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Vaccination against helminth parasites – the ultimate challenge for vaccinologists?

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Acknowledgements

The authors gratefully acknowledge financial support from the Wellcome Trust, the INCO-DC programme of the Commission of the European Communities, the Biotechnology and Biological Sciences Research Council and the Deutsche Forschungsgemeinschaft. We thank Judith Allen for critical comments on the manuscript, and Fred Finkelman for permission to quote unpublished work.

Summary: Helminths are multicellular pathogens which infect vast numbers of human and animal hosts, causing widespread chronic disease and morbidity. Vaccination against these parasites requires more than identification of effective target antigens, because without understanding the immunology of the host-parasite relationship, ineffective immune mechanisms may be invoked, and there is a danger of amplifying immunopathogenic responses. The fundamental features of the immune response to helminths are therefore summarised in the context of vaccines to helminth parasites. The contention between type-1 and type-2 responses is a central issue in helminth infections, which bias the immune system strongly to the type-2 pathway. Evidence from both human and experimental animal infections indicates that both lineages contribute to immunity in differing circumstances, and that a balanced response leads to the most favourable outcome. A diversity of immune mechanisms can be brought to bear on various helminth species, ranging from antibody-independent macrophages, antibody-dependent granulocyte killing, and non-lymphoid actions, particularly in the gut. This diversity is highlighted by analysis of rodent infections, particularly in comparisons of cytokine-depleted and gene-targeted animals. This knowledge of protective mechanisms needs to be combined with a careful choice of parasite antigens for vaccines. Many existing candidates have been selected with host antibodies, rather than T-cell responses, and include a preponderance of highly conserved proteins with similarities to mammalian or invertebrate antigens. Advantage has yet to be taken of parasite genome projects, or of directed searches for novel, parasite-specific antigens and targets expressed only by infective stages and not mature forms which may generate immunopathology. With advances under way in parasite genomics and new vaccine delivery systems offering more rapid assessment and development, there are now excellent opportunities for new antihelminth vaccines.

Introduction

Helminths are multicellular pathogens which currently infect a startlingly high proportion of the global human and domesticated animal populations (Fig. 1). Recent estimates for the prevalence of the gut-dwelling nematode *Ascaris lumbricoides*, for example, are that over 1 billion people are infected with this one species (1). While chemotherapy is available for major helminth diseases, individuals remain highly susceptible to reinfection. The high levels of infection betray the immune system's inability to eliminate helminths under natural conditions.

All eukaryotic parasites have elaborate life cycles in comparison to prokaryotes and viruses (Fig. 2), providing correspondingly more complex interactions with the host immune system. There are often a succession of developmental stages within the one host, which may bear stage-specific antigens (permitting escape from immunity to earlier stages), or which may allow early stages to predetermine the character of responses to subsequent stages. The three taxonomic groups of helminths (nematodes, cestodes and trematodes) diverged many hundreds of millions of years ago (Fig. 3) and presumably present the immune system with a highly diverse set of challenges.

Helminth parasites occupy a panoply of niches, intestinal (gut nematodes), epithelial (lungworms), vascular (schistosomes), lymphatic and skin (filarial nematodes), and a range of internal tissues (larval tapeworms). Each of these sites is a distinct immunological compartment. Helminth immune evasion mechanisms are likely to be finely tuned to each environment, to each host species, and to the particular requirements for transmission of each parasite.

Vaccination is the route of choice to eradicate helminth infections, because although chemotherapy provides temporary respite for patients, reversion of individuals and communities to high levels of transmission usually occurs without continued treatment. Vaccination is far preferable to preventative long-term chemotherapy. Control of vector species (mosquitoes for lymphatic filarial nematodes) or intermediate hosts (snails for schistosomes) has proved extremely difficult, so that eradication of these major tropical diseases requires new approaches. Socio-economic improvements in housing and water management have not materialised in large parts of the afflicted countries, so that geohelminth infestation has not abated. Vaccination therefore offers the prospect of a simple, one-step procedure to interrupt transmission and prevent parasitic disease.

There are some substantial obstacles to vaccine development to helminths, both logistical and scientific. There are few good animal models for human helminths, and even where the human-parasitic species (such as *Schistosoma mansoni*) infects rodents, there are radical differences in dynamics of infection between the laboratory and the field which obscure correlations. More generally, there is a lack of understanding of the basic events in helminth infection which permit parasites to invade and endure in the face of a fully armed immune system.

The crucial scientific problems are those of: a) selecting individual vaccine antigens without understanding the host-parasite relationship (particularly using antibody reactivity as a criterion for a candidate antigen); b) understanding

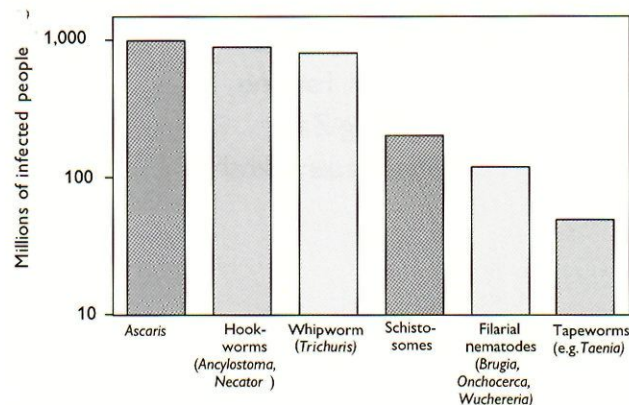
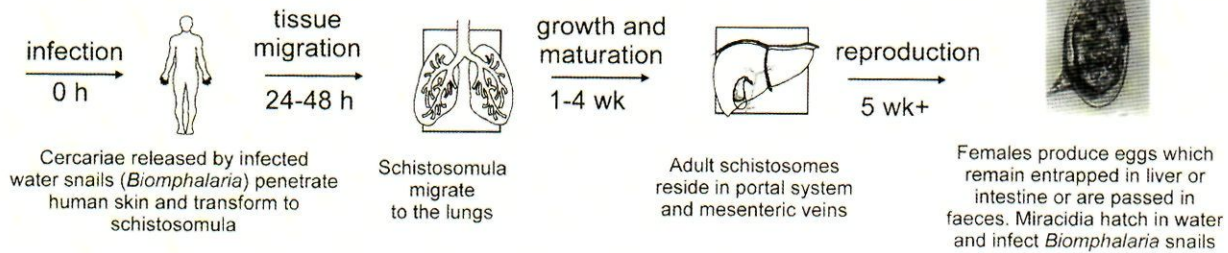
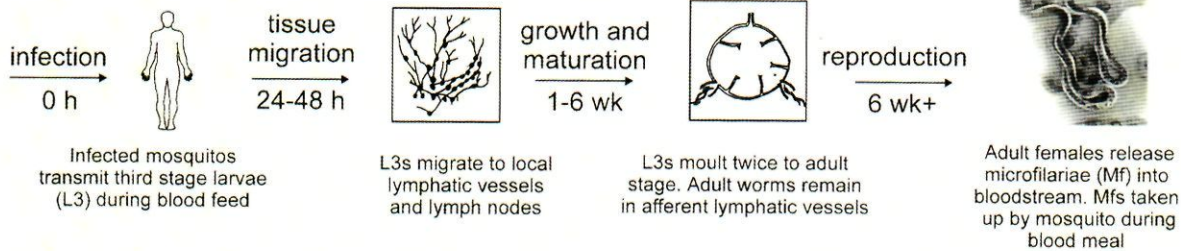
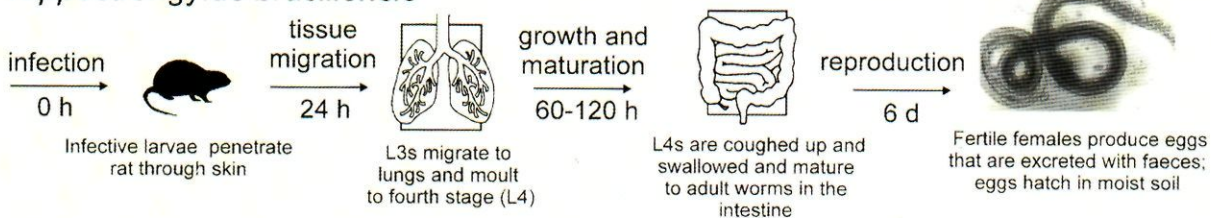


