



Helminths at mucosal barriers—interaction with the immune system

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Abstract

Helminth parasites are the cause of very significant morbidity, mortality and economic losses in man and domestic animals. Most parasitic helminths infect their hosts via the oral route, and live either at the mucosal surface of the gastro-intestinal tract (GIT), or cross this mucosal barrier on their way to predilection sites. Many helminths live at mucosal surfaces, typically the gut or respiratory tract, and some cross these barriers, either temporarily, spending a period of time in the mucosa before returning to the mucosal surface, or to access other tissues and sites in the host. Typically, helminths induce strongly polarised Th2 responses, which are often effective in mediating protective immunity against those parasites living at mucosal surfaces, but less so in protecting against tissue-dwelling parasites. Induction of strongly-polarised Th2 responses may impair the ability of parasites hosts to eliminate other pathogens. Control of helminth infections relies largely on chemotherapy, together with management and environmental measures designed to keep hosts away from infective stages. Drug resistance has become a significant problem in some helminth populations, and this has promoted interest in the development of immunoprophylactic strategies. However, despite intensive research efforts, helminth vaccines have not become part of regular control strategies. In addition to the considerable technical difficulties posed in the production of vaccines against these complex organisms, further difficulties in securing acceptance for anti-helminth vaccine by regulatory authorities and by users, will be encountered. Such vaccines need not result in sterile immunity, as is required of anti-bacterial and anti-viral vaccines. Recent evidence indicates that while helminths are responsible for disease, immunopathology and impairment of immunity to other pathogens, a complete absence of helminth infection during early life may be a predisposing factor for the development of auto-immune pathology. © 2004 Elsevier B.V. All rights reserved.

Keywords: Helminth; Mucosal immunity; Vaccine; Immunopathology

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1. Introduction

Helminth parasites are responsible for significant ill-health world-wide. The World Health Organisation estimates that soil-transmitted worms including hookworms, ascarids and whipworms pose a health risk to 2 billion people (one third of the world's population) and that water-borne helminths such as schistosomes pose a health-risk to between 500 and 600 million people (http://whqlibdoc.who.int/hq/2001/WHO_CDS_CPE_SMT_2001.8.pdf). Trematodes of the genus *Fasciola* are also regarded as important causes of human disease, with an estimated 24 million people infected across 61 countries [1]. In veterinary medicine, helminth parasites are of constant concern to livestock producers and, indeed to owners of companion animals, and this is reflected in the fact that 27% of sales of veterinary medicines are anti-parasitic drugs and this market was worth \$3.1 billion in 2002 (<http://www.ifahsec.org>).

Most helminths infect their hosts via the oral route, and therefore, either live at the mucosal surface of the gastro-intestinal tract (GIT), or traverse this mucosal barrier on their way to their predilection site. Major helminth parasites of man and animals which inhabit or traverse the mucosa of the GIT are shown in Table 1.

While a wide range of effective anthelmintic drugs are available, their use is associated with diverse problems such as pathology arising before treatment, acquisition of drug resistance, and, in veterinary med-

icine, drug residue issues. The development of effective anti-helminth vaccines would provide a solution to these problems. However, the development of such vaccines has been slow due to the complexities of helminth life-cycles, and the ability of these parasites to evade and modulate host immune responses. The increased availability of helminth parasite genomic information, together with new and improved methods of methods of gene expression (reviewed in [2]), promise to provide enhanced capabilities for helminth vaccine development.

It has recently become clear that helminth pathogens are important not only because of the pathogenic effects directly attributable to them, but because they interact with the host immune system in ways which may impair the response to other pathogens and/or cause immunopathology. In this review, we present an overview of the biology of parasitic helminths, and of the mechanisms whereby they cause intestinal disease. We describe the specific details of some interactions of intestinal helminths with their hosts, the characteristics of immune responses to such parasites, and the outlook for new prophylactic and therapeutic interventions.

2. Basic helminth biology

Helminths (worms) are classified zoologically as roundworms (nematodes) or flatworms (tapeworms or cestodes, and flukes or trematodes). Amongst the nematodes, only a minority are parasitic. The free-

Table 1
Helminth parasites of the GIT which are of major medical or veterinary importance

| Parasite group | Host location | Significance |
|----------------------------|--|---|
| Ascarids | Small intestine, some with migratory larval stages | <i>Ascaris lumbricoides</i> infecting large numbers of people in underdeveloped countries, ascarids of domestic animals causing production losses, and some, such as <i>T. canis</i> , causing zoonotic disease |
| <i>Fasciola</i> spp. | Liver flukes, which migrate across intestinal mucosa and peritoneal cavity to gain access to hepatic tissue | Large numbers of people in some developing countries such as Bolivia, Egypt, are infected. Major pathogen of ruminants in both temperate (<i>F. hepatica</i>) and tropical (<i>F. gigantica</i>) areas |
| Hookworms | Small intestine | Mouthparts adapted for bloodsucking, with the more pathogenic species causing severe anaemia in man and animals |
| Lungworms | Airways or lung parenchyma | Those species living at the airway mucosa are generally more pathogenic than those living in lung parenchyma. Immune responses are often protective against challenge but may also cause immunopathological lung damage |
| Schistosomes | Blood flukes whose main predilection site is mesenteric veins, thus allowing eggs to be laid into the intestinal lumen and pass out with host faeces | Major cause of morbidity and mortality in man and animals in tropical areas. Definitive hosts become infected by skin penetration by infective stages (cercariae) emerging from water snails |
| <i>Strongyloides</i> spp. | Small intestine, but sometimes also cause skin irritation | Large numbers of humans infected in developing countries. Also affects neonatal livestock |
| Tapeworms | Adults in small intestine, intermediate stages in a variety of tissues | Intestinal stages generally tolerated without significant pathology. Intermediate stages may cause serious disease, for example neurocysticercosis by <i>T. solium</i> , and echinococcosis |
| <i>Trichinella</i> spp. | Adults with brief life-span in small intestine, but larvae in tissues remain viable for years and are a source of infection for carnivorous hosts | Human infection generally controlled satisfactorily by meat inspection, but still a cause of muscle pathology following consumption of game |
| Trichostrongylid nematodes | Various parts of the GIT | Of greatest importance in ruminants. Several species have developed drug resistance, thus stimulating vaccine studies |

