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Strongyloides infection in rodents: immune response and immune regulation

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SUMMARY

The human pathogenic nematode *Strongyloides stercoralis* infects approximately 30–100 million people worldwide. Analysis of the adaptive immune response to *S. stercoralis* beyond descriptive studies is challenging, as no murine model for the complete infection cycle is available. However, the combined employment of different models each capable of modelling some features of *S. stercoralis* life cycle and pathology has advanced our understanding of the immunological mechanisms involved in host defence. Here we review: (i) studies using *S. stercoralis* third stage larvae implanted in diffusion chambers in the subcutaneous tissue of mice that allow analysis of the immune response to the human pathogenic *Strongyloides* species; (ii) studies using *Strongyloides ratti and Strongyloides venezuelensis* that infect mice and rats to extend the analysis to the parasites intestinal life stage and (iii) studies using *S. stercoralis* infected gerbils to analyse the hyperinfection syndrome, a severe complication of human strongyloidiasis that is not induced by rodent specific *Strongyloides* spp. We provide an overview of the information accumulated so far showing that *Strongyloides* spp. elicits a classical Th2 response that culminates in different, site specific, effector functions leading to either entrapment and killing of larvae in the tissues or expulsion of parasitic adults from the intestine.

INTRODUCTION

Strongyloides sp., like all nematode infections, present unique challenges to the protective immune response. The *Strongyloides* sp. life cycle is complex, with multiple stages including infective larvae (L3i), parthenogenetic female adult worms, first stage larvae (L1) and autoinfective larvae (L3a), in the definitive host (Viney and Lok, 2015). There is a wide range in the sizes of these stages, all of which are far beyond the capacity of cells to phagocytize. L3 and parasitic adults display both stage specific and shared antigens as potential targets for the immune response (Soblik *et al.* 2011) The various stages reside in specific locations in the host and it is clear that the protective immune response must function differently to expel adult worms from the intestine as compared with killing larvae in the tissues. Therefore, it is hypothesized that a different protective immune response, in terms of specificity and mechanism, is required to control each of the life stages.

Another challenge for the study of protective immune responses during human strongyloidiasis is that the species infecting humans, *Strongyloides stercoralis*, only infects humans, primates, dogs and gerbils. This host range provides limited opportunities for performing hypothesis-based research. Therefore, several alternative approaches have been utilized to study the development and mechanisms of protective immunity to the various life stages of *Strongyloides* sp.

Although it was determined that *S. stercoralis* does not establish patent infections in immune competent mice (Dawkins and Grove, 1982*a*) it was established that the L3i and L3a of *S. stercoralis* could be recovered from mice if they were implanted within diffusion chambers (Abraham *et al.* 1995). After skin penetration, the L3 migrate throughout the body thus making accurate recovery of the parasites and analysis of the parasite microenvironment difficult. Diffusion chambers contain the parasites *in vivo* in the subcutaneous tissues, a natural habitat for the larvae. Analysis of the contents of the implanted diffusion chamber allows an accurate assessment of parasite survival in mice and provides a unique view of the innate and adaptive immune factors present in the parasite microenvironment. Use of the mouse/diffusion chamber model is limited to the study of larval stages of *S. stercoralis* and to the single environment in which the diffusion chamber was implanted. It does provide the opportunity of studying protective immunity to the species of *Strongyloides* that infects humans and in a host for which there are the tools required to dissect the precise mechanism of parasite control by the immune response.

Species of *Strongyloides* also infect rodents and these models provide the opportunity of studying

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the immune response in a natural host-parasite relationship including the mechanisms of eradication of parasitic adults from the intestine. A significant difference in the life cycle between *S. stercoralis*, the human pathogen, and *Strongyloides ratti* and *Strongyloides venezuelensis*, the rodent pathogens, is that hyperinfection and extremely chronic infections that are hallmarks of *S. stercoralis* infection in humans are absent in *S. ratti* and *S. venezuelensis* infections. Still, the majority of mouse strains tested, including BALB/c and C57BL/6 mice are fully susceptible to patent infection with *S. ratti* (Dawkins *et al*. 1980) and *S. venezuelensis* (Sato and Toma, 1990). C57BL/6 mice are more susceptible to infection with both *S. ratti* and *S. venezuelensis* as compared to BALB/c mice. *Strongyloides venezuelensis* L3 migrate predominantly via the lung (Takamure, 1995) while *S. ratti* L3 are present in lungs, cerebrospinal fluid and heads of infected mice (Dawkins and Grove, 1981*a*; Dawkins *et al*. 1982*b*; Eschbach *et al*. 2010). Both *Strongyloides* species have similar timing for the migratory path. L3 are detected in lung and head tissue of infected mice from day 1 to day 3 post infection (p.i.) and maximal L3 numbers are recorded at day 2 p.i. L3 arrive in the small intestine from day 3 p.i. onwards and molt within the intestine to the L4 (day 4 p.i.) and to female adults (day 5 p.i.) (Dawkins and Grove, 1981*a*) that reproduce by parthenogenesis (Viney and Lok, 2015). Either eggs (*S. venezuelensis*), or predominantly hatched L1 (*S. ratti*) are released with the feces by day 5 p.i. Adult *S. ratti* and *S. venezuelensis* reside in the mucosa of the small intestine. Light and electron microscopic studies suggest that they may move along the intestine creating tunnels between intestinal epithelial cells but never penetrate the basement lamina or enter the lamina propria below (Dawkins *et al*. 1983).

Immune competent mice terminate primary *S. ratti* and *S. venezuelensis* infections with similar kinetics within a month (Dawkins and Grove, 1981*a*; Sato and Toma, 1990). The numbers of *S. ratti* and *S. venezuelensis* adults decline rapidly after day 6–7 p.i., and adults are almost undetectable after day 10 p.i. Egg and larval excretion within the feces, assessed by microscopic analysis, is below detection limit by day 14 p.i., while the more sensitive detection of *Strongyloides* spp. DNA by quantitative polymerase chain reaction (qPCR) in the feces shows ongoing *S. ratti* infection in C57BL/6 (Eschbach *et al*. 2010) and BALB/c (Blankenhaus *et al*. 2011) mice until 4 weeks p.i.

A resolved primary infection with *S. ratti* and *S. venezuelensis* induced partial resistance to a second infection suggesting the establishment of an adaptive immune response (Dawkins *et al*. 1980; Dawkins and Grove, 1981*a*; Sato and Toma, 1990). Primary infections of mice with as few as 6 *S. ratti* L3i, reduced the larval output during a second infection by 97% (Dawkins and Grove, 1982*b*). Immunity was mediated by both cellular and humoral effectors, as protection could be transferred from immune mice to naïve mice by serum or mesenteric lymph node cells (Dawkins and Grove, 1981*b*). If immunity was induced by a primary subcutaneous (s.c.) infection with *S. ratti* or *S. venezuelensis* L3i, the number of migrating L3 in the tissue was reduced in the secondary infection, suggesting that predominantly the tissue migrating L3 was the target of the adaptive immune response (Dawkins and Grove, 1981*a*; Sato and Toma, 1990). However, if the primary infection was initiated by oral transfer of *S. ratti* adults, thus bypassing the tissue migration phase of L3, the number of migrating L3 in the second infection was not reduced. Importantly, oral infection of mice with *S. ratti* adults reduced the number of adults in the intestine and the release of eggs in the feces from a secondary infection. This observation suggests that there is an intestinal immune response that acts against the adults of *S. ratti* that functions independently of the L3 specific immune effectors elicited by presence of the tissue migrating L3 (Grove and Northern, 1989).

In summary, the protective immune response to infection with *Strongyloides* spp. should be studied through two separate lenses, one focused on tissue migrating larvae and the other on the intestine dwelling adults. It is acknowledged that the two responses will have many shared characteristics, especially in the induction phase of the immune response; however, the effector mechanisms will differ radically based on the targets and their locations. Analysis of control of tissue migrating larvae, including L3i and L3a, will be the focus of the section on *S. stercoralis* and analysis of elimination of adult parasites from the intestine will be the focus of the section on *S. ratti* and *S. venezuelensis*.

CONTROL AND ERADICATION OF MIGRATING L3 IN THE TISSUE – *S. STERCORALIS*

Innate immunity

Innate immunity in mice to larval *S. stercoralis* is highly efficient, with parasite elimination occurring within 5–7 days p.i. (Abraham *et al*. 1995). Analysis of the parasite microenvironment, within the implanted diffusion chamber, revealed the presence of infiltrated eosinophils, neutrophils and macrophages. If parasites were implanted in mice in diffusion chambers that prevented cell ingress, parasite killing did not occur. This suggested that contact between cells and the worms was required for parasite killing and that soluble factors from the host were insufficient to kill the worms (Abraham *et al*. 1995). Elimination of neutrophils and eosinophils from naïve mice by monoclonal

antibody treatment resulted in an increase in parasite survival (O'Connell *et al*. 2011*a*), suggesting that these cells are active participants in the protective innate immune response. Similarly, *S. venezuelensis* induced an increase of eosinophils and mononuclear cells in the blood, peritoneal cavity fluid and bronchoalveolar lavage fluid (Machado *et al*. 2007, 2010). Studies with *S. ratti* have revealed that cellular infiltrates, consisting of neutrophils, eosinophils and macrophages, were observed in the skin in response to the invading larvae in rats and mice during both primary and secondary responses (Dawkins *et al*. 1981; McHugh *et al*. 1989) and that granulocytes are crucial in the early defence against migrating larvae of *S. ratti* in mice (Watanabe *et al*. 2000).

Eosinophils. Eosinophils are directly recruited to larval *S. stercoralis* without the need for other host cell assistance. Chemoattractants derived from the larvae stimulate the same receptors and second messenger signals to induce eosinophil chemotaxis as used by host derived chemokines. A soluble parasite extract stimulates multiple receptors on the eosinophil surface, including CCR3, CXCR2 and CXCR4, and multiple factors from the parasite recruit eosinophils including both proteins and chitin. The redundancy of the chemotactic factors produced by the parasite and the multiple responding receptors on the eosinophils suggests that chemotactic receptors on eosinophils may have evolved to ensure a robust protective response to this infection (Stein *et al*. 2009). Alternatively, the parasite may recruit eosinophils for its own benefit as has been reported with other nematodes (Gebreselassie *et al*. 2012; Huang *et al*. 2014).

A direct role for eosinophils in killing larvae was suggested by the observation that survival of *S. stercoralis* was increased in naïve mice deficient in IL-5 and survival of the larvae was diminished in mice overexpressing IL-5 (Herbert *et al*. 2000). Similarly, treatment of mice with a monoclonal antibody to IL-5 reduced eosinophils in mice and concomitantly reduced the capacity to control the larvae of *S. ratti* in primary infections (Watanabe *et al*. 2003). Treatment of naïve mice with an anti-CCR3 monoclonal antibody specifically eliminated eosinophils and blocked innate protective immunity to infection with *S. stercoralis* (Galioto *et al*. 2006; O'Connell *et al*. 2011*a*). Surprisingly, larval killing was not diminished in naïve PHIL mice, that constitutively lack eosinophils. Treatment of PHIL mice with a monoclonal antibody to eliminate neutrophils resulted in a diminished protective innate immune response, indicating that in the complete absence of eosinophils, neutrophils were capable of controlling the infection (O'Connell *et al*. 2011*a*). Therefore, both eosinophils and neutrophils can kill the larvae in naïve mice (Galioto *et al*. 2006) and it was inferred that neutrophils are sufficient to compensate for the loss of eosinophils and kill the larvae in mice with a genetic deficiency in eosinophils. Eosinophil killing of the larvae was shown to be dependent on the granular protein major basic protein (MBP) and not eosinophil peroxidase (EPO) (O'Connell *et al*. 2011*a*). MBP, EPO, eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (EDN), purified from human eosinophils, were tested *in vitro* for their toxicity to the larvae of *S. stercoralis*. Only MBP and ECP were toxic to the host adapted larvae ($L3^{+}$), while survival of infective larvae remained unaffected (Rotman *et al*. 1996).

