ, Kamisato JK, Chikunguwo SM. Induction of T helper cell unresponsiveness to antigen by macrophages from schistosomal egg granulomas. A basis for immunomodulation in schistosomiasis? *J Immunol.* 1990; 145:2697–700. [PubMed: 2145363]
60. Colley DG, Montesano MA, Freeman GL, Secor WE. Infection-stimulated or perinatally initiated idiotypic interactions can direct differential morbidity and mortality in schistosomiasis. *Microbes Infect.* 1999; 1:517–24. [PubMed: 10603568]
61. Taylor JJ, Krawczyk CM, Mohrs M, Pearce EJ. Th2 cell hyporesponsiveness during chronic murine schistosomiasis is cell intrinsic and linked to GRAIL expression. *J Clin Invest.* 2009; 119:1019–28. [PubMed: 19258704]
62. Mossmann T, Coffman R. TH-1 and TH-2 cells: different patterns of lymphokine secretion lead to different properties. *Ann Rev Immunol.* 1989; 7:145–73. [PubMed: 2523712]
63. Dong C. Diversification of T-helper-cell lineages: finding the family root of IL-17-producing cells. *Nat Rev Immunol.* 2006; 6:329–33. [PubMed: 16557264]
64. Stockinger B, Veldhoen M. Differentiation and function of Th17 T cells. *Curr Opin Immunol.* 2007; 19:281–6. [PubMed: 17433650]
65. Bettelli E, Korn T, Kuchroo VK. Th17: the third member of the effector T cell trilogy. *Curr Opin Immunol.* 2007; 19:652–7. [PubMed: 17766098]
66. Iwakura Y, Nakae S, Saijo S, Ishigame H. The roles of IL-17A in inflammatory immune responses and host defense against pathogens. *Immunol Rev.* 2008; 226:57–79. [PubMed: 19161416]
67. McGeachy MJ, Cua DJ. Th17 cell differentiation: the long and winding road. *Immunity.* 2008; 28:445–53. [PubMed: 18400187]
68. Mills KH. Induction, function and regulation of IL-17-producing T cells. *Eur J Immunol.* 2008; 38:2636–49. [PubMed: 18958872]
69. Kolls JK, Linden A. Interleukin-17 family members and inflammation. *Immunity.* 2004; 21:467–76. [PubMed: 15485625]
70. Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang YH, Wang Y, Hood L, Zhu Z, Tian Q, Dong C. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat Immunol.* 2005; 6:1133–41. [PubMed: 16200068]
71. Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, Weaver CT. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol.* 2005; 6:1123–32. [PubMed: 16200070]