living nematode *Caenorhabditis elegans* has been used extensively in the fields of basic nematode biology and genomics. All flatworms adopt a parasitic life-cycle. Where there has been a co-evolution of helminths with their hosts, and where parasite burdens are not extreme, it is common for helminth infections to result in only very minor, if any, clinical disease. This is the case, for example, for infection of man

with the “beef tapeworm”, *Taenia saginata*. Typically, helminth infections become clinically important in individuals or populations when environmental conditions promote a build-up of infectious stages, and therefore, very large parasite burdens. Helminths may also cause disease when host populations previously unexposed to a particular species become infected, due to a change in the environment, or when individ-

uals are immunosuppressed or nutritionally compromised. In many cases, neonates are more susceptible to helminth infection than adults. In some cases, resistance develops as a result of active immunity, but there are also many examples where innate age resistance plays a role.

Most parasitic helminths have at least one stage of their life-cycle where they are free-living. With many nematodes, for example, eggs passed in the faeces of infected hosts develop through several larval stages in the environment, with infection of a new host taking place through ingestion of an infective larva (often the third larval stage). In the case of some roundworms and all flatworms, life-cycles are indirect, with at least two and possibly more hosts involved.

2.1. *Helminths as pathogens*

Helminths which live in the GIT of their hosts include a wide variety of taxonomic groups, across both flatworms and roundworms. In many cases, such as in most tapeworm infections, they are tolerated well and are responsible for few, if any, pathogenic effects. Where disease does occur as a result of intestinal helminthosis, it is often the case that significant pathology is only seen when parasite burdens exceed a certain threshold. Various pathogenic mechanisms can be involved including loss of blood due to parasite feeding [3], competition for nutrients or nutritional dysfunction [4], immunopathology [5,6], interference with gut motility and/or neuromuscular control [7] and blockage of the intestine [8].

Helminths often cross the mucosal barrier of the GIT to gain access to other sites within the host. Another mucosal site favoured by helminths is the respiratory tract. Typically, helminths arrive here after travelling through the blood vascular system and then crossing over to the airways at the lung capillaries.

2.2. *Helminths within the gut, and across the mucosal barrier*

It seems probable that parasitic nematodes evolved from free-living populations which were transported from their environmental niche (soil and water) into the GIT of higher organisms. Evi-

dence of such a pattern can be seen in some nematode groups (such as the genus *Strongyloides*) which are still at the borderline between a parasitic and free-living life-cycle. These nematodes can be parasitic in a variety of hosts, including man. In this genus both parasitic and free-living life-cycles can occur, depending on environmental conditions. When the latter are unfavourable, the existence of parasitic forms ensures the survival of populations, which can revert back to free-living forms when conditions improve. Infection of hosts can take place either orally, or through skin.

Helminths which live at mucosal sites such as the GIT are, strictly speaking, not truly living within the host organism, and are therefore, in some ways less exposed to host immune defence mechanisms than helminths living within host tissues. However, the typical Th2 immune response evoked by helminths, which among its effector mechanisms includes increased gut motility, may be particularly suited to the elimination of intestinal parasites.

Another evolutionary step may have occurred when helminths living within the GIT of their hosts crossed this mucosal barrier to continue their life-cycle elsewhere, within host tissues, in some cases returning to the intestine to provide an easy access for reproductive stages to the outside environment. There are many such examples among parasitic helminths—including the liver and blood flukes, *Schistosoma* and *Fasciola*, some ascarids such as *Toxocara canis*. The evolution of such migratory patterns among parasitic helminths may have been driven by requirements to evade immune defence mechanisms, targeted specifically at helminths inhabiting mucosal sites. In order to penetrate mucosal surfaces and host tissues, helminths use a variety of mechanical and chemical tools including tough modifications of the surface (hooks, lancets, spines) and proteolytic enzymes (reviewed in [9]).

3. Characteristics of immune responses to intestinal helminths

Host immune responses to helminths living in the GIT are well-defined. Some general principles in relation to such responses can be stated. Innate immunity, often age-related, is important in many

cases. This may be due to physico-chemical differences in the gut environment in adult as compared with young hosts. In dogs infected with the ascarid parasite *T. canis*, for example, adult worms are expelled from the intestine when the host is between 3 and 6 months of age, and new infections do not establish as adults, following egg infection, in dogs older than 6 months [10]. This phenomenon does not depend on prior exposure to the parasite.

Distinct immune responses often occur to antigens expressed at different stages of the parasitic life-cycle. This is especially true where some stages are migratory. Responses to gut-dwelling stages are often polarised Th2 responses, characterised by eosinophilia, mastocytosis and IgE production. One effect of such a response in the GIT is stimulation of smooth muscle, increased gut motility, and diarrhoea [11]. Clearly, this effect is likely to promote parasite expulsion, but diarrhoea is also one of the pathogenic effects associated with GIT helminth infection. This illustrates another common feature of mucosal helminth infections, namely, that pathology often results from the host's own immune response. Allied to this, polarisation of the immune response to Th2 also down-regulates the Th1 cell subset [12], which may have implications for the ability of hosts with helminth burdens to mount protective immune responses to intracellular pathogens.