Neutrophils. Proof that neutrophils have the capacity to kill the larvae of S. *stercoralis* was obtained from studies in which purified neutrophils were implanted with larvae within diffusion chambers and implanted in mice. Neutrophils can kill the larvae when implanted in naïve mice (Galioto *et al*. 2006) through a process that is dependent on the neutrophil specific granular protein myeloperoxidase (MPO) (O'Connell *et al*. 2011*a*). If neutrophil recruitment to the parasites in mice was blocked, either because of a defect in Gαi2 signalling (Padigel *et al*. 2007*b*) or in the expression of CXCR2 (Galioto *et al*. 2006), the capacity of mice to kill *S. stercoralis* larvae was significantly decreased. CXCR2 dependent recruitment of neutrophils to larvae occurs independently of IL-17 and molecules extracted from *S. stercoralis* are capable of directly recruiting neutrophils through CXCR2, using signalling pathways similar to those used by host chemokines. In addition, the *S. stercoralis* soluble extract also induced neutrophils to release the chemokines MIP-2 and KC, which further enhanced the recruitment of neutrophils. The finding that neutrophils produce increased amounts of neutrophil-recruiting chemokines following exposure to *S. stercoralis* soluble extract suggests an efficiently orchestrated system whereby a primary stimulus from a parasite causes an autocrine amplification of cell recruitment through release of host-derived chemokines. The efficiency of this recruitment strategy is further highlighted by the observation that the CXCR2 receptor has the ability to respond to both parasite- and host-derived factors resulting in highly efficient neutrophil recruitment and control of infection with *S. stercoralis* (O'Connell *et al*. 2011*b*).

One of the challenges to the effector cells of the innate immune response is achieving contact with their target. Although it has been shown that both eosinophils and neutrophils undergo chemotaxis to the soluble worm products, it does not explain how the cells come into contact with moving worms. It has been reported that the *S. stercoralis* larvae migrate through tissue at the rate of $10\ \mathrm{cm\ h^{-1}}$ (Napier, 1949) whereas neutrophils migrate

through tissue at a rate of 0·06 cm h^{-1} (Chtanova *et al*. 2008; Peters *et al*. 2008; Bruns *et al*. 2010). Extracellular traps, a fibrous network of nuclear DNA released from neutrophils after a cell death process called etosis, have been associated with control of several pathogens (Kruger *et al*. 2015). Mouse neutrophils were induced to release NET's, *in vitro* and *in vivo*, after exposure to live *S. stercoralis* larvae. Worms were seen trapped within the NET's, preventing their movement, yet the parasites were not killed by entrapment within the DNA snare (Bonne-Annee *et al*. 2014). It was hypothesized that the DNA trap restricted the movement of the larvae thereby allowing effector cells to migrate to the immobilized worm to kill it.

Complement. In addition to neutrophils and eosinophils, complement activation is required for innate protective immunity to larval *S. stercoralis* in mice. Complement component C3 was detected on the surface of the larvae (Brigandi *et al*. 1996) and eosinophils only killed the larvae if a source of complement was provided (O'Connell *et al*. 2011*a*). Mice deficient in C3 did not kill the parasite whereas mice deficient in C3a receptor did eliminate the worms, suggesting that C3b is the active component of C3 that is required to mediate larval killing (Kerepesi *et al*. 2006). C3b may function in the larval killing process as an adherence molecule for cells, or it may facilitate activation and degranulation of the cells. Live *S. stercoralis* larvae activated complement *in vitro* through both the classical and alternative pathways which promoted the adherence of human monocytes and neutrophils to the surface of *S. stercoralis* (de Messias *et al*. 1994). Complement also promotes the binding of cells to the larvae of *S. ratti* (Grove *et al*. 1985).

Antigen presenting cells (APC). The transition from the innate to the adaptive immune response requires the parasite to be: (1) killed, (2) dissociated into a phagocytosable form and (3) presented by APC to T cells. Eosinophils are efficiently and independently recruited to the parasite (Stein *et al*. 2009) where they have the capacity to kill the larvae through the release of MBP (O'Connell *et al*. 2011*a*), which leads to the complete disintegration of the larvae. Interestingly, eosinophils also have the capacity to act as APC inducing parasite specific Th2 responses, including IL-4 and IL-5, and both IgM and IgG antibody responses in both primary and secondary infections of mice with *S. stercoralis* (Padigel *et al*. 2006, 2007*a*). Therefore, eosinophils have the capacity to migrate to the parasite microenvironment, kill the parasite and then present the antigens to naïve T cells to induce adaptive immunity to the infection.

Innate immunity – summary. The proposed sequence of events leading to protective innate immunity to larval *S. stercoralis* starts with neutrophils coming into contact with live worms as the worms migrate through the tissues. This contact induces the neutrophils to die through the process of etosis thereby releasing DNA that traps the larvae and prevents their movement. Cells, including neutrophils and eosinophils, undergo chemotaxis to the trapped worms and upon contact release toxic molecules that kill the parasite in collaboration with complement component C3b. Finally, eosinophils have the added capability of acting as APC to initiate the adaptive immune response to the parasite (Fig. 1).

Adaptive immunity

The adaptive immune response in mice, induced by immunization with live larvae, is highly effective with greater than 90% of the challenge larvae killed within 24 h (Abraham *et al*. 1995). Several components have been identified that are integral to the protective adaptive immune response.

T cells. Attempts to infect mice with *S. stercoralis* have revealed that immunologically intact mice and mice deficient in T cells killed the infections within several days (Dawkins and Grove, 1982*a*) However, SCID mice, which have a deficiency in both T and B cells, could be infected with *S. stercoralis*, with low numbers of adult worms and first stage larvae developing in the mice after infection (Rotman *et al*. 1995). This indicates that *S. stercoralis* can develop in mice and that lymphocytes are part of the immune response involved in eliminating the infection. Protective adaptive immunity to *S. stercoralis* larvae in mice requires $CD4^+$ but not $CD8^+$ T cells (Rotman *et al*. 1997). Immunized mice treated with recombinant IL-12 demonstrated a pronounced shift from a Th2 to a Th1 response that blocked mice from developing protective adaptive immunity. Depletion of the Th2 associated cytokines IL-4 or IL-5 from immunized mice using monoclonal antibodies impaired larval killing (Rotman *et al*. 1997). Therefore, $CD4^+$ T cells, and in particular Th2 cells, are required for the protective adaptive immune response.

B cells and Antibody. Protective innate immunity in μMT mice, that lack mature B cells, was sufficient to eliminate all parasites, whereas immunized μMT mice with no detectable antibody did not kill challenge infections in the adaptive immune response. Xid mice, which lack B-1 cells, developed a modest level of parasite specific IgG with little IgM following immunization and did not kill worms in the adaptive immune response. These studies demonstrates that B cells, and specifically B-1 cells, are required for adaptive immunity, but not innate immunity, to *S. stercoralis* and suggest that IgM is

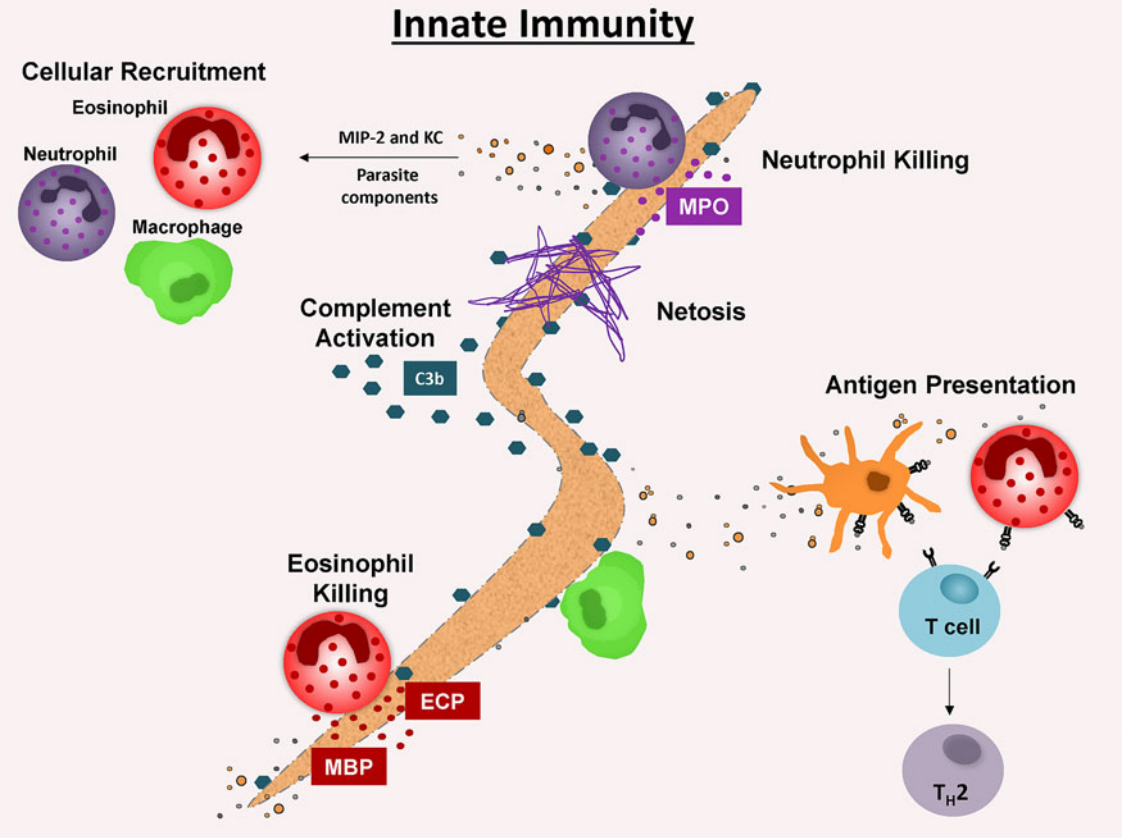

Fig. 1. Innate immunity to *Strongyloides stercoralis* L3 in mice. Neutrophil recruitment to the L3 microenvironment is parasite specific and results in neutrophil production of additional chemokines MIP-2 and KC. Eosinophils are also preferentially recruited by parasite components and are required for the innate but not adaptive immune response. Eosinophil granular proteins, human and mouse MBP and human ECP are toxic to L3. Eosinophils also act as APC, presenting parasitic antigens, resulting in the induction of Th2 cells. Neutrophils die by etosis and release NETs to trap larvae. Neutrophils utilize MPO to kill L3 during the innate and adaptive immune responses. L3 killing by granulocytes is C3 dependent. C3b potentiates adherence of effector cells to L3. Abbreviations: APC, antigen presenting cells; ECP, eosinophil cationic protein; MBP, major basic protein; MPO, myeloperoxidase.

required for adaptive immunity (Herbert *et al*. 2002*a*).