72. Cua DJ, Sherlock J, Chen Y, Murphy CA, Joyce B, Seymour B, Lucian L, To W, Kwan S, Churakova T, Zurawski S, Wiekowski M, Lira SA, Gorman D, Kastelein RA, Sedgwick JD. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature*. 2003; 421:744–8. [PubMed: 12610626]
73. Murphy CA, Langrish CL, Chen Y, Blumenschein W, McClanahan T, Kastelein RA, Sedgwick JD, Cua DJ. Divergent pro- and antiinflammatory roles for IL-23 and IL-12 in joint autoimmune inflammation. *J Exp Med*. 2003; 198:1951–7. [PubMed: 14662908]
74. Nakae S, Nambu A, Sudo K, Iwakura Y. Suppression of immune induction of collagen-induced arthritis in IL-17-deficient mice. *J Immunol*. 2003; 171:6173–7. [PubMed: 14634133]
75. Sonderegger I, Rohn TA, Kurrer MO, Iezzi G, Zou Y, Kastelein RA, Bachmann MF, Kopf M. Neutralization of IL-17 by active vaccination inhibits IL-23-dependent autoimmune myocarditis. *Eur J Immunol*. 2006; 36:2849–56. [PubMed: 17039570]
76. Yen D, Cheung J, Scheerens H, Poulet F, McClanahan T, McKenzie B, Kleinschek MA, Owyang A, Mattson J, Blumenschein W, Murphy E, Sathe M, Cua DJ, Kastelein RA, Rennick D. IL-23 is essential for T cell-mediated colitis and promotes inflammation via IL-17 and IL-6. *J Clin Invest*. 2006; 116:1310–6. [PubMed: 16670770]
77. Ye P, Garvey PB, Zhang P, Nelson S, Bagby G, Summer WR, Schwarzenberger P, Shellito JE, Kolls JK. Interleukin-17 and lung host defense against *Klebsiella pneumoniae* infection. *Am J Respir Cell Mol Biol*. 2001; 25:335–40. [PubMed: 11588011]
78. Happel KI, Dubin PJ, Zheng M, Ghilardi N, Lockhart C, Quinton LJ, Odden AR, Shellito JE, Bagby GJ, Nelson S, Kolls JK. Divergent roles of IL-23 and IL-12 in host defense against *Klebsiella pneumoniae*. *J Exp Med*. 2005; 202:761–9. [PubMed: 16157683]
79. Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, Hatton RD, Wahl SM, Schoeb TR, Weaver CT. Transforming growth factor-beta induces development of the T(H)17 lineage. *Nature*. 2006; 441:231–4. [PubMed: 16648837]
80. Huang W, Na L, Fidel PL, Schwarzenberger P. Requirement of interleukin-17A for systemic anti-*Candida albicans* host defense in mice. *J Infect Dis*. 2004; 190:624–31. [PubMed: 15243941]
81. Saijo S, Ikeda S, Yamabe K, Kakuta S, Ishigame H, Akitsu A, Fujikado N, Kusaka T, Kubo S, Chung SH, Komatsu R, Miura N, Adachi Y, Ohno N, Shibuya K, Yamamoto N, Kawakami K, Yamasaki S, Saito T, Akira S, Iwakura Y. Dectin-2 recognition of alpha-mannans and induction of Th17 cell differentiation is essential for host defense against *Candida albicans*. *Immunity*. 2010; 32:681–91. [PubMed: 20493731]
82. Korn T, Bettelli E, Gao W, Awasthi A, Jager A, Strom TB, Oukka M, Kuchroo VK. IL-21 initiates an alternative pathway to induce proinflammatory T(H)17 cells. *Nature*. 2007; 448:484–7. [PubMed: 17581588]
83. Zhou L, Ivanov II, Spolski R, Min R, Shenderov K, Egawa T, Levy DE, Leonard WJ, Littman DR. IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. *Nat Immunol*. 2007; 8:967–74. [PubMed: 17581537]
84. Matsuki T, Nakae S, Sudo K, Horai R, Iwakura Y. Abnormal T cell activation caused by the imbalance of the IL-1/IL-1R antagonist system is responsible for the development of experimental autoimmune encephalomyelitis. *Int Immunol*. 2006; 18:399–407. [PubMed: 16415102]
85. Sutton C, Brereton C, Keogh B, Mills KH, Lavelle EC. A crucial role for interleukin (IL)-1 in the induction of IL-17-producing T cells that mediate autoimmune encephalomyelitis. *J Exp Med*. 2006; 203:1685–91. [PubMed: 16818675]
86. Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, Cua DJ, Littman DR. The orphan nuclear receptor ROR γ directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell*. 2006; 126:1121–33. [PubMed: 16990136]
87. Rutitzky LI, Lopes da Rosa JR, Stadecker MJ. Severe CD4 T cell-mediated immunopathology in murine schistosomiasis is dependent on IL-12p40 and correlates with high levels of IL-17. *J Immunol*. 2005; 175:3920–6. [PubMed: 16148138]
88. Shainheit MG, Saraceno R, Bazzone LE, Rutitzky LI, Stadecker MJ. Disruption of interleukin-27 signaling results in impaired gamma interferon production but does not significantly affect immunopathology in murine schistosome infection. *Infect Immun*. 2007; 75:3169–77. [PubMed: 17403877]