3.1. Protective responses

There are many examples of intestinal helminth infections which are contained, and ultimately eliminated, by the classic "anti-helminth" Th2 response described above. In man, for example, such responses are involved in protection against hookworm infection [13]. Many studies on protective immunity to gastro-intestinal nematodes come from examples important in veterinary medicine, or from experimental animal models. One of the most extensively studied parasites in this respect is *Ostertagia ostertagi*, a nematode parasite which lives in the abomasum of cattle and causes serious economic losses to beef and dairy producers. Within a number of days following infection, and persisting for the duration of infection, there is a considerable increase in the size of regional lymph nodes draining the site of infection [14], and also an infiltration of lympho-

cytes at the site of infection [15]. This is accompanied by increased numbers of mast cells and eosinophils [16] and by increases in smooth muscle activity [17]. Despite these changes, immunity to *Ostertagia* infection develops slowly, so that animals during their first season of parasite exposure remain comparatively susceptible, and eliminate existing infections relatively slowly in spite of immunopathological consequences such as diarrhoea and loss of specialisation in the epithelial lining of the abomasum. Markers of developing protective immunity include lowered parasite egg production, stunting of worms, and increased parasite development times. The outcome for the animal is solid immunity, but only after several months of parasite exposure and, in cases where parasite burdens exceed a certain threshold, clinical disease. The outcome for the parasite is the establishment of relatively long-standing infections in young animals ensuring sufficient reproductive capacity for survival until the next generation of susceptible hosts becomes available, i.e. from one grazing season to another. Although eliciting similar immune-effector mechanisms, immunity to another gastro-intestinal nematode of veterinary importance, *Nematodirus battus*, is extremely rapid [18]. This parasite is a serious intestinal pathogen of lambs, but sheep over the age of about 6 months have a solid age-immunity. Infected lambs develop severe diarrhoea and a Th2-skewed immune response, with worm expulsion, associated with shedding of duodenal villi, occurring about 3 weeks post-infection [18].

The role of goblet cells and intra-epithelial $\gamma\delta$ -lymphocytes in immune-mediated expulsion of helminth parasites is receiving increasing attention. The higher numbers of goblet cells recruited to the GIT in helminth infection, associated with increased mucus production and in some cases parasite expulsion [19], may be under the control of $\gamma\delta$ T-cells [20]. The role of the latter as a bridge between the innate and adaptive immune systems is gaining currency, and new information on the existence of various subsets within the population promises to throw further light on this role (reviewed in [21]). A schematic showing some of the mechanisms that may contribute to Th2 mediated protective immunity against intestinal helminths is shown in Fig. 1.

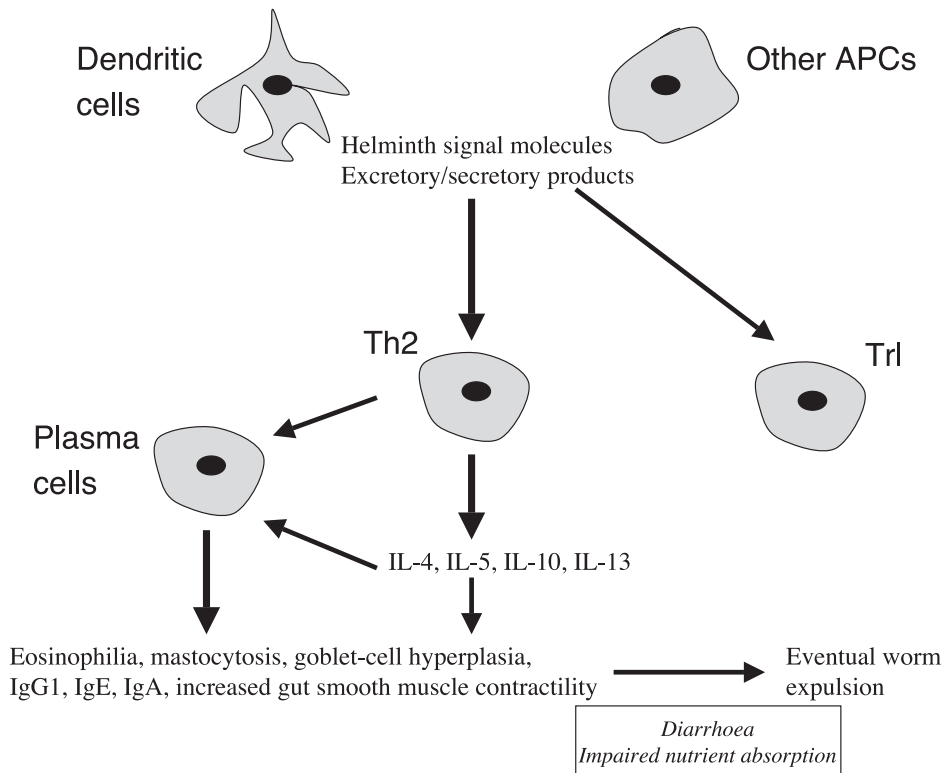


Fig. 1. A simple representation of how typical anti-helminth Th2 responses give rise to effector mechanisms which may lead to worm expulsion but at the same time lead to clinical signs such as diarrhoea and impaired nutrient absorption. In many cases the inflammatory changes accompanying helminth infection co-occur with moulting of larval stages or emergence of larvae from the gut mucosa into the lumen. Where very large numbers of larvae emerge synchronously, there may be dramatic changes leading to acute clinical signs, as seen in “Type-2” disease associated with nematode infection in horses and cattle.