Mice immunized with live larvae of *S. stercoralis* have elevated parasite-specific IgA, IgG1 and IgM levels in the serum (Abraham *et al*. 1995). IgM, recovered from mice 1 week after initial immunization, passively transferred protective immunity to naive mice through a mechanism dependent on granulocytes and complement (Brigandi *et al*. 1996). Both IgM and IgG recovered at three and 5 weeks post immunization could passively transfer immunity. IgG requires complement and neutrophils to kill the worms and functions through antibody-dependent cellular cytotoxicity (ADCC) based on studies in the Fc receptor gamma $(FcR\gamma)^{-/-}$ mice. This is in contrast to IgM from mice immunized with live larvae where protective immunity is ADCC-independent. Western blots were performed to determine what antigens the protective IgM and IgG recognized and it was determined that both antibody isotypes recognized some shared antigens, whereas other antigens were recognized independently by either protective IgG or IgM. Furthermore, IgM bound to the surface of the cuticle, basal cuticle-hypodermis, coelomic cavity and glandular oesophagus, whereas the IgG bound only to the basal cuticle-hypodermis and the coelomic cavity (Ligas *et al*. 2003). It therefore was concluded that while IgM and IgG antibodies are both protective against larval *S. stercoralis*, they recognize different antigens and utilize different killing mechanisms. Similarly, a role for antibody and cells in adaptive protective immunity to *S. stercoralis* in jirds has been observed (Nolan *et al*. 1995). IgG is required for antibody-dependent immunity to *S. ratti* (Murrell, 1981), and a correlation was observed between protective immunity to *S. ratti* and IgG in mice and rats (Dawkins and Grove, 1981*b*; Uchikawa *et al*. 1991; Bleay *et al*. 2007). Finally, passive immunization of mice with a IgM monoclonal antibody to HSP60 led to reduced numbers of migrating *S. ratti* larvae in lung and head (Ben Nouir *et al*. 2012).

Complement. The protective adaptive immune response in mice to larval *S. stercoralis* is dependent on

complement activation. In the initial studies, immunized mice treated with Cobra venom factor to deplete C3, were shown to be unable to kill the larvae (Brigandi *et al*. 1996). The necessity of complement was confirmed in immunized $C3^{-/-}$mice, where larval killing also did not occur. C3a receptor$^{-/-}$ mice killed larvae during the adaptive immune response as efficiently as wild-type mice thereby suggesting that C3b is the active complement component (Kerepesi *et al*. 2006). C3 has been observed to be on the surface of larvae recovered from immunized mice (Brigandi *et al*. 1996), and possibly serves as an anchor for cells to attach to the larvae in order to mediate killing of the parasite, as has been seen with human complement and cells in response to *S. stercoralis* (de Messias *et al*. 1994). Thus complement activation is an integral component of both protective innate and adaptive immunity to *S. stercoralis* in mice.

Eosinophils. Depleting IL-5 from mice immunized against infection with *S. stercoralis,* either by monoclonal antibody treatment (Rotman *et al*. 1996, 1997) or by genetically knocking out IL-5 (Herbert *et al*. 2000) resulted in decreased numbers of eosinophils and an absence of protective adaptive immunity. However, when eosinophils were specifically absent, either due to elimination by monoclonal antibody treatment (Galioto *et al*. 2006) or the use of PHIL mice which are genetically deficient in eosinophils (O'Connell *et al*. 2011*a*), it was determined that eosinophils were not required as effector cells in the adaptive immune response. Interestingly, immunized IL-5 deficient mice, that had severely reduced numbers of eosinophils, failed to establish protective immunity and had lower levels of parasite-specific IgM (Herbert *et al*. 2000). Reconstitution of immunized IL-5 deficient mice with wild-type eosinophils elevated the parasite-specific IgM levels and the mice were then able to eliminate challenge infections (Herbert *et al*. 2000). Similarly it has been reported that IgM induced by the adjuvant alum is compromised in mice genetically deficient in eosinophils and that transfer of IL-4 expressing eosinophils restored the production of antigen specific IgM (Wang and Weller, 2008), thereby confirming a role for eosinophils in IgM production. Immunized PHIL mice, which have no eosinophils but do have intact cytokine levels, did not have reduced IgM levels (O'Connell *et al*. 2011*a*). The immunized PHIL mice appear to have an alternative source for molecules required for the induction of IgM production that IL-$5^{-/-}$ mice do not have. Therefore, eosinophils function as effector cells in the innate immune response, antigen presenting cells and as sources of cytokines required for IgM production in the adaptive immune response.

Neutrophils. Studies were performed to determine the role of neutrophils in protective adaptive immunity to *S. stercoralis*. Using $CXCR2^{-/-}$ mice it was demonstrated that a reduction in recruitment of neutrophils resulted in significantly reduced adaptive protective immunity. Protective antibody developed in immunized $CXCR2^{-/-}$ mice, thereby demonstrating that neutrophils are not required for the induction of the adaptive protective immune response. Moreover, neutrophils from wild type and $CXCR2^{-/-}$ mice killed the larvae of *S. stercoralis* at the same rate, thus demonstrating that the defect in the $CXCR2^{-/-}$ mice was in recruitment of neutrophils and not in their ability to kill larvae (Galioto *et al*. 2006). Mice deficient in Gαi2 also failed to kill the larvae in a challenge infection with *S. stercoralis* despite developing an antigen-specific Th2 response characterized by increased IL-4, IL-5, IgM and IgG. Neutrophils from $G\alpha i2^{-/-}$ mice were competent in killing larvae; however, immunized $G\alpha i2^{-/-}$ mice had significantly reduced recruitment of neutrophils to the parasite microenvironment, as seen within the diffusion chamber (Padigel *et al*. 2007*b*). These data demonstrate that CXCR2 and Gαi2 are not required for the development of the protective immune responses against *S. stercoralis*; however, they are essential for the recruitment of neutrophils required for killing of larvae.

Toll-like receptors (TLRs) are pattern recognition receptors that recognize pathogen associated molecular patterns on pathogens and alert the immune response to the presence of invading pathogens. C3H/HeJ mice, which have a point mutation in the *Tlr4* gene, were used to determine the role of TLR4 in protective immunity to *S. stercoralis*. TLR4 was not required for killing the larvae during the innate immune response, but was required for killing the parasites during the adaptive immune response. No differences were seen in the T cell responses, antibody responses or cell recruitment to the parasite between wild type and C3H/HeJ mice after immunization. However, it was determined that neutrophils from the C3H/HeJ mice could not participate in killing the worms in the adaptive immune response. The *Tlr4* mutation severely alters the effector function, but not recruitment, of cells to the parasite microenvironment (Kerepesi *et al*. 2007). Finally, as in the innate immune response, neutrophils deficient in MPO had significantly decreased larval killing capacity (O'Connell *et al*. 2011*a*). Therefore, neutrophils require both MPO and TLR4 to kill the larvae of *S. stercoralis* in the adaptive immune response.

Macrophages. Macrophages can be separated into subsets, with classically activated macrophages associated with Th1 responses and alternatively activated macrophages associated with Th2 responses (Murray *et al*. 2014). Infection of mice with the larvae of *S. stercoralis* resulted in the induction of alternatively activated macrophages within the

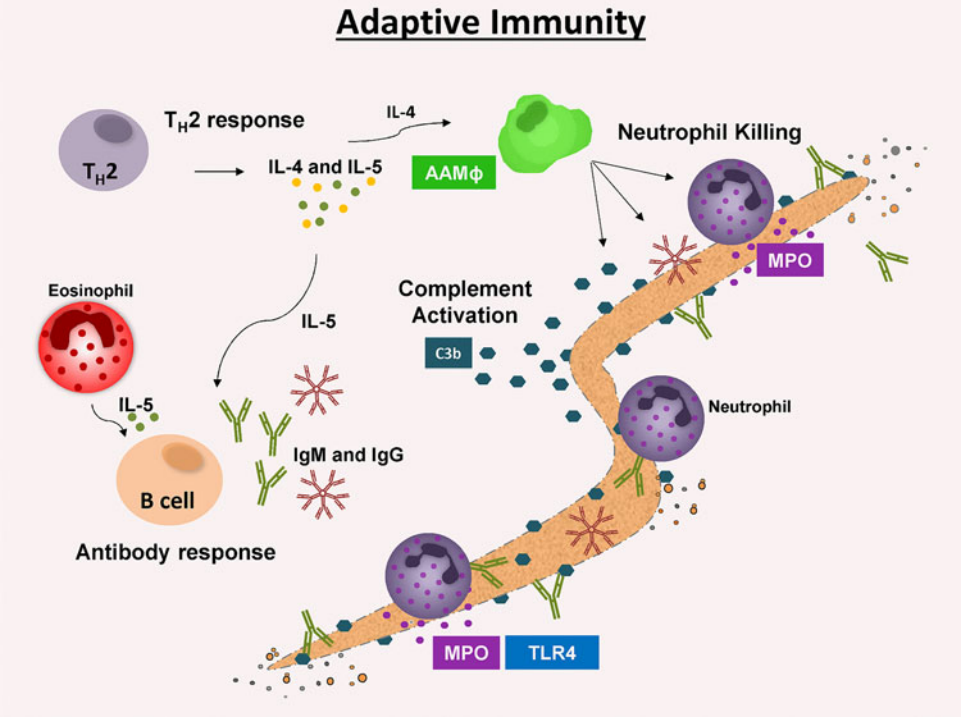

Fig. 2. Adaptive immunity to *Strongyloides stercoralis* L3 in mice. Immunization of mice with L3i results in Th2 cell derived secretion of IL-5 and IL-4. IL-5 is required for eosinophils, which collaborate with B cells to produce parasite specific IgM. IL-4 is required for the development of alternatively activated macrophages that interact with neutrophils, complement and IgM to kill during the adaptive immune response. Accelerated L3 killing by neutrophils during the adaptive response is mediated via MPO and complement component C3 and is IgG dependent. Abbreviation: MPO, myeloperoxidase.

peritoneal cavity. Induction of this subset of macrophages required a Th2 response and specifically the production of IL-4. Alternatively activated macrophages, but not classically activated macrophages, kill the larvae with neutrophils and complement *in vitro*. Using transwell culture plates, it was determined that both neutrophils and macrophages can kill the parasite with soluble help from the reciprocal cell. *In vivo* studies have demonstrated that purified neutrophils implanted with larvae within diffusion chambers can kill the larvae when implanted in naïve mice (Galioto *et al*. 2006). It is hypothesized that the neutrophils killed the worms in the *in vivo* studies with soluble help from macrophages located on the outside of the diffusion chamber. Finally, macrophages kill efficiently in the adaptive immune response in collaboration with parasite-specific IgM (Bonne-Annee *et al*. 2013).