Fig. 1. Worldwide prevalence of the main helminth parasitic infections. It is estimated that over 3.5 billion people are infected with intestinal and tissue helminths, causing disease in approximately 450 million cases. Multiple infections are common. Helminth parasites cause low mortality but high morbidity, and are a major cause of retarded growth, blindness and iron deficiency anaemia. Although direct mortality is low (60,000 per annum from ascariasis for example), the impact is enormous on the number of years of life lost due to disability and early mortality (disability-adjusted life years). More information is available at <http://www.who.int/ctd> (World Health Organization) and http://www.dpd.cdc.gov/dpdx/HTML/Para_Health.htm (Centers for Disease Control and Prevention).

effective immune mechanisms which kill parasites; and c) being able to preclude pathogenic responses and avoid exacerbation of disease. The complexity of helminths relative to prokaryotes renders the empirical approach unlikely to succeed, whilst the diversity of helminth life styles and niches requires us to appreciate the intricacies of each host-parasite relationship and the individual strategies which have evolved for immune evasion in each case. The first part of this review therefore discusses the basic immunology of helminth infection, leading into the subsequent discussion of existing and future vaccines against helminths.

The basic phenomena of infection with helminths are remarkably similar: i) high levels of T-helper 2 (Th2)-type responses are generated; ii) the major pathogens cause chronic infections characterised by long-lived parasites and ineffective immune responses; and iii) various forms of immune downmodulation are observed. It is arguable whether these characteristics are beneficial to the host or the parasite (2, 3).

Schistosoma mansoni*Brugia malayi**Nippostrongylus brasiliensis***Fig. 2. Life cycles of three helminth parasites.**

[We thank J. E. Allen for permission to reproduce the panel showing microfilarial parasites of *Brugia malayi*.]

Helminths drive Th2 responses

Infection with helminth parasites typically leads to a strong type-2 immune response characterised by IgE antibodies, eosinophilia and mast cell proliferation (4–8). Why should such a striking polarisation occur? It seems unlikely that each parasite species has evolved individual Th2-stimulating antigens, particularly those species for which a Th2 response protects the host. More probable is that generic invertebrate products signal through host receptors in a way that amplifies Th2 responses. There may be a helminth equivalent of the lipopolysaccharide (LPS) receptor, an early warning ('danger') signal from the innate immune system. In any event, the powerful and selective Th2 drive seen in helminth infections highlights a phenomenon of significance to basic immunology, with impact on allergy and clinical immunology in general.

The intensity of the type-2 response to helminth infections provided clear evidence for the T-helper 1 (Th1)/Th2 dichotomy in humans, which was confirmed by the isolation of

human Th2 cells specific for *Toxocara canis* antigens (9). The Th2 phenotype in human filariasis has been documented by a variety of approaches (10–13), with high frequencies of interleukin (IL)-4, IL-5 and IL-13 expression by patients' lymphocytes in response either to parasite antigen or mitogen. The resulting levels of type-2 cytokines in helminth infection are very striking: Fig. 4 compares IL-4 production in response to polyclonal stimulation by lymphocytes from *S. mansoni*-infected and *Brugia malayi*-infected with those from uninfected controls. Polyclonal Th2 activation may account for the high levels of total IgE in helminth-infected patients, which are comparable to those seen in cases of allergic disease in non-endemic countries (Fig. 5).

In general, the Th2 bias is antigen specific, so that for example the same individual's peripheral T cells mount a Th1 response to mycobacterial purified protein derivative (PPD) while producing Th2 cytokines in response to helminth antigens (9, 10, 14). In experimental models (with maximal par-

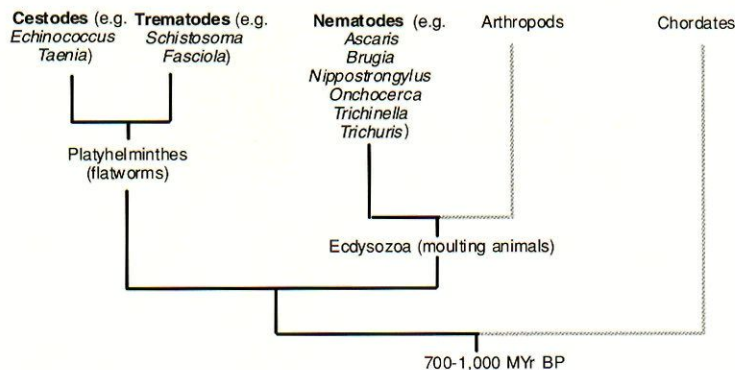


Fig. 3. Phylogenetic relationship of helminths. Based on recent analyses of molecular phylogeny (205) and the fossil record (206) of metazoa.

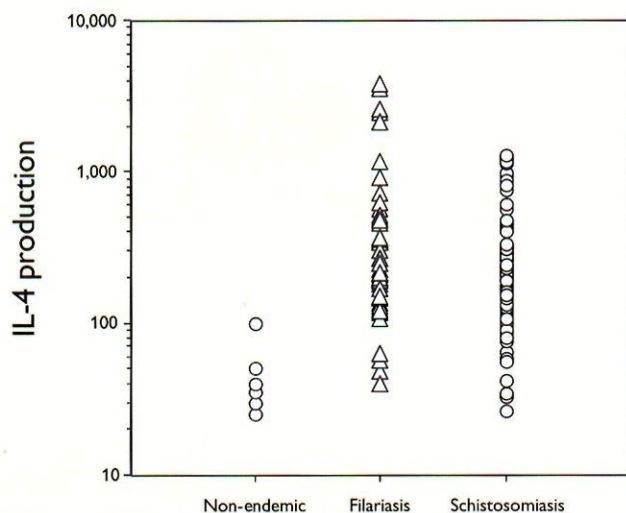


Fig. 4. Polyclonal IL-4 responses of T cells from helminth-infected humans. IL-4 (in units/ml) was measured in supernatants of mitogen-stimulated cultures of peripheral blood mononuclear cells. Each point represents a different patient.

asite loads), the Th2 skew is potent enough to convert responses to bystander antigens from Th0, or even Th1, to a Th2 pathway (15). An important and unexplored issue is whether vaccination against one pathogen is likely to interfere with or pre-empt an appropriate response to another. The propensity of some helminth-derived products to amplify Th2 responses may permit the design of vaccines which selectively induce Th2 differentiation, as discussed later in this article.

The immune system regulates pathology in chronic infection

Helminth parasites largely take their toll on human health by establishing long-term, chronic infections which often result in pathological damage to the host. Typical helminthiases present as spectral diseases, probably because of variation in immune responses to infection, and a minority (1–15%) suffer from the extremes such as elephantiasis or hepatosplenic schistosomiasis. A larger number have subclinical lesions and/or intermittent acute symptoms. Parasite lifespans can be surprisingly long – 5 years is a low estimate for filarial worms – and there will be a continuous challenge to the immune system of high levels of foreign antigens from resident worms, from their offspring, such as schistosome eggs, and from new waves of infectious organisms entering the body. A critical question is how the host deals with this unrelenting antigenic challenge, and how the immune system selects the optimum profile of responses to balance the cost of enduring parasitism with the cost of immunopathology. Chronic infection therefore requires immune regulation for host survival.