3.2. Ineffective responses

The chronicity of many infections with helminths in the GIT, resulting from slow acquisition of protective immunity, has been mentioned above. Recent evidence indicates that helminth-derived lipid molecules may be responsible for activating TLR2 pathways, thus inducing regulatory T-cells, and also stimulating dendritic cell (DC) orchestration of Th2 responses [22]. This phenomenon may explain how continual immune stimulation resulting from chronic nematode infection can be tolerated while still invoking strongly-polarised responses. In some cases, this may lead to persistence of tissue-dwelling, larval stages but elimination of intestinal adult stages (as with *T. canis* and *Trichinella spiralis*). In others, it may explain the inability of the immune system to contain and eliminate helminth infections which occur

at sites other than mucosal surfaces. Such helminths in many cases elicit the same, Th2-dominated, immune responses as those described above. A reasonable hypothesis is that such responses are unsuited to dealing with tissue-dwelling helminths, and that some helminths evolved a migratory life-cycle as a means of evading immune defences. One example of such a helminth is the trematode, *Fasciola hepatica* (liver fluke) which is an important cause of disease in both domestic animals (ruminants) and man. In fasciolosis the response in both mice and cattle is a characteristic, Th2-dominated, anti-helminth response [23,24]. However, cattle of all ages remain susceptible to infection in spite of repeated exposure [24]. In schistosomiasis, also, humans infected with these blood flukes develop strong Th2 responses (stimulated particularly by worm egg-laying activity) but infections remain chronic for many years and “immune” individuals

remain susceptible to new infection [25,26]. Further examples of typical anti-helminth responses being ineffective in the face of tissue-dwelling helminths comes from the filarial nematodes, where *Onchocerca volvulus* infection of man (the cause of river blindness), and *Dirofilaria immitis* infection of dogs (heartworm) result in typical Th2 responses, yet chronic infections are the norm and exposed individuals remain susceptible to new infection [27].

3.3. Immunomodulation by helminths

As part of their adaptation to a parasitic life-cycle, helminths have of necessity acquired the means of dealing with host immune responses. One such mechanism of immune evasion may have been the development of migratory strategies, away from mucosal immune defence mechanisms, as described above, coupled with induction of immunoregulatory cell subsets. There is also much evidence in the literature for specific modulatory mechanisms evoked by helminths to interfere with particular effector mechanisms evoked following induction of immune responses. Such mechanisms contribute to the chronicity of infections, and their persistence in adult populations, but they can also contribute to immunopathology, either by inducing potent inflammatory reactions which damage host tissues, or by impinging on host responses to other pathogens.

There are numerous mechanisms documented for helminth immunomodulation including down-regulation of Th1 responses [26,28] associated with the intense stimulation of Th2 responses, secretion of anti-effector molecules, inhibition of effector cells, inhibition of complement components, induction of non-specific proliferative responses, and inhibition of proliferative responses.

The complement system is an important effector of the innate and adaptive immune response. The system consists of a group of serum proteins that activate each other in a specifically ordered manner to generate biologically active molecules. These mediate the elimination of pathogens directly through cell lysis or indirectly through opsonisation and induction of inflammation. Following infection, helminth parasites typically activate immune recognition, represented by the complement pathway, which contributes to the host inflammatory response, specifically in the recruit-

ment of eosinophils and Non-T-Non-B cells. The juvenile stages of many parasites are susceptible to lysis by this cytolytic response [29–31]. However, following development to the adult phase of the life cycle, many parasites develop resistance to complement-mediated killing. It has been suggested that this may occur due to a number of immunoevasive strategies adopted by helminth worms. For example, schistosomes secrete proteases which cleave peptides anchoring the glycocalyx coat to the tegument of the worm. This results in the shedding, not only of the parasite coat but also of surface-bound components of the complement system [32].

Helminth parasites have also developed a number of immunomodulatory mechanisms, which inhibit complement killing via either the alternative or classical pathway. Complement activation of either pathway converges at the level of C3 with C3 convertase formation being critical to its function. Regulation of convertase enzyme activity is tightly controlled by a number of membrane-associated or secreted regulatory proteins, which prevent complement-mediated hemolysis by blocking the assembly or accelerating the decay of C3 convertase. Parasite worms, which spend a large proportion of their life cycle in the host vascular system, have developed the ability to acquire these regulatory proteins as part of their surface coat. In this way, the parasite inhibits the synthesis of enzyme, therefore, preventing induction of the classical complement pathway. For example, both *Echinococcus granulosus* and *Taenia taeniaeformis* sequester host-derived negative regulator, factor H, while *Schistosoma mansoni* adsorbs host decay accelerating factor (DAF) from erythrocytes [33,34]. Such acquisition of regulatory factors from the complement system ensures survival of the worm. However, this disruption of the C3 convertase enzyme, a pivotal component of both the classical and alternative pathway, undoubtedly affects the susceptibility of the host to concomitant bacterial or viral infections.

The alternative complement pathway requires the convertase activation of C3b, which binds to the surface of the invading pathogen preparing it for opsonisation. Two serine proteases isolated from *S. mansoni* have demonstrated the ability to cleave the C3b component of the human complement system [32], thereby inactivating the cascade of events leading to induction of the alternative pathway. While this

determines that the parasite is resistant to complement, it may also result in increased host susceptibility to concurrent infection by other pathogens or failure of vaccines to confer protection. It is widely accepted that C3d, a downstream product of C3b, is an effective adjuvant for inducing immune responses to a variety of antigens [35–37]. Therefore, immunomodulation by helminths resulting in the inactivation of C3b and consequential removal of its cascade products, may mediate susceptibility to the host towards infectious agents and reduce efficacy of a wide range of vaccines.