89. Rutitzky LI, Smith PM, Stadecker MJ. T-bet protects against exacerbation of schistosome egg-induced immunopathology by regulating Th17-mediated inflammation. *Eur J Immunol.* 2009; 39:2470–81. [PubMed: 19714576]
90. Rutitzky LI, Stadecker MJ. Exacerbated egg-induced immunopathology in murine *Schistosoma mansoni* infection is primarily mediated by IL-17 and restrained by IFN-gamma. *Eur J Immunol.* 2011; 41:2677–87. [PubMed: 21660933]
91. Shainheit MG, Smith PM, Bazzone LE, Wang AC, Rutitzky LI, Stadecker MJ. Dendritic cell IL-23 and IL-1 production in response to schistosome eggs induces Th17 cells in a mouse strain prone to severe immunopathology. *J Immunol.* 2008; 181:8559–67. [PubMed: 19050275]
92. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, Weiner HL, Kuchroo VK. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature.* 2006; 441:235–8. [PubMed: 16648838]
93. Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity.* 2006; 24:179–89. [PubMed: 16473830]
94. Acosta-Rodriguez EV, Napolitani G, Lanzavecchia A, Sallusto F. Interleukins 1beta and 6 but not transforming growth factor-beta are essential for the differentiation of interleukin 17-producing human T helper cells. *Nat Immunol.* 2007; 8:942–9. [PubMed: 17676045]
95. van Beelen AJ, Zelinkova Z, Taanman-Kueter EW, Muller FJ, Hommes DW, Zaat SA, Kapsenberg ML, de Jong EC. Stimulation of the intracellular bacterial sensor NOD2 programs dendritic cells to promote interleukin-17 production in human memory T cells. *Immunity.* 2007; 27:660–9. [PubMed: 17919942]
96. Ghoreschi K, Laurence A, Yang XP, Tato CM, McGeachy MJ, Konkel JE, Ramos HL, Wei L, Davidson TS, Bouladoux N, Grainger JR, Chen Q, Kanno Y, Watford WT, Sun HW, Eberl G, Shevach EM, Belkaid Y, Cua DJ, Chen W, O'Shea JJ. Generation of pathogenic T(H)17 cells in the absence of TGF-beta signalling. *Nature.* 2010; 467:967–71. [PubMed: 20962846]
97. Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. *Annu Rev Immunol.* 2009; 27:485–517. [PubMed: 19132915]
98. Sacks D, Noben-Trauth N. The immunology of susceptibility and resistance to *Leishmania major* in mice. *Nature Reviews Immunology.* 2002; 2:845–58.
99. Locksley RM, Heinzel FP, Sadick MD, Holaday BJ, Gardner KD Jr. Murine cutaneous leishmaniasis: susceptibility correlates with differential expansion of helper T-cell subsets. *Ann Inst Pasteur Immunol.* 1987; 138:744–9.
100. van Die I, van Vliet SJ, Nyame AK, Cummings RD, Bank CM, Appelmelk B, Geijtenbeek TB, van Kooyk Y. The dendritic cell-specific C-type lectin DC-SIGN is a receptor for *Schistosoma mansoni* egg antigens and recognizes the glycan antigen Lewis x. *Glycobiology.* 2003; 13:471–8. [PubMed: 12626400]
101. van Liempt E, van Vliet SJ, Engering A, Garcia Vallejo JJ, Bank CM, Sanchez-Hernandez M, van Kooyk Y, van Die I. *Schistosoma mansoni* soluble egg antigens are internalized by human dendritic cells through multiple C-type lectins and suppress TLR-induced dendritic cell activation. *Mol Immunol.* 2007; 44:2605–15. [PubMed: 17241663]
102. Zhang Y, Chen L, Gao W, Hou X, Gu Y, Gui L, Huang D, Liu M, Ren C, Wang S, Shen J. IL-17 neutralization significantly ameliorates hepatic granulomatous inflammation and liver damage in *Schistosoma japonicum* infected mice. *Eur J Immunol.* 2012; 42:1523–35. [PubMed: 22678906]
103. Mbow M, Larkin BM, Meurs L, Wammes LJ, de Jong SE, Labuda LA, Camara M, Hermelijn H Smits, Polman K, Dieye TN, Mboup S, Stadecker MJ, Yazdanbakhsh M. Th17 cells are associated with pathology in human schistosomiasis. *The Journal of Infectious Diseases.* 2012
104. Josefowicz SZ, Lu LF, Rudensky AY. Regulatory T cells: mechanisms of differentiation and function. *Annu Rev Immunol.* 2012; 30:531–64. [PubMed: 22224781]
105. Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. *Cell.* 2008; 133:775–87. [PubMed: 18510923]
106. Goerdts S, Orfanos CE. Other functions, other genes: alternative activation of antigen-presenting cells. *Immunity.* 1999; 10:137–42. [PubMed: 10072066]