Adaptive immunity – summary. In summary, the adaptive immune response is initiated by eosinophils functioning as APC stimulating $CD4^+$ T cells to produce the Th2 cytokines, IL-4 and IL-5. B cells produce IgM, which collaborates with macrophages, and IgG, which collaborates with neutrophils, to kill the larvae. Eosinophils do not participate in killing the parasites in the adaptive immune response, but are required for inducing the production of IgM. Complement activation, specifically C3b, is required for the accelerated killing in the adaptive immune response. Neutrophils kill through a process dependent on TLR4 and MPO. In addition to the antibody dependent killing, macrophages that have been alternatively activated by IL-4 also kill the parasites as part of a triad composed of macrophages, neutrophils and complement (Fig. 2).

Vaccine development. Studies were performed to identify antigens from *S. stercoralis* that would induce protective immunity and thereby be components of a vaccine against the infection. Mice immunized with soluble antigens derived from *S. stercoralis* larvae, administered with alum as the adjuvant, had a 50% reduction in larval survival. Purified IgG from mice immunized with the soluble antigens passively transferred immunity to naïve mice and was ADCC independent. Immunization of mice with the small pool of antigens specifically recognized by the protective IgG induced a level of parasite killing comparable with live larval immunization (Herbert *et al*. 2002*b*). These studies demonstrated that a limited pool of native antigens, identified by mouse protective antibody, were capable of inducing a high level of protective immunity to *S. stercoralis* in mice.

Passive transfer studies were performed using human serum with antibodies to diagnostic *S. stercoralis* antigens. Protective immunity developed in mice receiving the human serum. Using protective purified

human IgG, seven proteins were recognized in the pool of soluble *S. stercoralis* antigens, but only three were identified in the *S. stercoralis* EST database. The three proteins, tropomyosin (Sstmy-1), Na^+-K^+ATPase (Sseat-6) and LEC-5 (Sslec-5) were constructed into DNA plasmids. Sseat-6 was the only plasmid that induced a limited, but statistically significant, level of protective immunity against the *S. stercoralis* larvae (Kerepesi *et al*. 2005), showing that the DNA encoding a single antigen could induce the development of protective immunity.

Single recombinant purified protein antigens were tested for efficacy as a vaccine against *S. stercoralis*. Ss-EAT-6, Ss-TMY-1 and Ss-LEC-5 were selected as they were recognized by human IgG and there was success with Ss-eat-6 using DNA immunization (Kerepesi *et al*. 2005). In addition, the recombinant diagnostic antigens Ss-NIE-1 and Ss-IR (Krolewiecki *et al*. 2010) were included in the study. Immunization with the recombinant antigens in alum revealed that only immunization with the diagnostic antigen Ss-IR stimulated high and reproducible levels of protective immunity to infection. IgG from mice immunized with Ss-IR could transfer protective immunity and was found to bind to the larval surface and to the granules in the glandular esophagus. Interestingly, this is the same location that the protective human IgG bound to the worms (Kerepesi *et al*. 2004; Abraham *et al*. 2011).

AUTOINFECTION AND HYPERINFECTION

Strongyloides stercoralis infections in humans are extremely long-lived (Robson *et al*. 2009; Prendki *et al*. 2011), through a process of autoinfection, whereby L1 develop in the intestine into small, morphologically distinct infective larvae termed L3a. L3a penetrate the wall of the lower ileum or colon or the skin of the perianal region, enter the circulation again, travel to the lungs as well as other routes, and then to the small intestine, thus repeating the cycle. This maintains the life cycle for decades in the human host with infection levels moderated to limit pathogenicity. However, if infected individuals develop changes in the immune system secondary to diabetes, hematologic malignancies, malnutrition, hypogammaglobulinemia, use of immunosuppressive drugs such as corticosteroids, chronic obstructive pulmonary disease or malignancy, organ transplantation and HTLV-1, there is an increased risk of hyperinfection with *S. stercoralis* (Weatherhead and Mejia, 2014). This results in uncontrolled over-proliferation of larvae with spread to organs, including the lungs, liver and brain. Systemic sepsis is a common complication owing to translocation of enteric bacteria accompanying larval invasion of the gut wall (Greaves *et al*. 2013).

It is interesting that *S. stercoralis* infected SCID mice, lacking T and B cells, do not develop hyperinfection which suggests that control of hyperinfection is not T or B cell dependent in mice (Rotman *et al*. 1995). Treatment of *S. venezuelensis* infected BALB/c mice with the immune suppressive glucocorticoid Dexamethasone resulted in a generalized increase in tissue and intestinal parasite burden and prolongation of infection (Machado *et al*. 2011). However, although limited numbers of larvae were observed in the tissue up to day 37 p.i., this model may not resemble the uncontrolled and often lethal dissemination of large larval numbers observed in the human hyperinfection syndrome. Although mice are not susceptible to infection with *S. stercoralis*, Mongolian gerbils are susceptible, supporting development of all life stages. Treatment of infected gerbils with methylprednisolone acetate resulted in the development of hyperinfection characterized by the presence of large numbers of L3a and the death of the animals (Nolan *et al*. 1993). Neonatally infected gerbils produce a burst of autoinfection that amplifies the adult worm population but eventually control the production of L3a in most cases. This short burst of autoinfection in naive hosts suggests that the production of L3a is downregulated by the host's immune responses and exposure to L3i during the initial infection may prime the host and prevent development of autoinfection under normal circumstances (Nolan *et al*. 1999*a*). To test this hypothesis, parasitic adult worms were orally transmitted to naïve gerbils to determine if L3a would develop in the absence of an immune response directed at the L3i. Oral transfer of parasitic adult worms produced autoinfection in gerbils. If the transplanted adult worms were derived from infected gerbils treated with methylprednisolone acetate, L3a were observed in low numbers from day 5–9. If the adult worms came from untreated gerbils the number of L3a was negligible. Mice, infected orally with the adult worms, did not have any L3a. SCID mice were also infected orally with adult worms and did not develop L3a. This suggests that the development of L3a is not under the control of T and B cells in mice (Nolan *et al*. 1999*b*). Finally, gerbils infected with a large number of L3i developed high numbers of adult worms. L3a were seen in these animals for as long as 7 months and although a mechanism was not defined, it was suggested that the large number of L3a was a reflection of the large numbers of the precursor stage, the L1. Finally it was determined that these gerbils had a strong anti-L3i immune response, while allowing the L3a to remain alive in the tissues (Nolan *et al*. 2002).

The observation in gerbils, suggesting that the immune response that is effective at killing the L3i was not effective against the L3a was tested in mice. Immune responses generated by immunizing mice with live L3i were directed at the host activated, tissue migrating third stage larvae $L3^+$ (Brigandi *et al*. 1998). Combining this observation

with the susceptibility of the $L3^+$ to MBP (Rotman *et al*. 1996), suggests that L3i are resistant to immune attack whereas the $L3^+$ is the susceptible stage. Antigenic differences were seen between the L3i, $L3^+$ and L3a. Immunity generated with L3i and directed at $L3^+$ did not kill the L3a (Brigandi *et al*. 1997). This might explain how infections persist in human hosts for decades. Incoming L3i would be targeted by the adaptive immune response and this immunity would prevent overwhelming infection with the parasite. The L3a would survive in the face of this immune response, thereby perpetuating the parasite within the host. Furthermore, production of L3a is apparently controlled by the immune response, based on their uncontrolled development in immunosuppressed individuals. The net result is that the immune response controls L3i and L3a through different mechanisms which results in infections with *S. stercoralis* persisting for the lifetime of the host, yet causing only minor pathological effects in most cases.

CONTROL AND ERADICATION OF PARASITIC ADULTS FROM THE INTESTINE – *S. RATTI* AND *S. VENEZUELENSIS*

Surprisingly little is known about the nature of effector cells and mechanisms mediating intestinal control and eradication of *Strongyloides* spp. parasites compared with the detailed information regarding eradication of tissue migrating L3 that was reviewed in the section "Control and eradication of migrating L3 in the tissue – *S. stercoralis*". One problem lies in the differentiation of immune mechanisms relevant for control of migrating L3 *vs* adults. Any change in intestinal parasite burden of mice that were infected by s.c. injection of L3i could be the result of changed immunity to tissue migrating L3 alone, changed immunity to the parasitic adult in the intestine alone or a combination of both. Although this issue can be addressed either by performing oral infections thus bypassing the tissue migration phase or by quantification of tissue migrating L3 and adults in the intestine, the differentiation between tissue and intestinal immunity is not always clear and relevant immune effectors certainly overlap.

Innate intestinal effector cells

Several lines of evidence suggest that mucosal mast cells are the central effector cells mediating expulsion of *S. ratti and S. venezuelensis* from the intestine. Mast cells were induced in the small intestine during *S. ratti* and *S. venezuelensis* infection in mice (Khan *et al*. 1993) and rats (Shintoku *et al*. 2013). Mouse mast cell protease 1 (mMCP1), a protease that is specific for mucosal mast cells (Reynolds *et al*. 1990), was elevated in the serum of *S. ratti* and *S. venezuelensis* infected mice (Sasaki *et al*. 2005; Eschbach *et al*. 2010). Similarly rat mast cell protease II was elevated in intestinal tissue of *S. ratti* infected rats (Wilkes *et al*. 2007).

WBB6F1-$Kit^{W/W-v}$ (W/W^v) mice carry a mutation in the stem cell factor (SCF) receptor Kit that results in mast cell deficiency. Release of *S. ratti* (Nawa *et al*. 1985) and *S. venezuelensis* (Khan *et al*. 1993) eggs and L1 in the feces was prolonged in W/W^v mice. Reconstitution with wild type bone marrow cells partially reverted the phenotype suggesting a contribution of mast cells to intestinal immunity. Nawa *et al*. also described increased numbers of L3 in the head of *S. ratti* infected W/W^v mice during first infection (Nawa *et al*. 1985) and numbers of migrating *S. venezuelensis* L3 in W/W^v mice were not recorded (Khan *et al*. 1993). Therefore a formal differentiation between mast cell function in immunity to migrating larvae and parasitic adults in W/W^v mice is missing. Since W/W^v mice suffer from several other haematological and non-haematological deficiencies including a drastic reduction in the number of basophils, it is difficult to definitively attribute their phenotype to mast cell deficiency alone (Reber *et al*. 2012).

Support for a role of intestinal mast cells in expulsion of parasitic adults originates from two studies using Notch 2 and phosphatidylinositol-3 kinase (PI3K) subunit p85α deficient mice. Conditional Notch 2 deficient mice show a changed intestinal mast cell distribution (Sakata-Yanagimoto and Chiba, 2015). Accumulation of mast cells predominantly in the lamina propria instead of the intestinal epithelial layer was associated with increased fecal egg release during *S. venezuelensis* infection (Sakata-Yanagimoto *et al*. 2011). $PI3Kp85\alpha^{/-}$ mice lack gastrointestinal and peritoneal mast cells while still containing dermal mast cells. These mice displayed prolonged fecal release of *S. venezuelensis* eggs and prolonged presence of parasitic adults in the intestine (Fukao *et al*. 2002). Still, as PI3Kp85α deficiency results in impaired Kit signalling that was most likely causing their mast cell deficiency, these mice do not represent a Kit independent mouse model for mast cell deficiency either.