The activation of complement through the classical pathway requires C1q for initiation. Helminths have adapted a wide range of mechanisms to inhibit the function of this component of the classical pathway. The gut-associated antigen, CAA of *S. mansoni* behaves like a receptor for C1q preventing activation of the precursor C1 complement component thus protecting the schistosome gut from complement mediated attack [38]. Paramyosins from *S. mansoni* and *T. solium* inhibit C1 function by binding to C1q, resulting in the inactivation of C4, an essential element in the induction of inflammation [39]. Transgenic mice deficient for the C1q component are known to lack the classical pathway of complement activation. We hypothesise that a similar immune deficiency may, therefore, exist during infection with helminth parasites, leading to increased susceptibility to bacterial or viral infections. It has been demonstrated that C1q is a vital element in protection against bacteria. Mortality of C1q deficient mice to polymicrobial peritonitis was 83% compared to 42% for wild type. The same strain of mice were found to be significantly more susceptible to *Salmonella* infection, reflected in increased hepatic and splenic bacterial counts [40,41]. These observations further support our suggestion of helminth-induced predisposition to concurrent bacterial infection.

Parasitic helminths possess mechanisms to disable the short-range offences of effector cells, such as toxic reactive oxygen products of leucocytes (eosinophils and neutrophils) and macrophages or reactive nitrogen intermediates generated by macrophages. *S. mansoni* and *F. hepatica* are highly resistant to killing by reactive nitrogen intermediates generated by lipopolysaccharide (LPS)-stimulated macrophages and by chemically-generated reactive oxygen intermediates

(ROI). Inactivation of ROI involves oxidant-scavenging enzymes such as superoxide dismutase (SOD), glutathione peroxidase and glutathione-S-transferase (GST) [42]. SOD is also found at the surface of adult *Brugia* worm where resistance to killing by superoxide is observed [43].

While phagocytosis by macrophages is primarily mediated by reactive oxygen and nitrogen intermediates, neutrophils contain an abundant array of molecules, which are involved in oxygen-independent killing of pathogens. Among these, α -defensins, elastase and neutral proteinases (cathepsin G) cause lethal damage to membranes by forming destabilising ion-channels and degrading the extracellular matrices of pathogen membranes. Serine proteinase inhibitors (serpins) secreted by pathogens play a vital role in inhibiting the activity of these enzymes. Serpins from viruses have been implicated in pathogen evasion of the host defence system. Recombinant Bm-SPN-2, a protein found in the microfilarial stages of *B. malayi* was found to specifically inhibit enzymatic activity of human neutrophil cathepsin G and human neutrophil elastase [44]. It is possible that Bm-SPN-2 could function as a stage-specific serpin in the blood stream protecting the parasite from attack from neutrophils [44]. *F. hepatica* has also been shown to secrete a Kunitz-type serine proteinase inhibitor, which is expressed in the gut, parenchyma and tegument of the adult worm. This protein is thought to inhibit the activity of neutrophil elastase [45].

In order for T-cells to respond to foreign particles, the antigen must be broken into peptide fragments and presented via MHC molecules. The processing of peptides depends upon whether the antigen is exogenous (generally bacterial or viral origin) or endogenous (generally viral in origin). Antigen presenting cells that present exogenous antigens include macrophages, B-cells, DCs and epithelial cells whereas endogenous particles are generally synthesised by viruses within the cell and can be presented via all other nucleated cell types. Processing of exogenous antigens begins when antigens are internalised via endocytosis. Following endocytosis, endosome–lysosome fusion occurs and degradation of antigen commences within these acid vesicles. Initially the tertiary structure of the protein is destroyed by the reduction of disulphide bonds, thus making it more accessible to other degradative enzymes. Enzymes involved in the

degradation of proteins include cathepsin B, D and E. The antigen is further processed by endosomal proteases and the resulting peptides fragments form complexes with Class II MHC molecules that are expressed on the cell surface. Helminths are known to produce a number of protease inhibitors that interfere with processing of exogenous antigens. PI-3 is an aspartyl proteinase inhibitor, which is thought to interfere with cathepsin-E activity [46]. Cathepsin E is known to be involved in the processing of antigens and of invariant chains. It is a major aspartic proteinase in a murine antigen-presenting cell line, A20 [47] and is thought to play a major role in antigen processing. This enzyme is localised to a non-lysosomal compartment of the endosomal system in these cells. Functional studies using a highly specific inhibitor of cathepsin E have shown that this enzyme is essential for the processing of ovalbumin by this cell line [47].

During cell-mediated immunity, T-cells secrete cytokines which activate macrophages to become efficient phagocytes, with cells activated by Th1 pro-inflammatory cytokines, such as IFN γ , being critical in combating infection with intracellular micro-organisms. In contrast, Th2 cytokines, such as those observed following a helminth infection, activate macrophages toward a down-regulatory phenotype. In support of this, recent studies have described an innate response that leads to expansion of suppressor macrophage populations. These immunoregulatory cells, termed natural suppressor (NS) cells, originate from granulocyte–monocyte progenitors and are capable of inhibiting proliferative responses of naïve or activated T and B cells. Two different subclasses of NS have been described: (1) alternatively activated macrophages (AA), which are IL-4 dependent and (2) classically activated macrophages (CA), which are IFN γ -dependent. IL-4 dependent macrophages recruited to the peritoneal cavity in mice infected with *B. malayi* actively suppress the proliferation of lymphocytes co-cultured in vitro [49]. These AA macrophages block proliferation by cell-to-cell contact, implicating a receptor-mediated mechanism.