107. Anthony RM, Rutitzky LI, Urban JF Jr, Stadecker MJ, Gause WC. Protective immune mechanisms in helminth infection. *Nat Rev Immunol.* 2007; 7:975–87. [PubMed: 18007680]
108. Nair MG, Cochrane DW, Allen JE. Macrophages in chronic type 2 inflammation have a novel phenotype characterized by the abundant expression of Ym1 and Fizz1 that can be partly replicated in vitro. *Immunol Lett.* 2003; 85:173–80. [PubMed: 12527225]
109. Hesse M, Piccirillo CA, Belkaid Y, Prufer J, Mentink-Kane M, Leusink M, Cheever AW, Shevach EM, Wynn TA. The pathogenesis of schistosomiasis is controlled by cooperating IL-10-producing innate effector and regulatory T cells. *J Immunol.* 2004; 172:3157–66. [PubMed: 14978122]
110. McKee AS, Pearce EJ. CD25+CD4+ cells contribute to Th2 polarization during helminth infection by suppressing Th1 response development. *J Immunol.* 2004; 173:1224–31. [PubMed: 15240714]
111. Baumgart M, Tompkins F, Leng J, Hesse M. Naturally occurring CD4+Foxp3+ regulatory T cells are an essential, IL-10-independent part of the immunoregulatory network in *Schistosoma mansoni* egg-induced inflammation. *J Immunol.* 2006; 176:5374–87. [PubMed: 16622005]
112. Bazzzone LE, Smith PM, Rutitzky LI, Shainheit MG, Urban JF, Setiawan T, Blum AM, Weinstock JV, Stadecker MJ. Coinfection with the intestinal nematode *Heligmosomoides polygyrus* markedly reduces hepatic egg-induced immunopathology and proinflammatory cytokines in mouse models of severe schistosomiasis. *Infect Immun.* 2008; 76:5164–72. [PubMed: 18710859]
113. McGeachy MJ, Bak-Jensen KS, Chen Y, Tato CM, Blumenschein W, McClanahan T, Cua DJ. TGF-beta and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain T(H)-17 cell-mediated pathology. *Nat Immunol.* 2007; 8:1390–7. [PubMed: 17994024]
114. Ouyang W, Rutz S, Crellin NK, Valdez PA, Hymowitz SG. Regulation and functions of the IL-10 family of cytokines in inflammation and disease. *Annu Rev Immunol.* 2011; 29:71–109. [PubMed: 21166540]
115. Couper KN, Blount DG, Riley EM. IL-10: the master regulator of immunity to infection. *J Immunol.* 2008; 180:5771–7. [PubMed: 18424693]
116. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. *Annu Rev Immunol.* 2008; 26:677–704. [PubMed: 18173375]
117. Colley DG, Sasser LE, Reed AM. PD-L2+ dendritic cells and PD-1+ CD4+ T cells in schistosomiasis correlate with morbidity. *Parasite Immunol.* 2005; 27:45–53. [PubMed: 15813722]
118. Maizels RM, Yazdanbakhsh M. T-cell regulation in helminth parasite infections: implications for inflammatory diseases. *Chem Immunol Allergy.* 2008; 94:112–23. [PubMed: 18802342]
119. Jackson JA, Friberg IM, Little S, Bradley JE. Review series on helminths, immune modulation and the hygiene hypothesis: immunity against helminths and immunological phenomena in modern human populations: coevolutionary legacies? *Immunology.* 2009; 126:18–27. [PubMed: 19120495]
120. Maizels RM. Infections and allergy - helminths, hygiene and host immune regulation. *Curr Opin Immunol.* 2005; 17:656–61. [PubMed: 16202576]
121. Wilson MS, Taylor MD, O’Gorman MT, Balic A, Barr TA, Filbey K, Anderton SM, Maizels RM. Helminth-induced CD19+CD23hi B cells modulate experimental allergic and autoimmune inflammation. *Eur J Immunol.* 2010; 40:1682–96. [PubMed: 20306466]
122. Hubner MP, Shi Y, Torrero MN, Mueller E, Larson D, Soloviova K, Gondorf F, Hoerauf A, Killoran KE, Stocker JT, Davies SJ, Tarbell KV, Mitre E. Helminth protection against autoimmune diabetes in nonobese diabetic mice is independent of a type 2 immune shift and requires TGF-beta. *J Immunol.* 2012; 188:559–68. [PubMed: 22174447]
123. La Flamme AC, Ruddenklau K, Backstrom BT. Schistosomiasis decreases central nervous system inflammation and alters the progression of experimental autoimmune encephalomyelitis. *Infect Immun.* 2003; 71:4996–5004. [PubMed: 12933842]
124. Khan WI, Blennerhasset PA, Varghese AK, Chowdhury SK, Omsted P, Deng Y, Collins SM. Intestinal nematode infection ameliorates experimental colitis in mice. *Infect Immun.* 2002; 70:5931–7. [PubMed: 12379667]