The best paradigm for chronic infection with helminths, and particularly of the immune factors associated with the development and resolution of pathology, is the granuloma around schistosome eggs in the liver of infected hosts (16). In mice experimentally infected with *S. mansoni*, hundreds of eggs begin to be produced by mature females 5 or 6 weeks post-infection, most of which remain entrapped in the liver or the intestine. These eggs elicit a strong granulomatous response, attracting eosinophils, macrophages, lymphocytes, neutrophils, mast cells and fibroblasts, leading to extensive liver fibrosis. The liver granulomas play a central role in the immunopa-

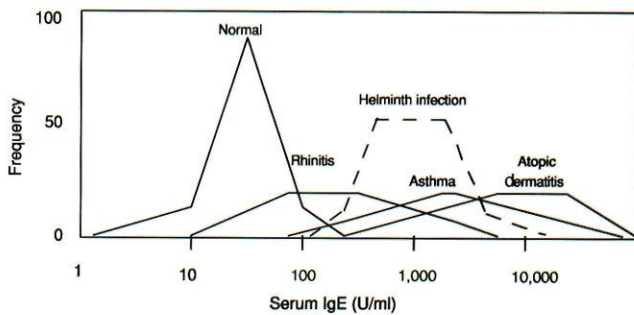


Fig. 5. Serum IgE levels in helminth infections and other disease states.

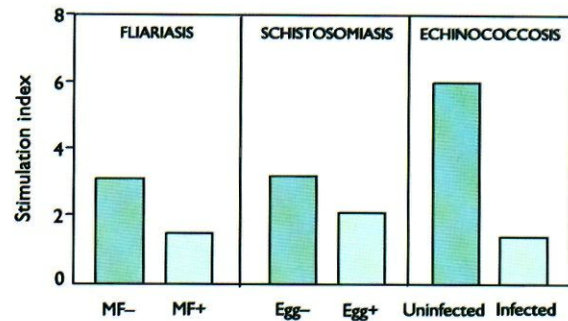


Fig. 6. T-cell hyporesponsiveness in helminth infection. Infected (dark shading) and uninfected or immune (light shading) individuals with filariasis, schistosomiasis or echinococcosis were studied by measuring peripheral blood PBMC stimulated *in vitro* with parasite antigen. Results represent the mean levels from extensive studies, as published for *B. malayi* filariasis (26), *S. haematobium* schistosomiasis (112) and *Echinococcus multilocularis* hydatid disease (207).

thology of schistosomiasis, a chronic problem, as schistosomes persist for years in untreated humans, and a single patient can carry several hundred schistosomes. Surprisingly, then, the granulomatous response may carry an immediate benefit to the host, as indicated by infection of T-cell-deficient mice in which the host's inability to respond with a granuloma results in toxic damage to hepatocytes (17, 18).

Granuloma formation requires CD4⁺ cells, but is not solely dependent on Th1 or Th2 responses. Rather, a key cytokine is tumour necrosis factor (TNF)- α , as provision of exogenous TNF- α rescues liver granuloma formation in severe combined immunodeficient (SCID) mice (18). The essential findings (reviewed in (16)) are that both type-1 and type-2 cytokines amplify the response. Granulomas form both in anti-IL-4-treated and in IL-4 knockout (KO) animals, but the Th2 contribution is demonstrated by significantly decreased granuloma size and collagen deposition in signal transducer and activator of transcription (Stat)-6 KO mice (which are profoundly deficient in Th2-cell function), and by the fact that recombinant IL-12 treatment greatly counteracts granuloma growth. In an adapted model in which lung granulomas are measured following intravenous inoculation of schistosome eggs, it has recently been shown that IL-13 is a major enhancing factor for granuloma size (19). The picture which emerges from all these studies is that granuloma formation in murine schistosomiasis results from both type-1 (TNF- α) and type-2 (IL-13) cytokines (16), and primarily involves mononuclear cells: anti-IL-5 inhibition of eosinophils has little impact on the outcome.

In the chronic phase of murine schistosome infection (18 weeks onwards), granulomas reduce in size (20). Macrophages from liver granulomas express lower levels of co-stimulator mol-

ecules, as a result of IL-10 production, and it is proposed that this loss of co-stimulation drives T cells into an anergic state (21). A more recent interpretation is that these macrophages are alternatively activated into a directly suppressive phenotype (22).

A wider perspective is provided by Brunet et al. (16, 23), who suggest that the interplay of type-1 and type-2 responses affects both immunopathology and host protection. Although type-2 cytokines increase the granulomatous response, thereby causing fibrosis and other related damage, they are also required to modulate the intensity of the Th1 response. Thus, IL-4-deficient C57BL/6 background mice die soon after the onset of egg-laying from an exaggerated type-1 reaction with an intestinal, rather than a hepatic, focus; the reaction can be alleviated with anti-TNF- α . This work implies that vaccines which stimulate untrammelled Th1 responses against helminths could in practice exacerbate pathology.

Helminth parasites induce T-cell hyporesponsiveness

In addition to downregulation of granulomatous reactions, there is a far broader range of immunomodulatory effects generated by helminth infections (24). Among the most striking are ablation of specific T proliferative responses in filariasis (25, 26) and schistosomiasis (27–29), as illustrated in Fig. 6. In onchocerciasis, unresponsiveness extends beyond parasite-specific determinants to bystander antigens and even mitogens (30). Moreover, interferon (IFN)- γ production by peripheral blood mononuclear cells (PBMC) in response to parasite antigen is also depressed in these diseases, while IL-4 is generally intact (31, 32). The loss of all proliferation is one sign that this is not a simple manifestation of a Th1 to Th2 switch; further evidence

is that IL-5 release is repressed in lymphatic filariasis (31), dissociating this response from another type-2 cytokine, IL-4. A key paradigm in filariasis is that patients with high parasite numbers are hyporesponsive and generally suffer mild forms of disease, while patients with few or no parasites and chronic pathology mount vigorous immune responses to the parasite (33, 34). The danger therefore is that vaccination may amplify or accelerate pathogenic immune responses, unless the target antigens of immunopathology are identified and excluded.

Hyporesponsiveness in helminth infections is not due to deletion of parasite-reactive T cells, as parasite-specific IL-4 is secreted by the same cell populations that fail to proliferate to parasite antigens. Moreover, proliferative responses are restored by chemotherapy (28, 35). One model postulates a state of T-cell anergy in these infections (33). Alternatively, high levels of IL-10 could account for the unresponsive phenotype (25, 29, 36), but a number of studies have failed to support these conclusions (37–39).

An interesting question is whether the downmodulation is directly induced by parasite molecules, which perhaps have evolved for this purpose as discussed below, or whether there is a self-limiting mechanism within the immune system to minimise immunopathological costs. Because downregulation is seen in a wide range of chronic infections from viruses to helminths, the latter possibility has some appeal. For example, in chronic lymphocytic choriomeningitis virus (LCMV) infection there is unresponsiveness not only in the CD8⁺ population (so permitting virus survival) but also in the CD4⁺ subsets too, apparently preventing the development of pathology (40). One possible source of immune modulation that has yet to be examined in helminth infections is the variously named regulatory T-cell subset(s) such as Th3 cells (41) and/or Tr1 cells (42).