Kit independent mast cell deficient mice were generated recently by heterozygous transgenic expression of Cre recombinase under the control of the mast cell specific carboxypeptidase A3 promoter ($Cpa3^{Cre/wt}$) that led to deletion of mast cells in mucosal and connective tissues via genotoxic mechanisms (Feyerabend *et al*. 2011). $Cpa3^{Cre}$ mice have reduced basophil numbers while other features of the immune system are normal. In line with the former studies using the mixed background WBB6F1-$Kit^{W/W-v}$ mice, mast cell deficient *S. ratti* infected $Cpa3^{Cre/wt}$ mice displayed higher parasite burden and prolonged fecal release of eggs and L1 compared with their mast cell competent $Cpa3^{wt/wt}$ littermates (Blankenhaus *et al*. 2014). Using mice that lack

mucosal and connective tissue mast cells (Feyerabend *et al*. 2011) or selectively connective tissue mast cells (Dudeck *et al*. 2011) or basophils (Ohnmacht *et al*. 2010), we are currently re-evaluating overlapping and separate roles of mast cells and basophils during *S. ratti* infection in mice. Mice lacking either connective tissue mast cells or basophils displayed an initial elevation in intestinal parasite burden but eventually terminated infection with wild type kinetics whereas mice that lacked both connective tissue mast cells and mucosal mast cells were unable to terminate *S. ratti* infection for more than 150 days. Here, immunity to tissue migrating L3 was not affected since differences in parasite burden were restricted to the intestinal life stage of *S. ratti*. These results show that basophils and connective tissue mast cells contribute to early intestinal immunity but are dispensable for final expulsion of parasitic adults whereas mucosal mast cells represent essential terminal effector cells that mediate the final expulsion of *S. ratti* from the intestine (Reitz and Breloer unpublished results).

Eosinophils and neutrophils predominantly mediate immunity to tissue migrating L3 as reviewed in the section "Control and eradication of migrating L3 in the tissue- *S. stercoralis*" and are dispensable for intestinal immunity to *S. ratti* and *S. venezuelensis*. Although eosinophils expanded in the intestinal tissue of *S. ratti* and *S. venezuelensis* infected mice, abrogation of this eosinophilia by anti-IL-5 treatment or in IL-5-deficient mice did not abrogate intestinal immunity (Korenaga *et al*. 1991*a*; Ovington *et al*. 1998; Watanabe *et al*. 2003). Increased eosinophilia in IL-5 transgenic mice did not accelerate termination of infection but reduced numbers of parasitic adults after oral infection (El-Malky *et al*. 2003). However, it should be noted that analysis of intestinal immunity to *S. ratti* and *S. venezuelensis* infection in eosinophil deficient mice has not been performed. Depletion of $Ly6G^+$ neutrophils and $Ly6C^+$ monocytes by anti-Gr1 (RB6–85C) treatment increased numbers of *S. ratti* L3 in the tissue and subsequent intestinal parasite burden (Watanabe *et al*. 2000). If $Gr1^+$ cells were depleted after the completion of the tissue migrating phase i.e. at day 3 p.i., the intestinal parasite burden did not change but fecal egg release increased. Thus, granulocytes are more important in controlling migrating L3 in the tissue as demonstrated for *S. stercoralis* (Galioto *et al*. 2006) than for eradication of adults from the intestine although they may contribute to parasite control by affecting fecundity.

Adaptive immune response

$RAG1^{-/-}$ mice that lack T and B cells displayed comparable numbers of parasitic adults in the intestine at day 6 p.i. (Breloer *et al*. 2015) demonstrating that initial control of *S. ratti* can be maintained in the absence of adaptive immunity. However, termination of infection clearly depends on a concerted action of innate effectors and the adaptive immune system, as infection was drastically prolonged for up to 1 year in T cell deficient nude mice (Dawkins *et al*. 1982*a*). $CD4^+$ T cells were the dominant cells contributing to timely termination of infection, since MHC-$I^{-/-}$ mice which lack $CD8^+$ T cells displayed an unchanged course of *S. venezuelensis* infection whereas MHC-$II^{-/-}$ mice, which lack $CD4^+$ T cells, displayed a delay in clearance of infection by 1 week (Goncalves *et al*. 2008; Rodrigues *et al*. 2009, 2013). As the authors did not quantify tissue migrating larvae, the net effect of absent $CD4^+$ T cells could reflect specifically a less efficient eradication of migrating L3 as shown before in $CD4^+$ T cell depleted mice for *S. stercoralis* (Rotman *et al*. 1997) or a combination of less efficient eradication of tissue migrating L3 and expulsion of parasites from the intestine.

The canonical T cell response to helminth infection is the Th2 response that is characterized by production of the cytokines IL-3, IL-4, IL-5, IL-9 IL-10 and IL-13 and the subsequent induction of antibody isotype switch to IgG1 and IgE (Allen and Maizels, 2011). Infection of rats (Wilkes *et al*. 2007; Chiuso-Minicucci *et al*. 2010) or mice (Machado *et al*. 2007; Eschbach *et al*. 2010; Blankenhaus *et al*. 2014) with *S. ratti* and *S. venezuelensis* provoked all features of this canonical Th2 response. Thereby the magnitude of Th2 cytokine production was positively correlated to the infection dose in *S. venezuelensis* and *S. ratti* infected mice (Eschbach *et al*. 2010; Schilter *et al*. 2010) and rats (Bleay *et al*. 2007) while single or repeated infection doses both elicited comparable Th2 responses (Paterson *et al*. 2008).

Evidence for a contribution of the Th2 immune response to anti-*Strongyloides* immunity is supported by several studies using mice with defective Th2 polarization. Signal transducer and activator of transcription (STAT) $6^{-/-}$ mice showed prolonged fecal egg release by 11 days compared with wild type mice (Sasaki *et al*. 2005). Injection of IL-27 or transgenic overexpression of IL-27 in *S. venezuelensis* infected mice resulted in reduced IgE and IL-4 production and a 10 day prolongation of egg release in the feces (Yoshimoto *et al*. 2007).

Transgenic overexpression of IL-12 was shown to abrogate immunity against *S. stercoralis* L3 in the tissue (Rotman *et al*. 1997). The reciprocal promotion of Th2 polarization in IL-$12^{-/-}$ C57BL/6 mice elevated several aspects of *S. venezuelensis* specific Th2 response such as eosinophilia, the production of IL-13, IL-10, IL-3, IL-5 as well as IgG1 and IgE response but resulted only in a slight decrease in parasitic adult numbers in the intestine day 7 p.i. in one study (Machado *et al*. 2009). Unchanged parasite burden in the intestine on days 7, 10 and 14 p.i. and unchanged fecal egg

release were reported in another study where IL-12$^{-/-}$ mice were infected with a lower dose of *S. venezuelensis* L3i (Negrao-Correa *et al*. 2006).

Interestingly, the induction of a Th1 driven anti-*S. ratti* HSP60 response induced by vaccination with *Sr*HSP60 and characterized by antigen-specific production of IFNγ and elevation of *S. ratti* specific IgG2b and IgG2c with simultaneous absence of IL-13 production, did not protect mice from subsequent infections (Nouir *et al*. 2012). By contrast, susceptibility of vaccinated mice was increased leading to elevated numbers of adults in the intestine and increased fecal release of *S. ratti* DNA. However, if the anti-*Sr*HSP60 response was biased towards Th2 by vaccination with alum precipitated *Sr*HSP60, antigen-specific IgG1 and IL-13 were induced, IFNγ was absent and mice were protected from subsequent infection thus highlighting the importance of intact Th2 response for control of *S. ratti* infection.

Type 2 innate lymphoid cells (ILC2) that are activated in response to tissue derived alarmins play an important role in the initiation of Th2 immune responses (Licona-Limon *et al*. 2013*b*). *Strongyloides venezuelensis* infection triggered production of the alarmin IL-33 by alveolar epithelial type II cells (ATII) in the lung day 5 and 7 p.i. (Yasuda *et al*. 2012). The authors provide evidence that IL-33 induced expansion of IL-5 and IL-13 producing ILC2 in the lungs of infected mice days 7–15 p.i. IL-33$^{-/-}$ mice showed no ILC2 induction, reduced eosinophilia and reduced local induction of IL-5 and IL-13 mRNA upon *S. venezuelensis* infection. Parasite burden recorded as fecal release of eggs was increased, clearance of infection was delayed by 1 day and intestinal mMCP1 concentrations at days 7–14 were reduced. Increased *S. venezuelensis* fecal egg release in IL-33$^{-/-}$ mice was partially restored by intranasal treatment with IL-33. Interestingly, nasal application of chitin also induced IL-33 in ATII cells, suggesting that presence of nematode parasites such as *S. venezuelensis* can be sensed by conserved "pathogen associated molecular patterns".

As parasite burden was quantified by fecal egg release and the number of migrating L3 in the lung was not assessed, discrimination between IL-33 mediated eradication of L3 and adults remains difficult. On the one hand IL-33 mediated eosinophilia occurred in the lung, suggesting that immunity to L3 in the lung was improved. On the other hand IL-33 was not detected during the tissue migration phase of *S. venezuelensis* day 1–3 but was elevated after day 4, when *S. venezuelensis* has already reached the intestine. Thus IL-33 induced ILC2 that promote type 2 immunity may affect the intestinal life stage as well.

Cytokines

Mice lacking the IL-4Rα chain, part of the IL-4 and IL-13 receptor, showed delayed eradication of *S. venezuelensis,* although initial parasite burden were alike (Negrao-Correa *et al*. 2006). Using bone marrow chimeras, the authors provide evidence that IL-4Rα expressed on lymphocytes was dispensable for timely termination of *S. venezuelensis* infection while mice that were deficient for IL-4Rα selectively on non-hematopoietic cells displayed the same delay in *S. venezuelensis* expulsion as complete IL-4Rα$^{-/-}$ mice. A comparable impact of IL-4Rα signalling on non-hematopoietic cells was shown for control of *Nippostrongylus brasiliensis* infection in the intestine, possibly via activation of intestinal smooth muscle cells or intestinal epithelial cells that express IL-4Rα (Finkelman *et al*. 2004). It is conceivable that IL-4 and IL-13 trigger IL-4Rα mediated signalling redundantly since both cytokines are induced with comparable kinetics during *S. ratti* infection (Eschbach *et al*. 2010) and neither IL-13 neutralization nor IL-4 deficiency delayed parasite expulsion from the intestine (Watanabe *et al*. 2001; Blankenhaus *et al*. 2014). A significant elevation of *S. ratti* eggs passed with feces in IL-4$^{-/-}$ mice was recorded at day 6 p.i. only, while fecal egg release was unchanged at day 5 and at days 7–13.

IL-5 neutralization or deficiency predominantly compromised immunity to migrating L3 in tissues as reviewed in the section "Control and eradication of migrating L3 in the tissue- *S. stercoralis*", but neither changed kinetics of parasite expulsion during first infection nor interfered with immunity to the intestinal life stage in immune mice during second infection (Korenaga *et al*. 1991*a*; Ovington *et al*. 1998; Watanabe *et al*. 2003). Since overexpression of IL-5 promoted expulsion of adults 1 day or 3 days after implantation (El-Malky *et al*. 2003), IL-5 may contribute to intestinal immunity but apparently is not essential.