125. Elliott DE, Metwali A, Leung J, Setiawan T, Blum AM, Ince MN, Bazzone LE, Stadecker MJ, Urban JF Jr, Weinstock JV. Colonization with *Heligmosomoides polygyrus* suppresses mucosal IL-17 production. *J Immunol.* 2008; 181:2414–9. [PubMed: 18684931]
126. Grainger JR, Smith KA, Hewitson JP, McSorley HJ, Harcus Y, Filbey KJ, Finney CA, Greenwood EJ, Knox DP, Wilson MS, Belkaid Y, Rudensky AY, Maizels RM. Helminth secretions induce de novo T cell Foxp3 expression and regulatory function through the TGF-beta pathway. *J Exp Med.* 2010; 207:2331–41. [PubMed: 20876311]
127. Walk ST, Blum AM, Ewing SA, Weinstock JV, Young VB. Alteration of the murine gut microbiota during infection with the parasitic helminth *Heligmosomoides polygyrus*. *Inflamm Bowel Dis.* 2010; 16:1841–9. [PubMed: 20848461]
128. Gaboriau-Routhiau V, Rakotobe S, Lecuyer E, Mulder I, Lan A, Bridonneau C, Rochet V, Pisi A, De Paepe M, Brandi G, Eberl G, Snel J, Kelly D, Cerf-Bensussan N. The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. *Immunity.* 2009; 31:677–89. [PubMed: 19833089]
129. Ivanov II, Frutos Rde L, Manel N, Yoshinaga K, Rifkin DB, Sartor RB, Finlay BB, Littman DR. Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. *Cell Host Microbe.* 2008; 4:337–49. [PubMed: 18854238]
130. Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, Wei D, Goldfarb KC, Santee CA, Lynch SV, Tanoue T, Imaoka A, Itoh K, Takeda K, Umesaki Y, Honda K, Littman DR. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell.* 2009; 139:485–98. [PubMed: 19836068]
131. Ochoa-Reparaz J, Mielcarz DW, Ditrio LE, Burroughs AR, Foureau DM, Haque-Begum S, Kasper LH. Role of gut commensal microflora in the development of experimental autoimmune encephalomyelitis. *J Immunol.* 2009; 183:6041–50. [PubMed: 19841183]
132. Lee YK, Menezes JS, Umesaki Y, Mazmanian SK. Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci U S A.* 2011; 108(Suppl 1):4615–22. [PubMed: 20660719]
133. Perona-Wright G, Lundie RJ, Jenkins SJ, Webb LM, Grecis RK, MacDonald AS. Concurrent bacterial stimulation alters the function of helminth-activated dendritic cells, resulting in IL-17 induction. *J Immunol.* 2012; 188:2350–8. [PubMed: 22287718]
134. Dessein AJ, Hillaire D, Elwali NE, Marquet S, Mohamed-Ali Q, Mirghani A, Henri S, Abdelhameed AA, Saeed OK, Magzoub MM, Abel L. Severe hepatic fibrosis in *Schistosoma mansoni* infection is controlled by a major locus that is closely linked to the interferon-gamma receptor gene. *Am J Hum Genet.* 1999; 65:709–21. [PubMed: 10441577]
135. Dessein AJ, Marquet S, Henri S, El Wali NE, Hillaire D, Rodrigues V, Prata A, Ali QM, Gharib B, de Reggi M, Magzoub MM, Saeed OK, Abdelhameed AA, Abel L. Infection and disease in human schistosomiasis mansoni are under distinct major gene control. *Microbes Infect.* 1999; 1:561–7. [PubMed: 10603573]
136. Rutitzky LI, Hernandez HJ, Yim YS, Ricklan DE, Finger E, Mohan C, Peter I, Wakeland EK, Stadecker MJ. Enhanced egg-induced immunopathology correlates with high IFN-gamma in murine schistosomiasis: identification of two epistatic genetic intervals. *J Immunol.* 2005; 174:435–40. [PubMed: 15611268]
137. Smith PM, Shainheit MG, Bazzone LE, Rutitzky LI, Poltorak A, Stadecker MJ. Genetic control of severe egg-induced immunopathology and IL-17 production in murine schistosomiasis. *J Immunol.* 2009; 183:3317–23. [PubMed: 19675160]
138. Teuscher C, Doerge RW, Fillmore PD, Blankenhorn EP. eae36, a locus on mouse chromosome 4, controls susceptibility to experimental allergic encephalomyelitis in older mice and mice immunized in the winter. *Genetics.* 2006; 172:1147–53. [PubMed: 16299394]
139. Morel L, Tian XH, Croker BP, Wakeland EK. Epistatic modifiers of autoimmunity in a murine model of lupus nephritis. *Immunity.* 1999; 11:131–9. [PubMed: 10485648]
140. Roberts LJ, Baldwin TM, Curtis JM, Handman E, Foote SJ. Resistance to *Leishmania major* is linked to the H2 region on chromosome 17 and to chromosome 9. *J Exp Med.* 1997; 185:1705–10. [PubMed: 9151907]