There are three major implications for vaccination strategies. First, induction of regulatory T cells may be highly desirable as an antipathology vaccine. Secondly, if vaccine-induced immunity reverses hyporesponsiveness, there is a danger of inducing pathology. Thirdly, we have no insight into how vaccination would work in patients who are already infected and exhibit a downmodulated state.

Immune evasion

Successful helminth parasites, colonising largely extracellular niches in the tissues and mucosal surfaces, would require a battery of immune evasion mechanisms (6). MacDonald et al. describe the paradox of children with hundreds of intestinal parasites (*Trichuris trichiura*) embedded in the mucosa with no sign of any host inflammatory response (43). Potential immune

evasion mechanisms are only now beginning to be revealed, but a good indication can be gained by comparison to viral (44) and bacterial (45) strategies, which are expressed by simpler organisms with much smaller genomes. For example, the bacterium *Helicobacter pylori* expresses a VacA protein able to directly interrupt processing of tetanus toxoid (45); we have found a similar effect with the cystatin-2 product of *B. malayi* (B. Manoury, C. Watts, W. F. Gregory, R. M. Maizels, unpublished results). Parasites may also influence antigen-presenting cells to modulate co-stimulation or alter the profile of cytokines secreted. Helminths have been demonstrated to modulate B cells (46) and/or digest immunoglobulins (47), and to countermand many effector mechanisms, for example with peroxidases and dismutases (48) against reactive oxidants, and protease inhibitors against leukocyte enzymes (49). In addition, a number of more general immunomodulatory factors released by helminths, which interfere with proliferation or chemotaxis, have been described (reviewed in (6, 24)).

These concepts of host–parasite interactions in immune modulation are encapsulated in a model system in which *B. malayi* filarial nematodes are implanted into the mouse peritoneal cavity (50). Within 7 days, the parasite induces a host population of macrophage/granulocyte type cells which exert a powerful anti-proliferative effect on T cells, while leaving their capacity to produce cytokines unabated (50, 51). Induction can be reproduced with secretions from the filarial parasite and the suppressive effect is not mediated by known downmodulatory molecules such as IL-10, prostaglandins, nitric oxide or transforming growth factor (TGF)- β (51). The exciting prospect is that a novel regulatory mechanism is at play, expressed by host cells but induced by parasitic infection.

Mechanisms of immunity: how are helminths killed?

Vaccination usually assumes the stimulation of protective antibodies and/or T cells, together with activation of elements of the innate immune system. One of the great issues in helminth immunology is that the nature of these components, and even whether they are necessary and sufficient, has not been resolved. We are also relatively ignorant of where the tissue-migrating parasites are killed: only painstaking biological studies have established that most larval schistosomes are killed in the lung rather than at the cutaneous site of invasion, or in the vascular niche of adult worms (52).

Our concepts of how the immune system kills helminth parasites are based on a range of sources: *in vitro* culture and assay systems, experimental rodent models, and statistical association in human studies. From these data, two general models

Table 1. Normal levels of resistance (○) or increased susceptibility (●) to a primary infection in gene-deficient mice

	Gene deficiency								
	SCID	μMT	FcεRI	IL-3	IL-4	IL-4Rα	IL-5	IL-13	Stat-6
<i>Brugia malayi</i>	● (208)	○ (209)	—	—	○ (80)	—	—	—	—
<i>Heligmosomoides polygyrus</i>	● (70)	⊕ (69)	○ (69)	—	● (69) ^a	● (210)	—	—	—
<i>Nippostrongylus brasiliensis</i>	● (70)	—	—	○ (89)	○ (211)	● (72)	—	● (77)	● (72)
<i>Schistosoma mansoni</i>	● (18)	◆ (212) ● (213)	○ (66, 67) ● Δ (64)	—	◆ (23)	—	—	—	—
<i>Strongyloides</i>	● (214)	—	—	● (89)	—	—	● (215)	—	—
<i>Trichuris muris</i>	● (216)	—	—	—	● (73)	—	—	● (73)	—

It is important to note that any one gene deletion may exhibit contrasting phenotypes when combined with different backgrounds, particularly BALB vs B6 genotypes.

^a F. D. Finkelman, J. F. Urban, unpublished observations.

◆ signifies that KO has normal levels of resistance to primary infection but increased pathology.

⊕ signifies that KO has normal levels of resistance to primary infection but increased susceptibility to secondary infection compared to wild-type.

Δ - IgE gene deficient.

have emerged. *In vitro*, many different effector cells such as eosinophils (53) or platelets (54), acting through antibody-dependent receptors and aided by a variety of mediators kill helminths. Alternatively, activated macrophages can kill helminths in an antibody-independent manner, most usually through nitric oxide attack (55). Often, the same parasite species has been shown to be killed by a variety of cells and mediators *in vitro*, and such experiments have not convincingly established which cell types or modes of attack are most important *in vivo*. More recent experiments have implanted parasites *in vivo* in chambers: these arguably provide more authentic conditions in which to assess parasite killing (56), but in one recent comparison with the filarial nematode *Acanthocheilonema viteae*, vaccinated animals which could kill 81–100% of a normal challenge killed only 13–48% of parasites transplanted within a chamber (57). *In vivo* experiments with infected animals provide the strongest body of data, although proposed mechanisms of parasite killing are then based on inference rather than direct observation. Most instructive in this regard have been the recent series of rodent experiments with cytokine-deficient animals (Table 1). Evidence from human studies is discussed later in this review.

Role of antibodies

Antibodies appear to play a secondary role in killing helminths, and many infections show similar courses in wild-type and

B-cell-deficient animals, and in animals with FcR deletions (Table 1). Passive transfer experiments demonstrate that serum antibodies can mediate killing of helminths (58–60), although consistent reports from murine models are rare. It is also clear that IgE (61–63) and IgA (58) isotypes play particular roles. Although IgE is not necessary for expulsion of a primary *Trichinella spiralis* infection, it is involved in the rapid response to a challenge infection with parasites (61), and IgE levels correlate with clearance of adult filarial nematodes in the cat (62), and of schistosomes in the baboon (63). However, IgE-deficient mice are not greatly different from wild-types in susceptibility to *S. mansoni*; three independent systems of depletion or gene knockout produced small increases (64), decreases (65) or no change (66) in parasite establishment. Similarly, FcεRI deletion does not result in enhanced susceptibility to this parasite (67) (Table 1).

Type-2 cytokines promote immunity to intestinal nematodes

Experimental infection of mice with gastrointestinal nematodes provides a remarkably clear picture of the pathways of immune expulsion (4, 68). Expulsion of intestinal nematodes requires type-2 cytokines such as IL-4, although not necessarily Th2 lymphocytes themselves (4, 68, 69). However, each parasite species is differentially susceptible to various immune components.

Nippostrongylus expulsion is stimulated by either IL-4 or IL-13. In fact, administration of either cytokine induces expulsion in lymphocyte-cell deficient SCID or RAG-2 knockout mice (70, 71). Signalling through the IL-4R α chain (common to IL-4R and IL-13R) is required as complete ablation of expulsion can be achieved by anti-IL-4R α antibody treatment as well as deletion of the IL-4R α gene (71, 72). Receptor ligation by either cytokine leads to phosphorylation and translocation of the Stat-6 transcription factor. Thus, Stat-6 KO mice have been tested, and found to be unable to expel *N. brasiliensis* (72).

A different situation pertains in *T. spiralis* and *Trichuris muris* infections, as it appears that both IL-4 and IL-13 are necessary for expulsion. Deletion of either IL-4 or IL-13 (73) prevents expulsion of *T. muris*, as does administration of anti-IL-4R α antibody (74). *T. muris* also provides a consistent system in which the type-1 cytokines IFN- γ and IL-12 block expulsion (74, 75).