Following successful migration to the lymphoid tissue, immune responses to pathogens are initiated when DCs present MHC class II antigen peptide complexes to CD4⁺ T cells. In combination with co-stimulation, this signal drives T cells to produce IL-2

and enter the cell cycle. There is increasing evidence that microbes drive the development of protective Th1 or Th2 cells via their effects on APC with suggestions that DC's carry a signal determining the polarisation of naïve T cells into either Th1 or Th2. Exposure of DC to Th2-inducing soluble egg antigen of schistosomes was found to be sufficient to induce the ability to promote Th2 responses when subsequently injected into mice or co-cultured with naïve CD4⁺ cells in vitro [48]. This indicates that pathogens, or their signature molecules, can induce biased immune responses by direct priming of DC's, and fully matured DC's are resistant to repolarisation by microbial stimuli. Carbohydrate ligands are increasingly recognised as important in helminth immuno-regulatory activity [50]. These observations have implications in the field, where populations endemic for Th2-inducing parasitic worms may be unable subsequently to prime effective subsequent Th1 response essential for defence against bacterial/viral infection and protection by vaccination.

Helminths are also known to suppress lymphocyte responsiveness directly. This has been shown for example in *F. hepatica* in a *Bordetella pertussis* mouse infection model [51], in onchocerciasis [52] and in schistosomiasis [53].

4. The influence of helminth infections on resistance to other diseases

4.1. Impaired responses to bacterial and viral infections

There is growing epidemiological evidence to support the hypothesis that helminth parasites cause impaired immune responses to bystander bacterial and viral infections. Studies in South Africa, for example, demonstrated a significant correlation between total serum IgE levels, anti helminth-specific IgE and the incidence of tuberculosis [54]. In countries such as Asia and South America, co-infection with schistosomes and hepatitis B virus is a frequent event with viral clearance dependent upon the intra-hepatic production of Th1 cytokines [55–57]. In addition, individuals co-infected with HIV and *S. mansoni* have higher viral loads than individuals from non-endemic regions [58]. Moreover, when anti-helminth chemo-

therapy was administered to *S. mansoni* infected individuals a reduced HIV viral plasma load, similar to that of individuals from non-endemic regions was observed [58,59]. This evidence suggests that treatment of helminth parasites will restore the immune response and result in clearance of infection or retarded progression of diseases such as HIV. Similar observations have been made in animals co-infected with *F. hepatica* and *Salmonella dublin* where spontaneous clearance of the latter was observed following anti-helminth therapy.

In addition, infection with these parasites also impairs the protective immune response to vaccination. *S. mansoni* infected patients, particularly those with hepato-intestinal disease, showed a diminished ability to mount an immune response to *Salmonella typhi* after immunisation with a typhoid vaccine [60]. Similarly, populations harbouring pre-existing trematode or nematode infections have diminished protective immunity to tuberculosis induced by vaccination. Significant improvement in the bacterial-specific immune responses occurs following anti-helminth therapy [61,62]. Concurrent infection with helminths such as *Ascaris*, *Onchocerca* and *Schistosoma* also diminished the magnitude of the Th1 immune response to tetanus, diphtheria and cholera toxins which was restored following helminth specific chemotherapy [63,64]. It is suggested that large-scale programs to eradicate helminths would have a significant impact on the incidence of bacterial and viral infections throughout the third world.

Studies in experimental animals have supported these observations, with many authors describing a generalised imbalance of the Th1/Th2 immune response following helminth infection affecting responses to unrelated antigens. For example, mice infected with *S. mansoni* displayed reduced Th1 cytokine response to sperm myoglobin [65]. Similarly, co-infection of mice with *S. mansoni* and recombinant vaccinia virus expressing HIV gp160 envelope glycoprotein, exhibited delayed clearance of the vaccinia virus, which was associated with the suppression of IFN- γ secretion from CD8⁺T cells [66]. Moreover, mice infected with *F. hepatica* and *B. pertussis* exhibit delayed bacterial clearance from the lungs, directly associated with inhibition of *B. pertussis*-specific IFN γ production [51]. This immune modulation has been further characterised by a variety of studies

demonstrating impairment of immune responses in specific circumstances. These include, for example, delayed rejection of skin grafts, modulation of *Helicobacter*-induced gastritis and absence of delayed type hypersensitivity responses which were restored by specific anti-helminth therapy [67,68]. Recently O'Neill et al. [69] demonstrated that a single purified enzyme Cathepsin L, secreted by *F. hepatica*, down-regulates Th1 immune responses to a non-parasitic bacterial pathogen. Furthermore, it was demonstrated that suppression of the bacterial-specific Th1 immune response was partially dependent upon IL-4. Cathepsin L is a proteolytic enzyme that plays a vital role in the acquisition of nutrient and migration within the host and was also shown to prevent antibody mediated eosinophil attachment.

4.2. Absence of helminth infections as a possible factor in the aetiology of immune-mediated disease

In addition to inducing increased susceptibility to some intracellular pathogens and impairing vaccine responses, there is increasing evidence that anti-helminth responses, or a lack of them, may be responsible for the increased prevalence of certain diseases in affluent, western societies where intestinal helminth infection is not widespread. Included under this heading are diseases such as asthma, atopy, type-1 diabetes and inflammatory bowel disease (IBD). The so-called "hygiene hypothesis" states that since gastro-intestinal helminths are among those pathogens likely to have co-evolved over the longest period of time with their hosts, lack of exposure to these organisms early in life in developed societies may predispose certain individuals to immunopathological conditions such as those listed. There are strong epidemiological correlates between low exposure to helminths and high levels of allergic/immunopathological conditions within individual societies [70–72]. The mechanisms involved are not yet clear, although there are pieces of experimental evidence beginning to emerge which offer at least partial explanation for this phenomenon. For example, the non-obese diabetic (NOD) mouse develops type-1 diabetes when raised under SPF conditions [73]. However, development of the disease can be prevented by infection with *S. mansoni* [74].

