Perspective: Prospects for Development of Vaccines against Human Helminth Infections

James S. McCarthy and Thomas B. Nutman

The global prevalence of infection with parasitic worms or helminths likely exceeds that of any other infection. One-third of the world’s population is estimated to harbor infection with intestinal helminths (Trichuris trichuria, Ascaris lumbricoides, Strongyloides stercoralis, or hookworm) [1]; between 200 and 300 million people are believed to be infected with one of the three species of schistosomes (Schistosoma mansoni, Schistosoma haematobium, Schistosoma japonicum) [2], and >150 million are infected with one of the pathogenic filarial parasites (Wuchereria bancrofti, Brugia malayi, Onchocerca volvulus, Loa loa) [3, 4]. The burden of helminth infection rests largely on the population of the developing world, where many persons carry infection with more than one of these parasites [5].

While nematode infection uncommonly leads to severe acute illness or death, a growing body of evidence indicates that chronic infection leads to patterns of morbidity and economic hardship not generally appreciated. For example, while heavy and chronic hookworm infection is a well-recognized cause of anemia, recent intervention studies have revealed that one course of anthelmintic treatment of schoolchildren leads to a sustained improvement in growth [6] and in academic performance [7]. Blinding eye disease is responsible for only part of the adverse impact of onchocerciasis, with chronic skin disease leading to major reduction in quality of life and economic productivity [4].

Helminth infection differs in a number of important ways from most other infectious diseases — differences that have a major impact on possible strategies for disease control. First, with few exceptions, helminths do not multiply within the human host but require passage through the environment and an arthropod vector or other invertebrate host (such as a snail) to reproduce and complete the life cycle. This offers the possibility of vector control or other environmental measures as means of disease control. Second, helminth infection may persist for years, an important consideration when control by chemotherapy or vector control is contemplated. For example, it is necessary to maintain onchocerciasis control programs using ivermectin (which is inactive against the adult worm) and vector control for 10–15 years (the life span of the adult worm). Third, the clinical effects of infection are not directly related to the presence or absence of infection but depend rather on a complex interplay of host and parasite factors, including the overall worm burden, the duration of infection, and the immune response of the host [8]. Fourth, data from epidemiologic surveys in a variety of helminth infections indicate that infection is not uniformly distributed in a population, with significant variation observed in the intensity of infection, particularly in younger age groups [8]. This variation in infection intensity is incompletely understood, with genetic, environmental, nutritional, and behavioral factors possibly contributing to the observed pattern; such variations necessitate consideration when control strategies are planned. Finally, the intensity and prevalence of infection generally fall with increasing age, suggesting the acquisition of immunity as exposure to infection accumulates (see below).

Strategies for Disease Control

Chemotherapy. Control of helminth infection by chemotherapy (either targeted to individuals identified as infected or through mass drug distribution) relies on a handful of drugs (praziquantel for schistosomiasis and other cestode parasites, ivermectin and diethylcarbamazine [DEC] for filariasis, and the benzimidazoles [e.g., albendazole] for geo- and helminths). While examples exist of successful control programs using chemotherapy (e.g., DEC-fortified table salt and mass distribution of the drug in southern China [3]), logistic and financial issues have impeded the effective implementation of control strategies based on chemotherapy. In addition, experience suggests that once such programs are relaxed, there is a rapid reemergence of infection [9]. Finally, despite the absence of well-documented resistance to any of these drugs and theoretical predictions that acquisition of such resistance is unlikely, the widespread resistance to the benzimidazole anthelmintic agents in veterinary practice [10] and the paucity of alternative chemotherapeutic agents is a cause for concern, particularly given patterns of resistance now seen in malaria and bacterial infection and recent reports of praziquantel-resistant schistosomiasis [11].

Vector control or environmental alteration. While the tools exist for the control of transmission of helminth infections (e.g., molluscicides for control of schistosomiasis, vector control for onchocerciasis, and improving disposal of human waste for geo- and helminths), control strategies based on these methods have been difficult to implement. Impediments include the environmental toxicity and expense of both manufacture and application of insecticides and molluscicides, as...
well as acquisition of resistance to these agents. Improving economic conditions—resulting in improved infrastructure and personal standard of living—effectively interrupts transmission of geohelminths and schistosomiasis. However, progress has been slow in many regions, particularly in times of rapid population growth, when social dislocation occurs through war, or when climatic change or altered environmental conditions lead to the emergence of new areas of endemicity. For example, the recent damming of the Senegal River in West Africa led to an explosive outbreak of schistosomiasis [12].