The efficacy of exogenous IL-4 or IL-13 in inducing expulsion in immunodeficient mice demonstrates that type-2 cytokines must stimulate non-lymphoid cells. Two key changes in the parasitized gut are expanded mucosal mast cell numbers and expansion of epithelial goblet cells which secrete mucins, and the latter population is strongly implicated in expulsion of *N. brasiliensis* (76). Significantly, goblet cell hyperplasia and increased mucin secretion are lost in IL-13 KO mice which show delayed expulsion of this parasite (77). In general, the mucus may trap parasites in the gut lumen and destabilise their anchorage to the epithelium; in addition, IL-4 and leukotrienes can directly act to raise peristalsis, providing a mechanical aid to expulsion of otherwise perfectly viable parasites (78). Thus, in order to achieve elimination of intestinal helminths, one would predict that both IL-4 and IL-13 need to be stimulated in the human system.

Cytokines in immunity to tissue helminths

The intestinal nematodes cannot be used as a paradigm for all helminth infections, however. Tissue infections with schistosomes (16) or filarial nematodes (79) obey quite different rules. In particular, the contributions of type-1 and type-2 cytokines are not as clear-cut. With filariae for example, IL-4 KO mice are equally able to kill infective *B. malayi* as wild-type mice (80), while mouse strains with high Th-2 responses are the most susceptible to *Litomosoides sigmodontis* (81). Likewise, IL-12 enhances the protective immunity of mice to schistosomes (82).

In murine schistosome infections, both type-1 and type-2 responses can exert some level of protection (83), and succes-

sive vaccination with irradiated parasites (see below) results in a switch from a protective Th1 to a protective Th2 response (84). The absence of Th2 cells altogether can result in fatal immunopathology (23) so that both types contribute to a healthy outcome (16). In cestode infections, a Th1 response is protective: rIL-12 confers protection against *Echinococcus multilocularis* in mice (85), while in *Taenia* infections, the early protective type-1 response is lost but type-2 responses remain as parasites progressively multiply (86). To conclude, a spectrum of Th1 to Th2 responses are involved in resistance to and recovery from helminth infections. It is likely that different combinations of cell subsets and mediators operate in the varied locales discussed here, and it may be that the protective Th1 and Th2 responses to schistosomes operate in different sites. In this case, a vaccine which induces both pathways of immunity might provide the vaccinated host with a redundancy of protection which could prove optimally effective.

Mast cells mediate immunity to some intestinal nematodes

The mast cell occupies an interesting position in immunity to parasites, because this cell is found at every stage from the initial reaction to infection (87) to the final stages of expulsion in the gut (76). There is good evidence of mast cells playing a crucial role in immunity to some parasites, but not others. Thus, giving IL-3 to nude mice allows them to expel *Strongyloides ratti* (88), and conversely *S. venezuelensis* expulsion is delayed in IL-3 KO mice (89). Overexpression of IL-9 amplifies mucosal mastocytosis and confers on mice the ability to rapidly expel *T. spiralis* (90), while Stat-6 KO mice which are susceptible to *T. spiralis* fail to exhibit host protective intestinal mastocytosis and mast cell degranulation (F. Finkelman, J. Urban, personal communication). Mast cell dependence of immunity has been observed only in infections with *Strongyloides* and *Trichinella*, parasites that survive the mucus-mediated reaction which expels *N. brasiliensis*. Other experiments aimed at elucidating the importance of mucosal mast cells are more difficult to interpret. A natural mutation (Wv) in the c-kit/stem cell factor receptor greatly reduces the mucosal mast cell population, but also impacts on the maturation of other cell types in the gut, such as intraepithelial lymphocytes, and interstitial cells of Cajal which co-ordinate smooth muscle contraction (69).

Eosinophils intercept tissue-migrating larvae

Eosinophils have been seen to be associated with helminth infection for over a century (91), and are heavily implicated in immune attack on parasites (53). The dependence of eosino-

phils on IL-5 has permitted experimental studies of the role of this cell, but results vary according to the parasite in question. For example, in anti-IL-5 treated mice the survival of *Angiostrongylus cantonensis* larvae in the brain is enhanced (92), as is that of *Onchocerca lienalis* microfilariae in the skin (93). Moreover, interception of *S. venezuelensis* en route to the gut is lost in anti-IL-5 animals, but expulsion from the gut itself is not compromised (94). Beyond these examples, however, IL-5 KO mice are no more susceptible to other helminths, despite their deficiency in eosinophils, as was originally shown by anti-IL-5 depletion experiments (95).

Studies using IL-5 transgenic mice (IL-5T), which have a life-long eosinophilia, serve to further illustrate differing modes of immunity depending on the parasite niche. IL-5T mice and wild-type littermates infected with *N. brasiliensis* clear infection with identical time frames (96), however, fecundity, size and number of worms are reduced in IL-5T mice (97). By contrast, recovery rates of *Toxocara canis* larvae were identical in IL-5T and wild-type control mice (97).

Naturally acquired immunity to helminths in humans

Human studies have had two main objectives: to establish the patterns of natural immunity to helminths, and to identify immunological parameters associated with that immunity. The most comprehensive studies on antihelminth immunity in humans have been carried out in schistosomiasis. Infection typically peaks at 13–16 years of age, followed by a decline in older subjects, from which it was concluded that immunity to incoming cercariae develops gradually, making adults resistant to infection (reviewed in (98)). Indeed chemotherapeutic intervention studies have shown that while children are susceptible, adults are immune to reinfection. Recent studies on whole populations newly exposed to *S. mansoni* have found that within a period of 18 months the age–intensity profile is typical of an established community (99, 100). This indicates that immunity to schistosomes does not need a period of more than ten years to develop and also points towards age-related resistance factors, perhaps non-immunological in nature, in schistosomiasis (101).

Naturally acquired immunity to other helminth parasites has also been demonstrated, but in every case protection is either partial or operates in a manner termed concomitant immunity, in which established parasites are unharmed by a response which blocks newly invading organisms (reviewed in (6)). The outcome of extensive work over many years is that natural immune protection can be mounted by the human immune system, but that this immunity appears to limit rather

than eliminate infection, and that immunity depends on a major age-dependent component which has yet to be identified. The latter point makes it difficult to declare that any particular helminth vaccine will be effective in children, who are the group most at risk from schistosomiasis and geohelminth infestation (102).

Human immunity: are type-2 responses responsible?

Human schistosomiasis was the first disease for which concerted efforts were made to test whether eosinophils and/or IgE-mediated resistance to infection, by comparing worm burdens at periods after chemotherapeutic clearance of parasites. Individuals classified as 'resistant' to reinfection carry approximately twice as many eosinophils as 'susceptible' cases (103). In addition, a number of independent studies have associated high levels of IgE with resistance to reinfection (104–108). For example, Hagan et al. showed that the lowest quintile in terms of serum IgE harboured on average 10 times more schistosomes than the highest quintile (104). IgE responses in hookworm infection correlate not with lower parasite numbers, but with reduced size and weight of the adult worms residing in the gut (109). In lymphatic filariasis, high IgE levels are found in all cases except those with high numbers of circulating microfilariae, but in this case the association is interpreted as parasite downregulation of IgE rather than clearance mediated by this isotype (11). The general question has been raised that an immune pathway mediated by IgE may not be one which we would wish to amplify by vaccination, for fear of stimulating adverse hypersensitivity reactions in a subset of patients (102).