Repeated injection of IL-3 reduced parasite burden and reciprocally increased the number of intestinal mast cells and mMCP1 concentration in serum and intestinal tissue of nude mice and immune competent C57BL/6 mice (Abe and Nawa, 1988; Abe *et al*. 1993). As IL-3 injection did not reduce parasite burden in W/W^{v} mice, IL-3 apparently acted via activation of mast cells or other effector cells that are absent or dysfunctional in W/W^{v} mice. The numbers of L3 recovered from heads of infected mice were unchanged in the presence or absence of IL-3 while numbers of parasitic adults were reduced in IL-3 treated mice even after oral infection with adult worms. Thus IL-3 activated effector cells attacked the adult parasites in the intestine rather than the migrating L3 in the tissues. The accelerated expulsion of adults in orally infected mice was observed as early as 6 h p.i., suggesting that initial establishment and not survival of adults in the intestine was compromised by IL-3 treatment (Abe *et al*. 1993). Supporting

these findings, IL-3 deficient mice displayed delayed expulsion of *S. venezuelensis* that was correlated to reduction in mast cell and basophil expansion during infection (Lantz *et al.* 1998, 2008). In summary, these studies provide direct evidence that IL-3 supports expulsion of *S. ratti* and *S. venezuelensis* from the intestine. Interestingly, W/W^{v} mice and IL-3$^{-/-}$ mice terminated infection with a delay of maximal 8 days whereas W/W^{v} mice, that additionally lacked IL-3, displayed prolongation of egg release for more than 50 days, suggesting a certain level of redundancy in IL-3 mediated effects.

Several lines of evidence suggest that IL-9 acts in concert with IL-3 to induce mast cell activation and *S. ratti* expulsion from the intestine. IL-9 is a cytokine with pleiotropic function that was shown to promote mast cell expansion and recruitment (Noelle and Nowak, 2010). Injection of recombinant IL-9 reduced numbers of *S. ratti* adults in the small intestine of BALB/c and C57BL/6 mice whereas neutralization of endogenous IL-9 increased intestinal parasite burden (Blankenhaus *et al.* 2014). Increased parasite burden in anti-IL-9 treated mice was correlated to reduced mMCP1 concentrations in the serum, indicating reduced activation of mucosal mast cells in the absence of endogenous IL-9. Injection of recombinant IL-9 alone did not trigger mMCP1 release as shown for IL-3 (Abe *et al.* 1993) but synergistically increased the mMCP1 release induced by recombinant IL-3 if both cytokines were injected together (Sasaki *et al.* 2005).

IL-9 production was further elevated in *S. ratti* infected mice that were depleted of Foxp3^{+} regulatory T cells (T_{reg}) (Blankenhaus *et al.* 2014) or were deficient for a negative co-regulatory receptor (B and T lymphocyte attenuator, BTLA) or its ligand (herpes virus entry mediator, HVEM) (Breloer *et al.* 2015). Increased IL-9 production was correlated to reduced parasite burden in the intestine and reduced release of *S. ratti* L1 in the feces, while numbers of migrating L3 in head and lung were not reduced. Thus elevated IL-9 in T_{reg} depleted or BTLA deficient mice improved immunity to the intestinal life stage of *S. ratti*. As only mast cell competent Cpa3$^{wt/wt}$ but not mast cell deficient Cpa3$^{Cre/wt}$ littermates benefited from elevated IL-9 in T_{reg} depleted mice, this study provides direct evidence that IL-9 functions as additional activator of mast cells during *S. ratti* infection (Blankenhaus *et al.* 2014). It should be noted that an additional putative function for IL-9 as enhancer of smooth muscle contractility that was shown to promote expulsion of *Trichuris muris* (Klementowicz *et al.* 2012) has not been investigated so far.

IL-9 is part of the canonical Th2 response (Allen and Maizels, 2011) but the existence of a specialized Th9 cell is currently debated (Schmitt *et al.* 2014) and specifically Th9 and not Th2 cells were shown to protect mice against *N. brasiliensis* infection (Licona-Limon *et al.* 2013*a*). T cells derived from *S. ratti*-infected mice clearly secreted IL-9 upon antigen-specific stimulation *ex vivo,* but flow cytometric analysis revealed that IL-9 was produced by a low frequency of both CD4^{+} T cells and CD4^{-} cells *in vivo* (Blankenhaus *et al.* 2014). As several non-T cells, including ILC2 and mast cells can principally secrete IL-9, the possible sources of IL-9 during *S. ratti* infection still need to be elucidated.

An unexpected role in promoting intestinal immunity was demonstrated for IL-18 in concert with IL-2. Daily injections with a combination of IL-18 and IL-2 induced intestinal mastocytosis and elevation of serum concentrations of mMCP1, IL-3, IL-4, IL-9 and IL-13 (Sasaki *et al.* 2005). As mMCP1 induction by IL-18/IL-2 treatment was CD4^{+} T cell dependent and could be replaced by injection of IL-3 and IL-9, the authors suggested that IL-3 and IL-9 producing CD4^{+} T cells were induced and subsequently caused the elevation of mMCP1. IL-2/IL-18 treated mice rapidly expulsed *S. venezuelensis* adults that were implanted in the intestine showing that specifically intestinal immunity was increased. Accelerated expulsion in IL-2/IL-18 treated mice was correlated with increased mastocytosis and not observed in W/W^{v} mice. Supporting these findings, IL-18$^{-/-}$ mice and mice deficient for IL-18 receptor displayed a transient reduction of mMCP1 release at days 7 and 10 p.i. and a delay in termination of *S. venezuelensis* by 4 days. Induction of mMCP1 by IL-18 and IL-2 treatment was independent of functional STAT6, i.e. independent of a classic Th2 polarization. STAT6$^{-/-}$ mice also displayed a delayed termination of *S. venezuelensis* infection by 11 days but additional neutralization of IL-18 and IL-2 in STAT6$^{-/-}$ mice prolonged infection even further and reduced mMCP1 in serum beyond day 14. Thus it was hypothesized that two synergistic pathways for mast cell activation exist, one that depends on STAT6 mediated classic Th2 polarization and one STAT6 independent that functions via IL-18/IL-2.

Antibodies

A central function of *S. ratti*-specific IgM and IgG is promoting the eradication of migrating L3 in the tissue during first and second infection and in vaccinated mice, as reviewed in the section "Control and eradication of migrating L3 in the tissue- *S. stercoralis*". Still, evidence for additional contribution of B cells to intestinal immunity was provided using B cell deficient JHD mice (El-Malky *et al.* 2013). *Strongyloides venezuelensis* adults that were directly implanted in the intestine, thus bypassing the tissue migration phase, established better within B cell deficient mice compared with wild type mice. The number of remaining adults 5 days after

implantation was increased and egg release was prolonged. Numbers of intestinal eosinophils and mucosal mast cells were comparable, suggesting that activation and not recruitment of effector cells was compromised in B cell deficient mice. However, since indicators of mast cell degranulation such as serum concentration of mMCP1 were not recorded, this hypothesis was not formally tested.

The FcRγ subunit chain is associated with the function of several Fc receptors such as the high affinity receptor for IgG, FcγRI and the high affinity receptor for IgE, FcεRI. Mice lacking the FcRγ chain displayed no IgE mediated anaphylaxis *in vivo* and cross-linking surface associated IgE did not induce mast cell degranulation *in vitro* (Takai *et al*. 1994). *Strongyloides venezuelensis* expulsion from the intestine was delayed in FcRγ$^{-/-}$ mice upon s.c infection. (Onah *et al*. 2000). Since oral infection also led to prolonged egg release in FcRγ$^{-/-}$ mice compared with wild type mice during first and second infection, intestinal immunity was impaired (Onah and Nawa, 2004). Nevertheless mast cell numbers increased in the intestine at day 13 p.i. and mMCP1 concentrations were even elevated in FcRγ$^{-/-}$ mice (Onah *et al*. 2000). The authors concluded that protective immunity during first infection depended on IgE/FcεR mediated activation of intestinal effector cells, while recruitment of mast cells to the intestine of *S. venezuelensis* infected mice was independent of functional FcεR. However, it should be noted that the FcRγ chain is involved in signal transduction via several Fc receptors as well as C-type lectin receptors (Takai *et al*. 1994), thus the phenotype of the FcRγ$^{-/-}$ mice may not be caused by defective FcεR alone.

Direct evidence for contribution of both FcεR and FcγRIII in expulsion of *S. venezuelensis* from the small intestine was provided using mice deficient in activation-induced cytidine deaminase (AID) (Matsumoto *et al*. 2013). B cells cannot undergo affinity maturation and subsequent class switch in AID$^{-/-}$ mice resulting in intact *S. venezuelensis* specific IgM but defective IgG1 and IgE responses. Initial parasite burdens were comparable in AID$^{-/-}$ and wild type mice but final expulsion of *S. venezuelensis* was 8 days delayed. Transfer of wild type immune serum to AID$^{-/-}$ mice accelerated expulsion of *S. venezuelensis*. Thereby the serum was transferred after completion of the tissue migration phase at day 6/7 p.i., showing that antibodies targeted the parasitic adult in the intestine. IgG and IgE acted redundantly since both isotypes, purified from immune serum, promoted expulsion. Depletion of either isotype reduced the protective effect of the serum, while only simultaneous depletion of IgE and IgG abrogated protective efficiency. Furthermore, transfer of immune serum in mice lacking either FcγRIII or FcεR1α still promoted expulsion while purified *S. venezuelensis* specific IgG or IgE did not accelerate parasite expulsion in mice lacking the respective Fc receptor. Supporting these findings, timing of *S. venezuelensis* infections was unchanged in mice lacking either FcγRIII or FcεR1α (Matsumoto *et al*. 2013). Also neutralization of IgE by application of anti-IgE mAb did not prolong larval output during *S. ratti* infection although IgE depletion from the serum of infected mice was controlled to be complete from day 0 to day 28 p.i. (Korenaga *et al*. 1991*b*), thus emphasizing the redundant functions of IgE and IgG.

As transfer of *S. venezuelensis* specific IgG and IgE did not promote expulsion of *S. venezuelensis* adults in W/W^{v} mice Ig function was dependent on mast cells or other effector cells absent in W/W^{v} mice (Matsumoto *et al*. 2013). It should be noted that the authors did not compare the effect of *S. venezuelensis* specific Ig transfer in WBB6F1-W/W^{v} mice to WBB6F1$^{+/+}$ mice but to C57BL/6 mice as WBB6F1$^{+/+}$ mice displayed ten times lower parasite burden without Ig treatment highlighting the importance of the genetic background on susceptibility.

Mechanism of S. ratti *and* S. venezuelensis *expulsion from the intestine*

The data accumulated so far strongly suggest that mast cells, probably alongside with additional yet poorly characterized intestinal effector cells, promote final expulsion of *S. ratti* and *S. venezuelensis* from the intestine. Thereby IL-3, IL-9 and *Strongyloides* spp. specific antibodies of IgG and IgE isotype trigger mast cell activation. Mast cells contain a plethora of preformed effector molecules including proteases, lysosomal enzymes, cytokines, biogenic amines and proteoglycans that are released upon activation. Additionally the *de novo* synthesis of effector molecules such as lipid mediator prostaglandin and leukotriene and platelet activation factor is induced (Wernersson and Pejler, 2014).