**Vaccine development.** Vaccine development has been a crucial component of the successful control of many infectious diseases (e.g., smallpox, measles, diphtheria, and invasive *Haemophilus influenzae* infection), while control has been difficult for those diseases against which no good vaccines exist (e.g., tuberculosis, human immunodeficiency virus infection, and malaria). Criteria desirable to ensure feasibility of vaccine development for a given infectious disease include the following [13]: an understanding of the important effector mechanisms in clearance of infection; a recognizable marker of immunity (e.g., a specific antibody response); the presence of a good animal model; and readily identifiable antigens to serve as the source of B and T cell epitopes (these epitopes should not vary or be subject to antigenic drift or shift).

In addition, for helminth infections—infestations in which the adult parasite can live for decades in the human host and in which populations are chronically and continually exposed to new infection—the demonstration of naturally acquired immunity in human populations also is desirable when vaccine strategies are contemplated. The paucity of vaccines for human parasitic diseases reflects the fact that many of these criteria have not been fulfilled for most of these infections.

### Naturally Acquired Human Immunity

Support for the proposition that it may be possible to develop a vaccine for control of helminth infection comes from epidemiologic studies of human populations in which evidence of naturally acquired protective immunity has been found (table 1). Foremost is the observation, in both schistosomiasis and filariasis, that the adult worm burden generally increases through childhood and adolescence but then plateaus or falls in adulthood [8]. This observation can be explained by the acquisition of so-called concomitant immunity [25], in which an individual develops resistance to new infection while harboring adult parasites. Although other explanations have been offered for this phenomenon (including reduced exposure with increasing age [8] or pathophysiologic sequelae that affect the ability of the host to harbor further infection), concomitant immunity is supported by animal models of helminth infection (see below).

Evidence also exists that some persons with helminth infection, once treated, are resistant to reinfection despite ongoing exposure to infection [14–16]. It is proposed that such individuals develop concomitant immunity and, once cured of infection with adult worms, are resistant to new infection.

In filarial infection, a small group of individuals appears to be infection-free despite long-term residence in areas of high endemicity, the so-called “putative immune” or “endemic normal” group [18, 20, 21]; however, such individuals with sterile immunity are uncommonly identified. While it is possible that mechanisms other than classical immunity (such as genetic resistance) may account for the status of such individuals, an understanding of the mechanism of resistance to infection in such individuals may lead to the development of vaccines or other therapies to block infection.

### Immune Effector Mechanisms in Human Helminth Infection

The effector mechanisms responsible for immunity in human helminth infection are not fully understood, with studies in different systems suggesting an important role for different immune effector mechanisms (table 1), including eosinophils, humoral immune responses involving IgE, or a cellular immune response polarized toward either a T helper (Th) Th1 or Th2 response.

**Humoral immunity.** The balance of antiparasite antibody isotype and subclass response appears to be an important correlate of the level of immunity. For example, in schistosomiasis, antiparasite IgE levels (and eosinophilia) are associated with lower parasite burdens [16, 26] and resistance to reinfection following curative chemotherapy [15, 27], while levels of IgG4 (frequently identified as a blocking antibody) directly correlate with intensity of infection.

Studies evaluating the role of humoral immunity in filariasis by measuring the antibody response to parasite antigen have revealed conflicting results, some suggesting an important role for parasite-specific IgG and IgE [28, 29], a finding not confirmed in other studies [21, 22]. The hypothesized stage-specific nature of the protective immune response is supported by significant differences between infected and immune subjects in response to larval stages of filarial parasites [17, 30].

**Cellular immune response.** Studies of the cellular immune responses in human filariasis suggest that a Th1-type response to parasite antigen is associated with protection [21–24]. Lymphocyte proliferation in response to parasite antigen was greater in the putative immune groups studied [21, 22, 24], as was production of interleukin-2 (IL-2) [21, 22, 24] and interferon-γ (IFN-γ) [22, 24]. Further support of the stage-specific nature of immunity is provided by the positive correlation between proliferative response of peripheral blood mononuclear cells to larval-stage antigen and age of the individual studied, a finding not observed with antigens from other life cycle stages of the parasite [19].