The prominence of IgE naturally raises the issue of the relative importance of Th1 or Th2 cytokines in resistance to reinfection. Some studies have found higher levels of IL-5 (110), or of both IL-4 and IL-5 in response to schistosomes from individuals who are relatively resistant (111). This finding is not uniform even for schistosomiasis: one study in Gabon reported that individuals resistant to reinfection respond strongly with both IL-5 and IFN- γ in response to parasite antigens (112). In examining other helminth infections, such as lymphatic filariasis and onchocerciasis, there is essentially no support for the concept that archetypal Th2 responses mediate immunity in humans (3). In heavily parasitised cases, both IL-5 and IFN- γ are depressed (31) while individuals who appear by all criteria to be infection free have strong Th1 responses to parasites (12, 113).

The role of the immune response in pathogenesis is a major feature of disease. Thus, hepatosplenomegaly in schistosomiasis is associated with higher type-1 cytokines (IFN- γ and TNF- α), and lower type-2 (IL-5), indicating a strong skewing

towards proinflammatory cytokines (114). Likewise, chronic lymphatic disease in filariasis is associated with expression of type-1 cytokines (11). Immune function is also important in transmission of schistosomes. For example, in human immunodeficiency virus (HIV)-seropositive individuals in Kenya, egg output is 3-fold lower than in seronegative cases, and there is a significant negative correlation between egg numbers and CD4⁺ counts (115). As in animal studies, T-cell responses may therefore be essential for egg excretion. Praziquantel treatment to kill schistosomes was found to be effective in both HIV-positive and negative subjects with varying CD4 counts, arguing against a role for CD4⁺ T cells in drug-mediated clearance of parasites (116). Measurement of reinfection in these praziquantel-treated patient groups should illuminate the role of CD4 cells in resistance to reinfection.

A final and critical feature of human immunity is that mechanisms operate against parasite reproduction independently of any lethal action. Such antifecundity immunity is manifest in *S. japonicum* infections, in which egg counts but not adult numbers decay rapidly with age (117), and in hookworm infections where egg production is reduced in patients with high IgE responses (109). Induction of antifecundity immunity on its own may be sufficient to block transmission and be of significant benefit to community health. In addition, prevention of egg production by schistosomes would greatly limit the pathology suffered by infected patients.

An ideal antihelminth vaccine for human use would therefore a) induce both Th1 and Th2-type responses; b) be effective in children as well as adults; c) avoid any danger of inducing immunopathology or enhancing transmission; and d) attack the helminth's reproductive capacity as well as the viability of the parasite itself.

Are there resistance genes in humans?

An important question for any vaccine is whether there are polymorphic loci encoding resistance factors which may influence the outcome of infection. This issue has only been seriously addressed in schistosomiasis, in which a resistance gene termed SM1 (118), mapped to chromosome 5, showed significant association in two independent populations, in Brazil (119) and Senegal (120). Moreover, the same locus now appears to be associated with controlling parasitaemia in malaria (121, 122), which raises the interesting proposition of a common mechanism in immunity to these extremely different parasites.

There is less clarity surrounding the influence of host major histocompatibility complex (MHC) loci on immunity to

helminths. An association of MHC class II alleles with resistance to *S. japonicum* has been reported in one study (123), but requires confirmation in an unrelated group of subjects. Other MHC class II alleles have been associated in independent studies on *O. volvulus* infection with progression to pathology (124, 125), but no consistent linkage has been found in lymphatic filariasis (126, 127). This is an area where future efforts will need to be extended if helminth vaccination is to proceed in the human population.

Identification of protective antigens

By identifying immune humans, it may be possible to identify individual molecules which act as the target for immunity and which therefore would be first choice antigens for a vaccine. This approach need not assume that there is a qualitative difference in recognition, and indeed most studies have been conducted by statistically correlating magnitude of responses to a range of proteins with the disease status of the donors. So far, however, the entire search for antigens involved in protective immunity in humans has relied on antibody reactivity as the discriminatory criterion. Products which selectively stimulate T cells, or those which are normally immunologically silent (128) have yet to be fully explored. The ease of recombinant protein analysis and production has resulted in relatively little attention being given to carbohydrate and other non-protein determinants despite evidence that they can be important in generation of protective immunity (129).

Comparing responsiveness of resistant and susceptible individuals in Brazil (130) and Egypt (131), two independent groups have reported that recognition of the *S. mansoni* 37 to 42-kDa glyceraldehyde 3-phosphate dehydrogenase (G3PDH) is associated with immunity. Monoclonal antibody to G3PDH, a parasite surface antigen (130), confers marginal, but statistically significant, protection (38%) in mice against infection with cercariae (132). Immunisation with a gel-purified fraction containing G3PDH gave 40–61% protection in mice and hamsters (131), comparable with other purified proteins used in vaccination experiments (Table 2).

On an alternative track, proceeding from evidence that human immunity to schistosomes may be IgE-mediated, two 22 kDa antigens have been shown to be major targets of the IgE response in *S. mansoni*-infected subjects (133). IgE reactivities to the larger (22.6 kDa) protein showed a negative correlation with worm burden even after correction for age (134). Interestingly, there is a family of related antigens in trematodes, which are all these dynein-like tegumental proteins. Similar antigens, of 21.7 (135) and 22.6 kDa (136), have been identi-

Table 2. Purified helminth antigens showing vaccine potential

Species	Antigen	Host	Protection	Reference
<i>Acanthocheilonema viteae</i>	Tropomyosin	Jird	24–64%	(168)
<i>Brugia malayi</i>	BmA-2, 120 kDa	Jird	88%	(217)
	Paramyosin	Mouse	42–61% (Mf)	(218)
<i>Fasciola hepatica</i>	Fh-GST	Sheep	57%	(219)
	Cathepsin L2	Cattle	72%	(220)
	+ Haemoglobin			
<i>Haemonchus contortus</i>	H-11	Sheep	83%	(178)
	GA1 gut protein	Goat	60%	(221)
<i>Schistosoma mansoni</i>	GST (28 kDa)	Rat	72%	(222)
	Paramyosin	Mouse	33%	(223)
	p42 (G3PDH)	Mouse	40%	(131)
<i>Trichinella spiralis</i>	43 kDa ("48/55kDa")	Mouse	59–81%	(224)
<i>Trichostrongylus colubriformis</i>	18 kDa ES ag	Guinea pig	84%	(225)

Note that this table highlights a small proportion of the literature in which helminth antigens have been isolated and tested for vaccine potential. Further antigens have been designated as potential vaccine candidates on the basis that monoclonal antibodies to those antigens confer passive protection in animal models, for example *S. mansoni* triose phosphate isomerase (41–50% protection in mice (226))

fied in *S. japonicum* as targets of IgE, and there is a homologue in *Fasciola hepatica*. In mice vaccinated against *S. mansoni* (discussed in detail below), the 21.7 kDa antigen was clearly identified as the target of antibody responses (137).

In lymphatic filariasis one interesting study identified a 43-kDa chitinase as being selectively recognised by immune individuals (138). Curiously, the sequence is 46% identical to rickettsial (*Serratia marcescens*) chitinase and only 23% to a previously reported *B. malayi* chitinase, MF1 (139). It now seems likely that the 43 kDa protein is encoded by an endosymbiotic rickettsial-like organism, *Wolbachia*, present in tissues of *B. malayi* (140); this does not detract from it being a possible immune target during infection, but raises questions of how intact filarial parasites could be attacked by effector cells specific for chitinase and other internal proteins.