One possible explanation for autoimmune diseases in this scenario which is gaining currency is the

“gatekeeper” hypothesis [71]; lack of exposure to certain pathogens which the host has evolved to expect early in life may result in failure of regulatory T-cells (Tr1) to delete those clones which are specific to self-antigens but also cross-react with pathogen epitopes. This hypothesis could also explain the pathogenesis of auto-immune bowel disease even where there is a well-recognised association with sensitivity to one particular antigen, such as Crohn’s disease. Epidemiological links between type-1 diabetes and Crohn’s disease have been observed [75].

5. Immunopathology

The immune response is a two-edged sword when it comes to helminth infection. Although helminths can impair protective immune responses both to themselves and to other pathogens, they may also be associated with severe inflammatory reactions and immunopathology. The example of bovine ostertagiosis is as apt here as it is in discussing the genesis of protective immune responses to intestinal helminths. The clinical signs of this condition include diarrhoea, inappetance and weight loss, all of which can be traced to changes in the architecture and chemistry of the abomasal epithelium which result from the inflammatory and immune response to parasite challenge. Diarrhoea results, in part, from increased stimulation of smooth muscle contractility along the length of the GIT. It is also due to hyperplasia and loss of specialised cells in the abomasal glands, which in turn leads to an elevated pH because of impaired acid secretion, elevated levels of gastrin and continued overgrowth of non-specialised cells. These changes seem to be due to a response to parasite antigen is shown by the fact that the most severe changes coincide with a moult and emergence from the gastric glands of larval worms, and that some changes (elevated serum gastrin) occur in immune animals following secondary challenge [76]. An understanding of immunopathology as a consequence of immune responses to helminth infection has implications for the design of anti-helminth vaccines. Clearly, there is a risk that mimicking the host response to infection may carry the risk of inducing undesirable pathology. However, there are some indications that in some experimental infections at least, a distinction can be

drawn between the mechanisms underlying protection and immunopathology. Garside et al. [77] have shown that abrogation of the mediators of enteropathy such as iNOS and TNF does not prevent expulsion of *T. spiralis* in mice. In addition experimental vaccines against schistosomiasis, using IL-12 as adjuvant, are able to diminish the immunopathology associated with egg granulomata [78].

6. The outlook for mucosal anti-helminth vaccines

The first vaccine effective at protecting a target species against a helminth parasite has been available since the 1950s. Irradiated *Dictyocaulus viviparus* larvae, administered orally to calves, are effective in inducing protective immunity without contributing significantly to pasture contamination or inducing disease signs. On the advent of this vaccine, it was widely predicted that many others would follow, and that helminth vaccines would become a useful tool in the prevention of disease in man and animals. Unfortunately, however, it proved a much more difficult task to produce vaccines effective against other helminth parasites, and, to date, they have not made a major impact on medical or veterinary practice. One of the first successful attempts to produce a recombinant anti-helminth vaccine was described by Johnson et al. [79]. This vaccine was developed using a recombinant antigen (To45W) to protect sheep against infection with the larval stage (cysticercus) of a tapeworm, *Taenia ovis*. Although the vaccine was very effective it was not ultimately commercialised, due to lack of a sufficient market. Work is continuing, however, in using the same approach to produce effective vaccines against other tapeworms of greater pathogenic significance in animals and man, including *Taenia solium*, a tapeworm which cycles between pigs and man and causes the serious disease of neurocysticercosis (reviewed in [80]). Promising experimental vaccines to hookworms [81], schistosomes [25] and liver flukes [82] currently being developed are likely to yield at least some commercial products within the next decade. Vaccines against *F. hepatica* developed in our laboratory can reliably deliver substantial protection (55–72%) to cattle in terms of reduction in worm burden, coupled with an almost complete anti-embryonation effect [83]. These effects combine to render

the transmission-blocking efficacy of these vaccines equal to almost 100%. To date, however, these vaccines have not incorporated antigens specific to the intestinal stages of the flukes (newly excysted juveniles, NEJs). These traverse the intestinal mucosa and cross into the peritoneal cavity within 24 h of infection, so vaccines inducing responses targeted at these stages would need to evoke rapidly-active effector mechanisms at the mucosal surface.

Notably, vaccines effective against helminths living in the gastrointestinal or respiratory tract mimic the Th2 response induced by infection [81,84]. In contrast, protection can often be correlated with Th1 responses for vaccines which are protective against tissue-dwelling parasites including *F. hepatica* [83] and juvenile tapeworms [85].

A new impetus to the quest for anti-helminth vaccines has been provided by the increasing problems posed by acquisition of drug resistance by helminth populations, and by logistical difficulties and cost associated with the requirement for repeated drug administration as a control method. Recent increases in the amount of information available from parasite genome projects will also have a major impact on the rate of progress towards effective vaccines [2]. Gene discovery and identification of molecules for vaccine applications is already happening as a result of genome projects focused on schistosomes and *Onchocerca* spp. [86], while projects to sequence the genomes of other major pathogenic helminths including *F. hepatica*, are underway. Recent advances will allow us to use biotechnology to understand the parasite proteome and metabolome, and to use improved recombinant expression systems (yeast, baculovirus and even transfected helminth cell lines), to produce helminth proteins in as near as possible to their natural state. These new tools hold much promise for overcoming serious technical difficulties associated with helminth vaccines.

However, in order to reach the goal of commercial application, the hurdles to be overcome by helminth vaccines include not only technical and scientific problems, but also issues with regulatory agencies and public acceptance. Certainly for helminth vaccines for veterinary applications, appropriate criteria for measuring vaccine efficacy differ from those accepted for bacterial and viral vaccines, where sterile immunity is achievable. Where helminths are concerned, sterile

immunity may be neither desirable nor achievable. Vaccines capable of maintaining parasite burdens below pathogenic levels, and/or having a transmission blocking effect, while still maintaining a level of infection sufficient for immune challenge, represent the ideal. However, the regulation and acceptance of such vaccines will clearly require different standards from those applied to date. The prospects for vaccines for the control of helminth infections are further discussed in Ref. [87].