In contrast to filarial infection—and in agreement with the observed importance of eosinophilia and IgE in protective immunity in human schistosomiasis [14–16] and their regulation...
Animal Models of Immunity in Helminth Infection

While much information exists regarding immunity in helminth infections of veterinary importance [32], discussion will be limited to animal models of protective immunity in helminths closely related to those pathogenic for humans. Although there are significant variations in models studied (such as infection with various species into permissive, semipermissive, or nonpermissive animal hosts), immunity, albeit partial, can be induced in most systems. Generally greater protection has been found when live rather than dead parasites or parasite extracts are used as vaccines, with the best-characterized immunization strategies relying on immunization with radiation-attenuated parasites. Levels of protection of 70%-90% have been achieved in animal models of schistosomiasis, filariasis, strongyloidiasis, and hookworm (table 2). The so-called trickle-infection model perhaps most closely resembles the pattern of exposure to helminths seen in nature. In such experimental models, animals repeatedly exposed to small numbers of infective larvae of the parasite develop patent infection but become progressively more resistant to new infection [51, 52].

In addition to documenting a reduction in adult worm burden following immunization, several studies have demonstrated stunting or arrested development of larvae within immunized hosts [46, 53–60], again suggesting that the protection is mediated by the immune response to developing stages of the parasite.

The immune effector mechanisms identified to be of importance in animal models of human helminth infection are not always in agreement with those found in human studies, however. For example, in contrast to the association of IgE (the expression of which is dependent on Th2 cytokines) with immunity in human schistosomiasis, in a well-studied murine model of schistosomiasis, the Th1 cytokine IFN-γ was associated with clearance of schistosomula from the lungs of immunized mice [61]. Similarly, the association of a Th1 response with protection in human filariasis is not supported by data from animal models, which suggests an important role for Th2-type responses in immune-mediated killing of infective larvae [44, 45].

In a murine model of intestinal helminth infection with *Trichuris muris*, Th2 responses are associated with protection [62]. Neutralization of IFN-γ caused susceptible strains of mice to expel *T. muris*, while anti-IL-4 receptor antibody caused infection to persist in resistant strains. IL-4 administration to susceptible mouse strains resulted in expulsion of the worms [62].

Thus, the significant differences observed in the immune effector mechanisms responsible for protection in different species (e.g., human vs. animal models), and even between strains of the same species (e.g., different inbred strains of mice [63]), prevent the construction of a unifying theoretical framework to explain the mechanism of helminth immunity. However, the great variety in life cycle, ecologic niches, and the prolonged co-evolution of these parasites with their hosts provide likely explanations for these apparently conflicting data [64].

Nevertheless, available evidence indicates that concomitant immunity is a frequent outcome of chronic exposure to helminth parasites and that such immunity is not generally "sterile" but rather exists in a state of dynamic balance, in which newly infecting larvae face progressively stronger immune attack until the rate of successful establishment of new infection by incoming parasites is balanced or exceeded by the rate of attrition of adult worms. Many factors may influence this balance, including the activity and orientation of the immune response to the parasite, the major histocompatibility complex background of the host [65], and other less-well-understood genetic influences. Early, heavy, and prolonged infection may condition the immune response into a so-called hyporesponsive state, a phenotype typically seen in asymptomatic infected individuals and one unlikely to promote clearance of the parasite. Further, in utero exposure to parasite antigen may have a lasting effect on the pattern of immune responsiveness to helminth infection [66] and, thus, on the likely clinical outcome of infection.

Table 1. Studies indicating the existence of protective immunity in human helminth infection.