141. Fortin A, Stevenson MM, Gros P. Complex genetic control of susceptibility to malaria in mice. *Genes Immun.* 2002; 3:177–86. [PubMed: 12058252]
142. Jurynczyk M, Jurewicz A, Raine CS, Selmaj K. Notch3 inhibition in myelin-reactive T cells down-regulates protein kinase C theta and attenuates experimental autoimmune encephalomyelitis. *J Immunol.* 2008; 180:2634–40. [PubMed: 18250475]
143. Frazer KA, Eskin E, Kang HM, Bogue MA, Hinds DA, Beilharz EJ, Gupta RV, Montgomery J, Morenzoni MM, Nilsen GB, Pethiyagoda CL, Stuve LL, Johnson FM, Daly MJ, Wade CM, Cox DR. A sequence-based variation map of 8.27 million SNPs in inbred mouse strains. *Nature.* 2007; 448:1050–3. [PubMed: 17660834]
144. Yang H, Bell TA, Churchill GA, Pardo-Manuel de Villena F. On the subspecific origin of the laboratory mouse. *Nat Genet.* 2007; 39:1100–7. [PubMed: 17660819]
145. Guenet JL, Bonhomme F. Wild mice: an ever-increasing contribution to a popular mammalian model. *Trends Genet.* 2003; 19:24–31. [PubMed: 12493245]
146. Smith PM, Jacque B, Conner JR, Poltorak A, Stadercker MJ. IRAK-2 regulates IL-1-mediated pathogenic Th17 cell development in helminthic infection. *PLoS Pathog.* 2011; 7:e1002272. [PubMed: 21998578]

Figure 1. Gross and histological aspects of severe and mild hepatic immunopathology in murine schistosomiasis

Mice were infected i.p. with 80 cercariae of *S. mansoni*, Puerto Rico strain, and livers were examined after a 7-week infection. Top panels: Gross aspect of (A) severe immunopathology in a group of four mice, represented by large, often confluent egg granulomas. (B) Mild immunopathology, characterized by significantly smaller lesions. Bottom panels: The histology of (C) severe immunopathology consists of large, poorly circumscribed granulomas composed of mono- and polynuclear inflammatory cells aggregating around central schistosome eggs (white arrows). The poor circumscription is due to considerable inflammatory cell spillage into the hepatic parenchyma. In (D) mild immunopathology, the perioval granulomas are smaller and more sharply circumscribed. Hematoxylin-eosin stains, magnification 100x.