Successful vaccines: somatic and secreted proteins

Helminths are multicellular organisms occupying extracellular niches. The large majority of proteins they synthesise are destined to remain within the body of the parasite, whether as cytosolic, plasma membrane or internally secreted substances. While these will inevitably encounter immune recognition, only damaged or dead parasites are likely to release such antigens, arguing that immune responses against somatic proteins will be futile. This position receives some support from the fact that in general protein extracts from helminths fail to generate

protective immunity (141), although schistosomes are an important exception to this (55).

A more selective avenue has been to generate immune responses to the small subset of products released as 'excretory/secretory' antigens (141–143), either directly by collecting supernatant from parasite cultures, or indirectly as perhaps is occurring in the attenuated vaccines described below. As early as 1955, it was shown that mice immunised with *Trichinella* ES expelled parasites more rapidly (144). Subsequent testing in a wide range of parasites has proven ES proteins to be successful immunogens, but for most helminth parasites secreted proteins are available in such small quantities that this approach has not been widely adopted.

A combination of strategies has led investigators to purify native helminth antigens for vaccine testing. For example, schistosome somatic antigens, which can be protective when injected in some contexts, contains several potential vaccine candidates (Table 2). Likewise, purification of components in ES materials, and isolation of native antigens recognised by antibodies from immune donors, provides additional candidate vaccine antigens which have been tested in animal models (Table 2). Such candidates should be verified by testing with recombinant versions of the proteins in question; in some cases this has been achieved (Table 3), but a number of prominent candidates have yet to show active vaccination in recombinant form.

Radiation-attenuated vaccines

The first convincing demonstration that antihelminth vaccines are a realistic goal was the successful immunisation of animals with radiation-attenuated larval parasites. Irradiation levels which prevent developmental progress, but permit a degree of tissue migration and short-term viability, are optimal. This pathway has been successfully followed for most important helminth parasites, and constitutes the central paradigm for helminth immunology from which immune mechanisms and identification of vaccine antigens has followed.

Irradiated larval vaccines were developed commercially in the 1960s for use against the veterinary nematodes *Dictyocaulus viviparus* (145) and *Ancylostoma caninum* (146), and subsequently for a number of other nematode parasites (reviewed in (141, 142, 147)). In general, these vaccines reduce parasite establishment by 90% or more in a wide range of species from farm animals to rodents. In murine schistosomiasis, irradiated cercarial vaccines achieve lower levels of protection (60–80%), but offer a ready experimental system to analyse the basis for immunity (148, 149). The issues of parasite attenuation, migration, immune stimulation and mechanisms of immunity induced by schistosome vaccination have been fully described in recent reviews (148, 149). The most relevant questions for vaccination are the modes and targets of the protective immune response.

Surprisingly, the irradiated schistosome vaccine can induce both a protective Th1 and protective Th2 response, according to the time and number of immunisations. There is conclusive evidence that the protective effect of a single vaccination with irradiated schistosomula is dependent on type-1 responses, and that similar protection can be induced with somatic extracts together with Th1-promoting adjuvants such as Bacillus Calmette Guérin (BCG) (150) or IL-12 (151). In particular, IFN- γ R knockout mice have reduced levels of protection from the vaccine (24% reduction compared to 53% in wild-type mice) (152), while injection of IL-12 increases the efficacy of protection to 87% or more (82). When mice are multiply immunised (three injections at 28-day intervals), immunity operates through a Th2-associated pathway (84). However, even in the latter case, the Th1 arm remains strong, as IL-4 knockout mice are almost as immune as wild-types after 3 vaccinations (153).

There would appear to be a patent contradiction between the evidence for type-2 immune protection against human schistosomiasis, and a type-1 response mediating immunity in mice. However, once sufficiently amplified, Th2 responses can protect mice against schistosome challenge. Moreover, there is much stronger evidence of human immunity to filariasis being

dependent upon Th1 activation (12, 113). Thus, there is every possibility that, as in mice, both type-1 and type-2 responses can protect humans against helminths in the appropriate circumstances. It is important to remember that even if only type-2 responses are naturally mobilised against schistosome infection, that does not preclude vaccine-induced type-1 responses from being equally or more effective in promoting immunity from infection.

While irradiated live parasites constitute effective vaccines against most helminths, there are few which present a practical proposition for veterinary use, and none would be acceptable for humans. The focus therefore is increasingly to identify individual parasite antigens which may be applied to subunit vaccines, most probably based on recombinant proteins although the possibility of carbohydrate protective antigens should not be ignored.

Vaccine antigens for schistosomiasis

Based on antibody responses in schistosome-vaccinated mice, a set of six vaccine candidate antigens has been put forward for phase I trials. These are paramyosin (Sm97) (154), glutathione-S-transferase (GST) 28 kDa (155), rIrV5 (myosin-like 62 kDa fragment) (156); Sm23, integral membrane protein (157); 28-kDa triose phosphate isomerase (TPI) (132); and Sm14, fatty acid-binding protein (158). However, more extensive comparisons for these six antigens have been disappointing in laboratory immunisations of mice, with none reaching a threshold of 40% protection (159).

The selection of these six antigens for schistosomiasis has not been universally accepted (102, 160), as five are intracellular and only one a membrane protein. Moreover, all are clear homologues of known proteins from higher organisms. An important issue for vaccine antigens belonging to conserved gene families (e.g. enzymes, muscle proteins) is the risk of cross-reactions with host or environmental antigens. For example, the recombinant rIrV-5 *S. mansoni* vaccine candidate is 51% identical to human cardiac myosin heavy chain (156), while the 22 kDa schistosome vaccine antigen identified by human IgE recognition shares epitopes with seafood allergens (136). So far, very few schistosome membrane/secreted proteins have been identified by antibody screening, and if the optimal immunogen is a schistosome-specific membrane protein, it may very well have not yet been identified (102).

The case has been argued that alternative antigens, perhaps defined by means other than serological reactivities, should be considered (102, 160). Recently, a T-cell clone specific for *S. mansoni* adult worm antigen was generated, and by screening

Table 3. Recombinant vaccines against helminths

Species	Antigen	Host	Protection	Reference
CESTODES				
<i>Echinococcus granulosus</i>	EG95	Sheep	96%	227
<i>Taenia crassiceps</i>	KET7c	Mouse	30–73%	228
<i>Taenia ovis</i>	45W	Sheep	88%	201
<i>Taenia saginata</i>	TSA-9, -18	Cow	88–99%	229
NEMATODES				
<i>Acanthocheilonema viteae</i>	Ov-Tropomyosin	Jird	46%	175
<i>Ancylostoma caninum</i>	ASP-1	Mouse	79%	230
<i>Brugia malayi</i>	Bm-PMY	Jird	43%	170
	Chitinase	Jird	80% (Mf)	174
	SXP-1	Jird	97% (Mf)	174
<i>Onchocerca volvulus</i>	Ov-Tropomyosin	Mouse	48–62% (Mf)	175
<i>Trichinella spiralis</i>	43 kDa aa 40–80 ^a	Mouse	64%	231
TREMATODES				
<i>Fasciola hepatica</i>	Sm-FABP	Mice	100%	158
<i>Schistosoma mansoni</i>	Sm GST (28 kDa)	Rat	67%	232
	Sm14 (FABP)	Mouse	37–89%	158
	rIrV5	Mouse	32–75%	156

^a Synthetic peptide corresponding to sequence deduced from cDNA

Note that there is an extensive literature on recombinant helminth antigen immunisation, and this table provides highlighted examples of the major model systems rather than a comprehensive account. All experiments measure immunity to infective stage parasites, except where immunity to transferred microfilariae is tested, denoted by (Mf).