<table>
<thead>
<tr>
<th>Parasitic infection, finding</th>
<th>Immune effector mechanism implicated</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schistosomiasis</td>
<td>IgE, eosinophils, Th2 response</td>
<td>[14–16]</td>
</tr>
<tr>
<td>Individuals refractory to reinfection after curative chemotherapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphatic filariasis</td>
<td>Larval-specific immunity</td>
<td>[17]</td>
</tr>
<tr>
<td>Plateau in worm burden despite ongoing exposure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endemic uninfected (putative immune)</td>
<td>Th1 immune response</td>
<td>[18, 19]</td>
</tr>
<tr>
<td>Onchocerciasis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endemic uninfected (putative immune)</td>
<td>Th1 immune response</td>
<td>[20–24]</td>
</tr>
</tbody>
</table>
Table 2. Animal models of protective immunity in human helminth infection.

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Animal model</th>
<th>Protection (%)</th>
<th>Implicated immune effector mechanism</th>
<th>[Reference]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestinal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Trichuris muris</em></td>
<td>Mouse</td>
<td>90</td>
<td>Th2 responses</td>
<td>[33]</td>
</tr>
<tr>
<td><em>Strongyloides stercoralis</em></td>
<td>Mouse</td>
<td>0</td>
<td>IgM, complement, granulocytes</td>
<td>[34-36]</td>
</tr>
<tr>
<td><em>Ancylostoma caninum</em></td>
<td>Dog</td>
<td>80-90</td>
<td></td>
<td>[37]</td>
</tr>
<tr>
<td>Cestode</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Schistosoma mansoni</em></td>
<td>Mouse</td>
<td>90</td>
<td>IgG, Th1 responses</td>
<td>[38, 39]</td>
</tr>
<tr>
<td><em>Schistosoma haematobium</em></td>
<td>Baboon</td>
<td>76-84</td>
<td>Antibody dependant</td>
<td>[40-42]</td>
</tr>
<tr>
<td>Filarial</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Brugia malayi</em></td>
<td>Mouse</td>
<td>70</td>
<td>Th2 responses</td>
<td>[44]</td>
</tr>
<tr>
<td><em>Onchocerca volvulus</em></td>
<td>Mouse</td>
<td>64-70</td>
<td>Th2 responses</td>
<td>[45]</td>
</tr>
<tr>
<td><em>Acanthocheilonema viteae</em></td>
<td>Jird (gerbil)</td>
<td>75</td>
<td>Arrested development of larvae</td>
<td>[46]</td>
</tr>
<tr>
<td><em>Dirofilaria immitis</em></td>
<td>Dog</td>
<td>88</td>
<td></td>
<td>[47]</td>
</tr>
<tr>
<td><em>Brugia malayi</em></td>
<td>Monkey</td>
<td>71</td>
<td></td>
<td>[48]</td>
</tr>
<tr>
<td><em>Brugia pahangi</em></td>
<td>Cat</td>
<td>80</td>
<td>IgE</td>
<td>[49, 50]</td>
</tr>
</tbody>
</table>

Strategies to Identify Helminth Antigens as Possible Vaccine Targets

In "rational screening" strategies, antigens that have intuitive appeal as targets for immune attack are identified and studied. Such targets include molecules important for parasite invasion, growth, and development (e.g., molting-related proteins), metabolism (e.g., transporters or enzymes necessary for parasite survival), or protection against immune attack (e.g., antioxidants). In immunologic screening strategies, antibodies are derived from vaccinated or immune animals, monoclonal antibodies raised against parasite antigens, or from sera from "immune" human populations. These antibodies are then used to identify antigens, either directly by isolating the parasite protein or indirectly by screening recombinant expression libraries.

While the former approach has been aided by the recent gene discovery efforts as part of World Health Organization-sponsored parasite genome projects [67], such an approach relies on a priori assumptions about a particular target and thus may be unrewarding if the correct target is not selected. The latter approach is hampered by a number of potential problems, including definition of the "immune" sera and the likelihood that parasite antigens identified using this approach may be recognized by all individuals exposed to infection, regardless of their immune status, as well as the theoretical consideration that the antibody-mediated immune response may not be responsible for protection and, therefore, vaccine targets identified using this approach may not be appropriate.