Prostaglandin E2 (PGE_2) (Machado *et al*. 2010) and Leukotriene B4 (LTB_4) (Machado *et al*. 2005) were elevated in the lung and in the intestine of *S. venezuelensis* infected mice. Inhibition of LTB_4 synthesis using either an inhibitor (MK886) or mice lacking an enzyme involved in LTB synthesis (5-lipoxygenase) increased parasite burden in small intestines and fecal egg release suggesting a contribution of LTB_4 to parasite control. The phospholipid mediator platelet activation factor (PAF) has also been implicated in intestinal immunity since PAF receptor deficient mice displayed delayed parasite expulsion leading to increased adult numbers at day 12 p.i. (Negrao-Correa *et al*. 2004).

mMCP1 is a protease specifically expressed by mucosal mast cells (Reynolds *et al*. 1990) and elevation of mMCP1 concentration in the serum indicates mast cell activation. Elevated mMCP1 concentrations in the serum of *S. ratti* and *S. venezuelensis*

infected mice were associated with reduced intestinal parasite burden in several studies (Abe *et al*. 1993; Sasaki *et al*. 2005; Blankenhaus *et al*. 2011, 2014; Breloer *et al*. 2015). Reciprocal reduction in systemic mMCP1 concentration upon IL-9 neutralization was correlated with increased parasite burden (Blankenhaus *et al*. 2014), suggesting a causative link between mMCP1 and parasite expulsion. FcRγ deficient mice, by contrast, displayed a clear delay in *S. venezuelensis* expulsion while both mastocytosis and mMCP1 release were comparable or even increased in the absence of the FcRγ chain at later time points (Onah *et al*. 2000). Since mast cells can be partially activated, leading to secretion of some effector molecules without complete degranulation (Wernersson and Pejler, 2014), mMCP1 may be an indicator of mast cell activation that does not necessarily indicate complete degranulation and may not contribute to protection in all settings. It should be noted that the course of *S. ratti* or *S. venezuelensis* infection in mMCP1 deficient mice that display delayed expulsion of another gastrointestinal parasite, *Trichinella spiralis* (Knight *et al*. 2000), has not been investigated so far, thus direct evidence regarding the contribution of mMCP1 to expulsion of *Strongyloides* spp. is missing.

Strongyloides ratti does not invade the intestinal wall but rather embeds itself in the mucosa of the intestine tightly attached to the layer of intestinal epithelial cells (Dawkins *et al*. 1983). Oral implantation resulted in quick and stable adherence to the intestine after 4 h in naïve but not in immune mice. This intestinal immunity was inversely correlated to mast cell numbers and was compromised in W/W^{v} mice (Maruyama *et al*. 2000). *In vitro* incubation of *S. venezuelensis* adults with the mast cell products chondroitin sulphate (CHS) A and E or heparin reduced their ability to invade the mucosa after subsequent intestinal implantation. It is conceivable that CHS contributes to expulsion of parasitic adults by preventing adhesion to the intestinal mucosa. In support of this hypothesis, the increased intestinal parasite burden in FcRγ$^{-/-}$ mice upon oral infection was correlated with a reduction in free CHS present in intestinal washings (Onah and Nawa, 2004). Thereby, the number of mast cells in the intestine and release of mMCP1 were not changed (Onah *et al*. 2000) and total content of CHS in gut homogenates was actually increased, suggesting that mast cells were recruited but not fully activated in the absence of FcRγ chain. Differences in CHS release were also implicated in the reduced intestinal susceptibility of 129/SvJ mice to *S. venezuelensis* infection compared with C57BL/6 mice (Nakamura-Uchiyama *et al*. 2001). Therefore, the release of CHS by fully activated mast cells apparently promotes expulsion of parasitic adults from the intestine by preventing their adhesion to the intestinal epithelial cell layer.

Nitric oxide (NO) an inflammatory mediator that is produced by macrophages and granulocytes was induced in macrophages exposed to antigens derived from *S. venezuelensis* L3 and parasitic adults *in vitro* and during the tissue migration phase of *S. venezuelensis* infection *in vivo* (Ruano *et al*. 2012). Treatment of BALB/c mice with NO donors reduced numbers of L3 in the lung and in the intestine in normal mice whereas treatment with an inhibitor of the inducible NO synthase reciprocally increased parasite burden (Ruano *et al*. 2015). Of note, treatment with NO donors also reduced the drastically increased parasite burden and fecal egg release in immune suppressed mice. Since mice were infected by s.c. injection of *S. venezuelensis* L3i, the reduced number of parasites in the intestine could reflect the already reduced number of migrating L3. Thus the target of NO, L3 or adult, remains to be elucidated.

In light of the many effector molecules, including cytokines that are produced by intestinal effector cells or are present in mast cell granules (Wernersson and Pejler, 2014), the analysis of potential effector molecules that contribute to expulsion of *S. ratti* and *S. venezuelensis* from the intestine is rather fragmented to date. The data accumulated so far regarding the pathways contributing to parasite expulsion from the intestine are summarized in Fig. 3.

IMMUNE MODULATION

Immune evasion

Helminth parasites manage to establish chronic infections by actively dampening the immune response of their hosts (McSorley and Maizels, 2012). Rodent specific *S. ratti* and *S. venezuelensis,* by contrast, cause transient infections that are terminated after 3–4 weeks (Dawkins *et al*. 1980; Sato and Toma, 1990). Since parasitic females have the capacity to live for more than 1 year in the absence of adaptive immunity (Gardner *et al*. 2006) the timely termination of *S. ratti* and *S. venezuelensis* infection in wild type mice and rats apparently reflects efficient and unsuppressed function of the immune system. In spite of this, recent evidence suggests that active suppression of the host's immune response is required for *S. ratti* to survive even the first week within the intestine of immune competent mice (Blankenhaus *et al*. 2011, 2014; Breloer *et al*. 2015).

Infection of BALB/c and C57BL/6 mice induced transient expansion of Foxp3^{+} T_{reg} numbers first at day 2 p.i. in popliteal and inguinal lymph nodes and later on day 7–14 p.i. in MLN. The Foxp3^{+} T_{reg} population contracted to naïve levels by day 36 p.i., thus kinetics of Foxp3^{+} T_{reg} expansion and contraction resembled the parasites migration path

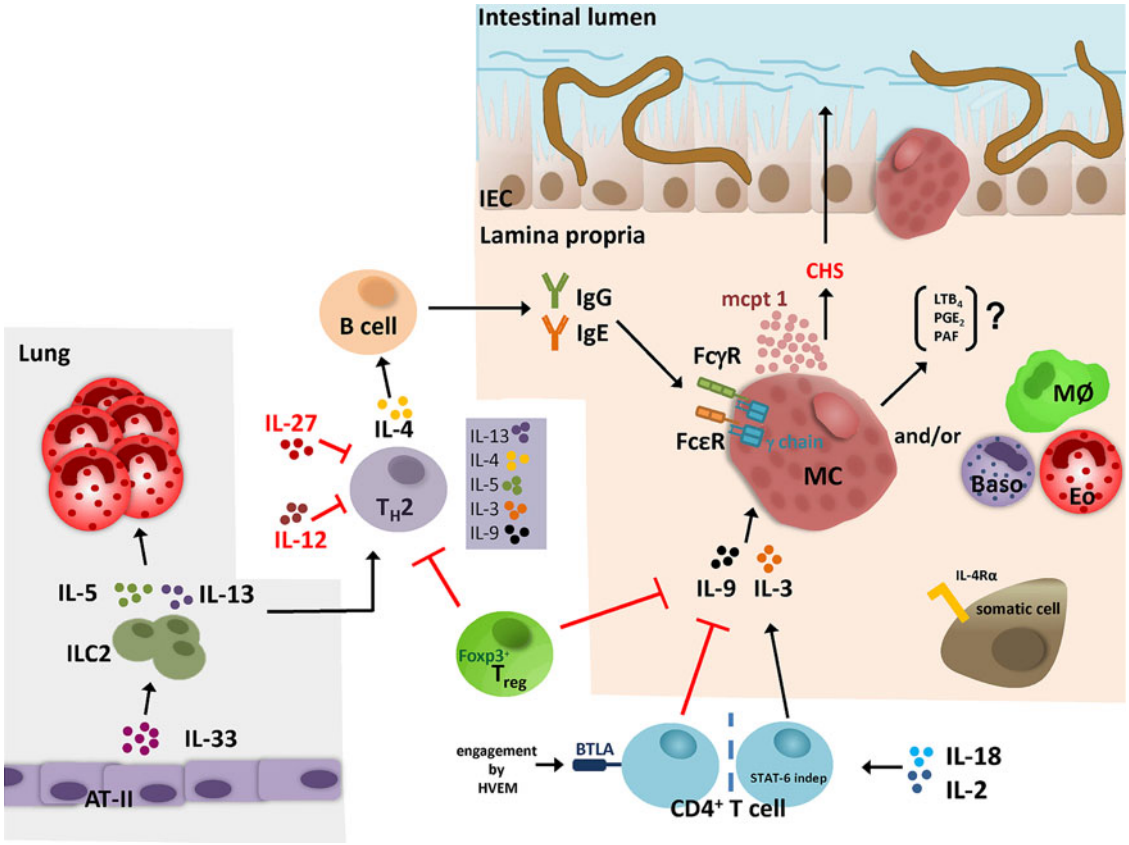

Fig. 3. Control and eradication of parasitic *S. venezuelensis* and *S. ratti* adults from the small intestine. Migrating *S. venezuelensis* L3 induce tissue-derived IL-33 in the lung. IL-33 triggers the expansion of IL-5 and IL-13 producing ILC2 that subsequently promote parasite control and initiation of Th2 response. Classical STAT6 dependent Th2 response results in production of IL-13, IL-4, IL-5, IL-3 and IL-9. Additional IL-9 may be produced by IL-18/IL-2 activated $CD4^+$ T cells and other undefined sources. IL-27 and IL-12 antagonize initiation of Th2. $Foxp3^+$ T_{reg} and BTLA-HVEM mediated signalling dampens IL-9 production during *S. ratti* infection. IL-4 triggers B cell differentiation to IgG1 and IgE producing plasma cells. IgG and IgE as well as IL-3 and IL-9 activate mast cells and probably other intestinal effector cells. Mast cell activation results in mMCP1 release with unknown contribution to parasite expulsion. The mast cell product CHS interferes with attachment of *S. venezuelensis* to IEC. Other potential mast cell products LTB_4, PGE_2 and PAF as well as IL-4 and IL-13 receptor engagement on somatic cells promotes parasite expulsion via unknown mechanisms. Abbreviations: BTLA, B and T lymphocyte attenuator; CHS, chondroitin sulphate; HVEM, herpes virus entry mediator; IEC, intestinal epithelial cells; LTB4, Leukotriene B4; mMCP1, mouse mast cell protease 1; PAF, platelet activation factor; PGE_2, Prostaglandin E2; STAT, signal transducer and activator of transcription.

through the host, with 2 days tissue migration and prolonged presence in the intestine until complete clearance after 1 month (Blankenhaus *et al.* 2014). The transient depletion of $Foxp3^+$ T_{reg} in BALB/c mice before s.c. infection with *S. ratti* L3i reduced parasite burden in the intestine and fecal release of eggs and L1 at any time point of infection until clearance. As T_{reg} depletion did not affect eradication of migrating L3 in lung and head tissue, specifically intestinal immunity was improved (Blankenhaus *et al.* 2011). T_{reg} depletion led to a generalized increase in Th2 associated IL-3, IL-5, IL-13 and IL-10 production by MLN cells and elevated IgE and mMCP1 concentration in the serum. However, the improved intestinal immunity in T_{reg} depleted mice was not due to increased Th2 response in general but was caused by increased IL-9 production and subsequent IL-9 driven accelerated activation of mucosal mast cells. Improved resistance and accelerated mMCP1 release in T_{reg} depleted BALB/c mice were abrogated by IL-9 neutralization while IL-13 neutralization had no effect (Blankenhaus *et al.* 2014). Additional deficiency of mast cells in T_{reg} depleted $Cpa3^{Cre/wt}$ mice increased parasite burden to wild type levels while IL-9 production was still elevated. Thus mast cells apparently represent one effector cell population that was first activated by IL-9 and subsequently contributed to improved *S. ratti* expulsion in T_{reg} depleted mice.