7. Exploiting helminths as therapeutic agents

Mirroring current interest in the role of helminth infection in immunopathological conditions, there have been some attempts to use helminths as tools to alleviate such diseases [88]. Given public perceptions of “worms” as associated with lack of hygiene, poverty and poor living conditions, it is probably unlikely that “worms on prescription” would ever become widely accepted, even as a therapy for diseases which have a devastating impact on sufferers’ quality of life. However, knowledge of the mechanisms whereby strong induction of Th2 responses can prevent immunopathology opens up possibilities of more acceptable novel therapies for IBD and other autoimmune conditions. Those which have been used in clinical situations to date include cytokines [89], immunomodulatory agents directed at pathogenic effector mechanisms [90], and probiotics which are capable of blocking or reducing such effector mechanisms [91].

The dramatic increase in such immune-mediated conditions in developed society as asthma and atopic allergy may mean, however, that a preventive approach on a population basis, rather than treatment of individual clinical cases, might be advisable. Such an approach might take the form of immunomodulatory vaccination in childhood, capable of providing the same type of stimulus as helminth infection. Clearly, given recent history of public mistrust and suspicion of childhood vaccinations, this approach would not be readily accepted. In veterinary medicine, there is widespread recognition that at least in production animals, “a few parasites is better than none”, from the point of view of acquisition of protective immunity, decreasing required dosing frequency and thereby

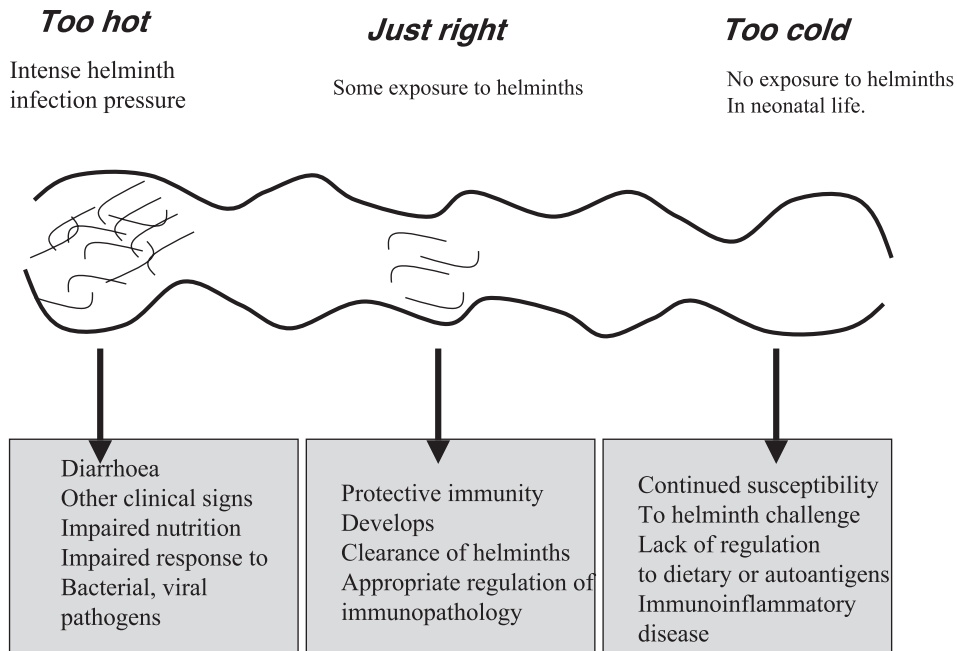


Fig. 2. The co-evolution of intestinal helminth parasites with their hosts may be responsible for immunopathological events where circumstances intervene to ensure that either very heavy infections, or none at all, take place. Generally, infections with a few parasites are compatible with the survival of both host and parasite. Large parasite burdens may build up where animals or people are crowded together in environments that facilitate faecal-oral transmission of infection, and as a result the hosts may respond sub-optimally to vaccination or to infection with other pathogens. In developed society, on the other hand, lack of exposure to helminth infection may predispose to auto-immunity, as proposed by the “hygiene hypothesis”. The immunopathological effects of too many or too few helminths, considered together, can be considered as the basis for the “Goldilocks hypothesis”, with an optimum level of exposure (not too much, not too little, but just right), conducive to immunological balance. The induction of regulatory T-cells by helminths and other pathogens may be crucial in achieving this balance.

slowing the rate of drug resistance in parasite populations. As the “hygiene hypothesis” and the “gateway hypothesis” have gained currency as possible explanations for immunopathological consequences of an absence of helminth infection, it is possible to provide a broader view of the relationship between helminth parasites, the immune system and disease by means of the “Goldilocks hypothesis” (Fig. 2).

8. Concluding remarks

Pathogenic helminths of man and animals, most of which have contact with the mucosa of the GIT for all or part of their life-cycle, have been among the most difficult category of pathogens to target with appropriate immunoprophylactic strategies. Rapid developments in parasite genomics, bioinformatics and

recombinant expression technology are now beginning to have a positive impact on the development of helminth vaccines. Throughout the history of immunology, and particularly since the advent of the Th1/Th2 paradigm, the study of immune responses to helminths has been rewarding in what it has revealed about immunoevasion and immunomodulatory mechanisms. It now seems that parasites have also something to teach us about immunoregulation, avoidance of immunopathology, and the benefits of an immune system which, like little bear’s porridge, is neither too hot not too cold, but just right.

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