Progress toward Development of Helminth Vaccines

While a number of helminth vaccines have been commercially developed for veterinary use [68, 69], no helminth vaccine candidates have yet reached human clinical trial. The greatest progress has been seen in schistosomiasis, in which several recombinant antigens have been extensively studied [70, 71] and human phase 1 trials are being contemplated for a subset [71]. Levels of protection induced in murine models have ranged from 30% to 70%, but when these vaccine candidates have been tested in nonhuman primates, lower protective efficacy has been found (25%-40%) [72-74]. A number of vaccine candidates have been identified in human filarial infection [75], and vaccination studies in murine and jird (gerbil) models have demonstrated levels of protection for some recombinant antigens that are comparable with results seen in schistosomiasis [76, 77].

Ghosh et al., in this issue of the *Journal* [78], report a vaccination study in a murine model of hookworm infection using a 42-kDa recombinant antigen, ASP-1. The molecule was first identified as a major protein secreted by *Ancylostoma caninum* larvae undergoing transition from the free-living to parasitic state [79], a developmental stage attractive for targeting by vaccine. Of interest, DNA sequence analysis revealed significant similarity of the protein to the major venom allergen of hymenopteran insects (fire ants and yellow jackets) as well as to a human testis-specific protein [79]. Vaccination of mice led to a 79% reduction in recovery of worms from the lung compared with that of unvaccinated controls, a figure comparable to that seen after repeated challenge with infective larvae. Although there is as yet no information regarding the immunogenicity of this molecule in the natural (canine) host of the parasite or any data on the role of this molecule or its homologues in human hookworm infection, further study of this molecule is warranted.

The sequence similarity of this molecule to some prominent allergens and to a human protein raises the issue of exacerbation of allergic disease, or induction of autoimmunity. While
sequence similarity has been noted between vaccine candidates for other parasitic diseases and human proteins and no adverse autoimmune consequences have been reported in animal models (e.g., [80]), such questions will require careful consideration prior to human trials.

Other Approaches to Vaccine Development

Among alternate strategies for development of vaccines for helminth infections are approaches targeting parasite reproduction. Depending on the parasite, the stage of its life cycle responsible for clinical disease in the human host, and the stage of reproduction blocked, the outcome may be an "altruistic" vaccine similar to the transmission-blocking vaccine of malaria [81] or one that may have disease-ameliorating effects. For example, vaccination with microfilarial chitinase generates an immune response that prevents microfilariae from escaping the peritrophic membrane of the mosquito vector [82]. An approach targeting receptors involved in mating of adult schistosomes [83] or otherwise interfering with schistosome egg production [72] would have the dual effect of reducing the damage to host tissues due to egg deposition and reducing the transmission of infection.

Another novel vaccine strategy showing recent promise seeks to alter the host immune response believed to cause much of the pathology in helminth infection [84]. Wynn et al. [85], in a murine model of schistosomiasis, administered the Th1 cytokine IL-12 in conjunction with schistosome eggs and observed inhibition of granuloma formation and a dramatic reduction of the tissue fibrosis induced by natural infection.

The recent demonstration that protective immunity can be induced in viral and bacterial infection [86] as well as in rodent malaria [87] by so-called DNA vaccines is an important advance, and work is under way to investigate the use of this technology in helminth infections [88]. Such vaccines offer some major advantages over standard vaccination strategies, including the ability to avoid adjuvants and obviating the requirement for expression and purification of recombinant proteins, thus providing an important alternative for rapid identification of promising vaccine candidates.

Conclusion

In addition to the impediments to the development of all vaccines—such as choice of adjuvant, schedule, and route of administration—vaccine development for parasitic diseases faces a number of obstacles not regularly encountered in viral and bacterial infections. These include the likelihood that sterile immunity is an uncertain outcome and the difficulties and expense of field trials to test vaccine efficacy (as recently illustrated by trials of SPf66, a vaccine candidate for malaria [89]). Further, since the clinical outcome of infection is not generally severe in many helminth infections, the possibility exists that disruption of the host-parasite relationship by vaccination may lead to more severe manifestations of infection (e.g., lymphedema in lymphatic filariasis or hepatic fibrosis in schistosomiasis) if the individual does become infected.

Just as our growing understanding of the biology of helminth infection and the nature of the host-parasite relationship increases our awareness of the potential obstacles to the implementation of a successful vaccine program, so this knowledge also may lead to the identification of antigens and vaccine strategies to subvert our longstanding fellow travelers.

Acknowledgments

We thank Alan Sher and Thomas Wynn of the Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, for their helpful comments.

References