The depletion of T_{reg} (DEREG) mouse model used for these studies causes only transient T_{reg} depletion (Lahl *et al.* 2007). T_{reg} numbers returned to normal levels day 6 p.i. and T_{reg} depletion at later time points after infection i.e. at day 4 p.i. did not reduce parasite burden (Blankenhaus *et al.* 2011) suggesting that suppression of IL-9 mediated

mast cell activation by Foxp3$^+$ T_{reg} was achieved during the first days of infection.

Comparison of BALB/c and C57BL/6 mice showed that T_{reg} expansion and phenotype during *S. ratti* infection was similar in both mouse strains. T_{reg} function was apparent in both strains as T_{reg} depletion elevated Th2 associated cytokine production and IgE concentration in C57BL/6 mice and BALB/c mice to the same extent (Blankenhaus *et al*. 2014). Also efficiency of T_{reg} depletion and repopulation kinetics was comparable in BALB/c and C57BL/6 DEREG mice. Strikingly, T_{reg} depletion in C57BL/6 mice did not reduce parasite burden in high dose or low dose infections and did not significantly increase mMCP1 release and IL-9 production. Thus, expanding Foxp3$^+$ T_{reg} mediate control of IL-9 production and subsequent mast cell activation in *S. ratti* infected BALB/c mice in a non-redundant manner while this is not the case in C57BL/6 mice. As immunity to *S. ratti* in C57BL/6 mice also depends on IL-9 (Blankenhaus *et al*. 2014) and C57BL/6 are even more susceptible to *S. ratti* infection than BALB/c mice, it was hypothesized that additional regulatory elements of the immune system maintain control of IL-9 production and subsequent mast cell activation in *S. ratti* infected C57BL/6 mice in the absence of T_{reg}.

Expression of the regulatory receptor BTLA (Murphy and Murphy, 2010) increased predominantly on CD4$^+$ T lymphocytes in MLN of day 7 *S. ratti* infected C57BL/6 mice (Breloer *et al*. 2015). Absence of either BTLA or its ligand HVEM reduced parasite burden in the intestine and fecal release of eggs and L1 throughout infection. Numbers of migrating L3 in lungs and heads of BTLA$^{-/-}$ and HVEM$^{-/-}$ mice were not reduced suggesting that intestinal immunity improved in the absence of BTLA-HVEM mediated signalling. Thereby specifically IL-9 production by MLN increased and mMCP1 release in the serum was accelerated in BTLA$^{-/-}$ and HVEM$^{-/-}$ mice. Thus BTLA-HVEM mediated signalling interfered with IL-9 driven mast cell activation and promoted *S. ratti* survival in wild type C57BL/6 mice.

Although expression of the regulatory receptor cytotoxic T lymphocyte attenuator 4 (CTLA4) increased on Foxp3$^-$ effector T cells in MLN of *S. ratti* infected BALB/c and C57BL/6 mice (Blankenhaus *et al*. 2011; and Blankenhaus unpublished results) it played only a minor role during immune evasion. Blockade of CTLA4 mediated signalling elevated *S. ratti* specific and polyclonal Th2 response in both mouse strains, but did not reduce fecal release of *S. ratti* eggs and L1. Intestinal parasite burden was significantly reduced in anti-CTLA4 treated BALB/c mice at day 7 p.i but not at day 6 or day 8 p.i., thus providing no consistent increase in resistance.

In summary these studies show that suppression of IL-9 driven mast cell activation via induction of Foxp3$^+$ T_{reg} predominantly in BALB/c mice or BTLA expression predominantly in C57BL/6 mice was central for establishment of even transient intestinal infection by *S. ratti* in immune competent mice.

Bystander suppression

Helminth-induced immune suppression spills over to bystander antigens and may change the outcome of allergy, vaccination or co-infections (McSorley and Maizels, 2012). In this context, the suppression of some aspects of Ovalbumin (OVA) induced allergy were reported in *S. stercoralis* infected mice (Wang *et al*. 2001). Implantation of L3 prior to immunization with OVA decreased eotaxin concentration in the lung as well as OVA-specific IgE production to intranasal challenge OVA exposure, although *S. stercoralis* did not induce a patent infection in BALB/c mice and L3 were never present in the lung. *S. venezuelensis* infection induced lung airway hyporesponsiveness (AHR) in the absence of other allergic stimuli (Silveira *et al*. 2002) but the more dramatic increase in AHR elicited by experimental OVA induced asthma was ameliorated by *S. venezuelensis* infection during OVA challenge in sensitized mice (Negrao-Correa *et al*. 2003).

Still the direct comparison of the immune modulatory effects induced by transient *S. ratti* infection to long lasting *Litomosoides sigmodontis* infection showed that chronic nematode infection caused a stable suppression of immune responses to bystander antigens, whereas transient *S. ratti* infection predominantly polarized bystander immune responses towards a Th2 phenotype. Mice that carried a chronic *L. sigmodontis* infection displayed drastically reduced IgG responses to model antigen vaccination affecting both Th1 associated IgG2 and Th2 associated IgG1 responses (Hartmann *et al*. 2011; Haben *et al*. 2014). By contrast, transient *S. ratti* infection suppressed Th1 associated IgG2 responses to model antigen vaccination more pronounced than Th2 associated IgG1 responses (Hartmann *et al*. 2012). Modulation of vaccination response was only observed if vaccination was performed during maximal *S. ratti* parasite burden. Also production of Th1 associated IFNγ by anti-CD3 stimulated spleen cells and MLN was only transiently suppressed during *S. ratti* infection while anti-CD3 engagement induced production of Th2 associated cytokines at the same time (Eschbach *et al*. 2010). Furthermore, concurrent infection with *L. sigmodontis* interfered with the induction of *Plasmodium berghei* specific cytotoxic lymphocytes by experimental vaccination whereas concurrent *S. ratti* infection did not abrogate vaccination efficacy under similar conditions (Kolbaum *et al*. 2012*b*).

In line with these findings, multiple infections with *S. venezuelensis* did not change the course of

experimental autoimmune encephalomyelitis in Lewis rats (Chiuso-Minicucci *et al*. 2011) and the pathology induced in a model of type I diabetes resulting in weight loss, hyperglycemia and islet infiltration was only mildly ameliorated by a regimen of previous *S. venezuelensis* immunization and infection (Peres *et al*. 2013). The beneficial effect of *S. venezuelensis* infection was correlated to transient induction of Th2 associated IL-5 and IL-10 suggesting that Th1 associated islet inflammation was attenuated by the pre-existing Th2 polarization rather than by active suppression. Chronic *L. sigmodontis* infection, by contrast, clearly prevented autoimmune diabetes in non-obese mice independent of a Th2 polarization by TGFβ mediated immune suppression (Hubner *et al*. 2012).

Co-infection

It is conceivable that the transient Th2 polarization observed in *Strongyloides* spp. infected mice would interfere with the initiation of protective immune responses to infection with parasites that are predominantly controlled by Th1 associated mechanism. However, *Plasmodium yoelii* and *Leishmania major* were efficiently controlled in *S. ratti* co-infected mice (Kolbaum *et al*. 2011, 2012*a*). Thereby the *S. ratti* induced Th2 response was outcompeted by the *P. yoelii* and *L. major* induced Th1 polarization although mice were first infected with *S. ratti* for 6 days to establish Th2 polarization before co-infection was performed. Despite the suppressed Th2 polarization in co-infected mice, the *S. ratti* infection itself was also efficiently controlled and terminated, even if *L. major* infections were performed 14 days before *S. ratti* infection to allow full establishment of Th1 polarization (Kolbaum *et al*. 2011). These studies suggest that the immune system is sufficiently elastic to cope with two infections requiring opposing types of immune response. Still, pre-existing *S. venezuelensis* infection interfered with an efficient response to intravenous *Mycobacterium bovis* co-infection 3 days later. Thereby bacterial load in lung increased at day 28 of *M. bovis* infection and lung concentration of IL-17 but not of IFNγ decreased (Dias *et al*. 2011). Also numbers of *S. venezuelensis* adults increased in co-infected mice and Th2 response recorded as concentration of IL-4 and IL-13 in lung and intestinal tissues decreased (Carmo *et al*. 2009). It should be noted that the course of *Mycobacterium tuberculosis* aerosol infection was not changed by pre-existing *S. ratti* infection (Stubbe, Breloer and Hölscher, unpublished results).

In summary these findings suggest that transient *S. ratti* and *S. venezuelensis* infections in mice predominantly polarize the immune system towards a Th2 phenotype and suppress IL-9 driven mast cell activation to delay their own expulsion from the intestine but do not display the long lasting and systemic suppression of bystander immune responses observed in chronic helminth infections. Still, some bystander suppression may occur in selected settings and modulation of IL-9 driven pathology by pre-existing *Strongyloides* spp. infections has not been investigated.

CONCLUDING REMARKS

Analysis of the immune response against a helminth parasite that displays both, tissue migrating and intestinal life stages reveals the flexibility of the mammalian immune system. While the initiation of a canonical Th2 immune response is a shared prerequisite of protective immunity to both stages the response culminates with the activation of different effector cells in tissue and intestine reflecting the different strategies of trapping and killing *vs* expulsion that evolved for eradication of parasites at these different locations.

Strongyloides spp. L3 are first sensed by conserved structures such as chitin and provoke tissue derived alarmin production and ILC2 induction. L3 are predominantly opsonized by complement, trapped by NETs and eradicated by neutrophils and eosinophils via MPO and MBP during first contact (Fig. 1). Eosinophils participate in induction of adaptive immune response by acting as APC. *Strongyloides* spp. specific IgM and IgG opsonize tissue migrating L3 in immune mice and lead to highly efficient killing by neutrophils and macrophages that reduces numbers of L3 by more than 90% (Fig. 2).

The expulsion of parasitic female adults from the intestine is dependent on mast cells that are activated via the cytokines IL-3 and IL-9 and via *Strongyloides* spp. specific IgE and IgG in association with FcεR and FcγR, but the exact mechanism of parasite expulsion still needs to be elucidated (Fig. 3). Although immune competent mice completely terminate infection after 1 month, the immune system is not working with maximal efficiency. Recent evidence shows *S. ratti* delays its own expulsion by dampening IL-9 driven mast cell activation via induction of $Foxp3^+$ T_{reg} and the regulatory receptor BTLA.

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