Figure 2. Development of CD4 T cell subsets in severe and mild forms of murine schistosomiasis
 In the case of severe immunopathology (top panel), egg Ag binding to pattern recognition receptors (PRR) on APC, such as dendritic cells (DC) and macrophages, followed by internalization, processing and presentation in context with MHC class II and costimulatory molecules, results in the production of cytokines that activate Ag-specific CD4 Th17, Th1 and Th2 T cells. The activated T cells produce lineage-specific cytokines that create an overall proinflammatory environment leading to vigorous leukocyte recruitment in response to tissue-trapped eggs and thus severe immunopathology. In this setting, macrophages undergo classical activation (CAM). In the case of mild immunopathology (bottom panel), possibly weaker egg Ag binding to fewer or even different PRRs on APC leads to Ag presentation in the context of coinhibitory rather than costimulatory molecules and results in the production of cytokines that induce and activate Ag-specific Th2 and T regulatory (Treg) cells. These T cells produce lineage-specific cytokines that create an overall antiinflammatory environment resulting in diminished leukocyte recruitment and tempered immunopathology. Under these conditions alternatively activated macrophages (AAM) predominate. Most examined mouse strains develop severe immunopathology with Th17 as well as Th1 and Th2 responses to varying degrees (top panel); a plain Th2-polarized response (bottom panel) is an exception that is only observed in low-pathology strains such

as BL/6. PRRs: pattern recognition receptors; TGF- α : transforming growth factor- α ; CSFs: colony stimulating factors; TNF- α : tumor necrosis factor- α .

Figure 3. Loci governing immunopathology and cytokine levels in murine schistosomiasis
 Depicted in the figure are the mouse chromosomes and the location, size and logarithm of odds (LOD) score of quantitative trait loci governing granuloma size (GS) and the levels of different cytokines. Colors correspond to the indicated high $\times$ low-pathology mouse strain crosses in which the loci were identified.

Table 1
Variations in immune response and immunopathology in multiple mouse strains infected with *S. mansoni*

Severity of immunopathology and immune responses typical of several inbred mouse strains at 7 weeks post infection with 80 cercariae of *S. mansoni*. Pathology is a reflection of granuloma size measured by morphometric analysis. Th1, Th17 and Th2 subset representation is an aggregate reflection of the corresponding signature cytokines IFN- γ , IL-17 and IL-4/IL-5 produced by stimulated lymphocytes from infected mice, and measured by ELISA, real time RT-PCR or intracellular staining.

Strain	Pathology	Th1	Th17	Th2
BL/6 (H2 ^b)	+	– to +/-	– to +/-	++
BL/6 + SEA/CFA	++++	+++	+++	– to +/-
CBA/C3H (H2 ^k)	+++	+++	++ to +++	+++
SJL (H2 ^s)	++++	+++	+++	++
Molf (H2 unknown)	++++	+++	+++	++
BL/10 (H2 ^b)	++	++	++	++

Box 1
The TCR Tg mouse specific for the major Sm-p40 egg Ag

Sm-p40	<p>Most abundant egg Ag</p> <p>Third most abundant secreted egg Ag</p> <p>354 aa; homology to α-crystallins, small heat shock proteins</p> <p>Immunodominant epitope: peptide 234–246 (PKSDNQIKAVPAS)</p>
Tg T cells	<p>Specificity: Sm-p40_{234–246}</p> <p>TCR usage: Vα11.3Vβ8</p> <p>MHC class II restriction: I-A^k</p> <p>Default T cell response to eggs, SEA, Sm-p40, Sm-p40_{234–246}; Th1/Th17</p> <p>Capable of mediating pathology in adoptive transfer</p>
Tg mouse	<p>CBA (H2^k)</p> <p>> 95% of T cells express Vβ8</p>