400 cDNA clones known to react with anti-*S. mansoni* antibodies, the protein calpain was identified as the antigen (161). Since the T-cell clone was isolated from mice vaccinated (with adult worm antigen + BCG) and carried the Th1 phenotype associated with immunity in mice, calpain may be considered to be a potential vaccine antigen. Dessein and colleagues (162) have isolated T-cell clones from human patients, and identified a dynein light chain-like 10 kDa protein as the cognate antigen (163).

A summary of the major achievements with recombinant vaccines against the major helminths, including schistosomes, is presented in Table 3. It is important to note that any realistic vaccine is likely to combine a number of effective immunogens, although there are few examples of synergistic or additive protective effects when multiple immunisations are given against helminths.

Vaccine antigens for filariasis

Vaccine research in filariasis has been hampered by the lack of a convenient rodent model (79, 164), and antigens have been

tested in a range of contexts: the susceptible jird (or gerbil, *Meriones unguiculatus*) or the resistant mouse, which quickly kills larvae under normal conditions. *Meriones* will accept several species which are more or less well-adapted to this host. Mice will harbour transplants of maturing stages or an intravenously transferred microfilaraemia. Thus, there has been no single experimental model of immunity on which to base the search for antigens. Perhaps the most coherent model is that of *Acanthocheilonema viteae*, which is a natural parasite of the jird and for which both irradiated larvae and secreted antigens generate high levels of immunity (165); immunity acts on the maturing larvae in vivo (166). Using this system, the major antigens recognised serologically are chitinase (167) and tropomyosin (168).

The major human filarial parasites are *B. malayi* and *Wuchereria bancrofti*. Only the former will successfully infect laboratory rodents, and although irradiated filarial larvae vaccinate jirds, the only antigen identified with antibodies from vaccinees is paramyosin (169). Thus, *B. malayi* paramyosin generated 43% reduction in worm load in one experiment, but the homologous protein from *Dirofilaria immitis* (98% amino acid identity) showed no protection whatsoever (170). The weakness of the animal model has encouraged other approaches of identifying surface proteins or larval-specific products without selecting according to antibody activity. We have identified a number of larval antigens from *B. malayi* by the criteria of abundance of mRNA in the mosquito-borne infective larva, including the abundant larval transcript family (ALT) (171).

Because pathology in filariasis is strongly linked to immune responsiveness to antigens associated with the adult stages (33, 34), there is a powerful argument for restricting filarial vaccine candidates to antigens expressed only in the mosquito-borne infective larvae and in early post-infective stages. The ALT family described above (171) may therefore be a good target antigen, and indeed preliminary data suggest these proteins can act as protective immunogens against *B. malayi* (W. F. Gregory, R. M. Maizels, unpublished data) and *O. volvulus* (172) infective larvae.

There is also potential in immunising against the microfilarial stage, which would act to block transmission rather than to prevent infection altogether. Several microfilariae-specific antigens have been considered. A 75-kDa surface-associated chitinase (173) is the target of protective monoclonal antibodies, and vaccination reduces microfilaraemia without affecting adult worm counts (174). Immunity to the microfilariae of *Onchocerca* can be generated in mice with tropomyosin (175), an antigen identified by reactivity of vaccinated cattle; in humans, levels of antitropomyosin antibody inversely correlate with microfilarial load (176).

Hidden antigens in vaccines against gastrointestinal nematodes

Substantial efforts have been made to generate new vaccines to nematodes of farm animals, in part because of the existing economic cost of these parasites, and in part because of the ability to test vaccines directly in the intended host species. This area has recently been reviewed (177). The present discussion will focus on the concept of 'cryptic antigens', those which are never normally exposed to the immune system and may therefore not have evolved evasive characteristics such as antigenic variation. A lead in this concept has come from studies on transmission blocking immunity in malaria, where antibodies to stages only developing in the mosquito blood meal can prevent onward transmission. A further extension has been in vaccines against ectoparasites, such as ticks and fleas, in which the target can be arthropod gut proteins. By analogy, therefore, we can vaccinate against blood-sucking nematode parasites by using components of the worm's gut brush border membrane. Studies in *Haemonchus contortus* have been pioneered by Munn and colleagues, first with contortin, a polymeric protein from the parasite's intestinal lumen, and more extensively with H11, an integral membrane protein on intestinal microvilli (177). Immunisation causes dramatic drops in egg counts (up to 99%) and 83% in adult worm numbers (177, 178).

New vaccine candidates from helminth genomics

In the past few years, the sequencing of a number of microbial pathogen genomes with sizes of 1–5 million base pairs has been completed and an entire field of functional genomics has developed around the use of these sequence data for deciphering pathogenesis and developing new pharmaceutical targets (179). At present two helminth parasite genome projects are well advanced: *B. malayi* (180) and *S. mansoni* (181). Because of the relatively large size of helminth genomes (100 million base pairs or more), these projects have concentrated on expression sequencing, providing single-pass sequence data on large numbers of cDNA clones from libraries representing different stages of the parasites. Some 16,000 expressed sequence tags (ESTs) are now available for *B. malayi*; and smaller numbers for *S. japonicum* (182), *Necator americanus*, *Onchocerca volvulus* and *Toxocara canis*. Although these projects do not describe the genomic environment, they are likely to have identified all major antigens from these parasites. We believe the opportunity has come to start exploiting this information to develop helminth parasite vaccines, in a manner now being pursued for malaria (183), and

to conduct EST projects for further helminth species for which new vaccines would be desirable.

For pathogens with small genomes, a feasible approach is expression library immunisation, in which a genomic library in plasmids is tested directly in animal models (184). This approach is problematic for eukaryotic parasites with large gene complements and intronic genes. An alternative approach, which we consider more practical, is to identify highly expressed stage-specific genes by analysis of EST information, and to assess these candidates in a murine model. Preliminary evidence in our own laboratory indicates that this track is promising, as we can induce strong immune responses with proteins encoded by dominant mRNAs expressed by *B. malayi* infective larval (171) and microfilarial (49) stages corresponding to >1% of ESTs and specific to each stage. Over the coming few years, we anticipate a closer integration of genomic information and immunological assays for vaccines.

New bacterial, viral and DNA vaccine vectors

The development of multivalent vaccines for human and veterinary use based on the expression of heterologous antigens in live attenuated bacteria such as *Salmonella* spp. has received considerable attention in the last decade. Attenuated bacteria carrying precise deletions in their genome for reducing virulence and immunogenicity and gene insertions which code for protective antigens from other pathogens can be delivered orally to re-establish a self-limiting infection persisting in the tissues for a few weeks before being cleared. During that time, they can evoke a broad spectrum of immune responses against their own antigens as well as recombinant heterologous antigens. A few helminth parasite antigens, from *S. mansoni* (185), *E. granulosus* (186) and *B. malayi* (187), have been tested by expression in attenuated bacteria (*Salmonella* or *Bordetella pertussis*) in animal models (see Table 4). These induce strong immune responses and, it is hoped, longer-lasting mucosal immunity appropriate for vaccines against intestinal helminths. Intranasal delivery of bacterial vaccines offers a further alternative: mice given a single attenuated (toxin-deleted) *Bordetella pertussis* carrying the *S. mansoni* GST (Sm28GST) gene, had significant reductions of worm burden (56%) and liver egg counts (60%) after parasite challenge compared with non-immunised mice (185).

Perhaps the most exciting development in vaccine technology in recent years, however, has been the development of naked DNA gene transfection techniques (DNA vaccines). DNA vaccines are attractive for several reasons: their simplicity, cir-

Table 4. Bacterial and DNA vaccines

Species	Antigen	Protection	Reference
Attenuated bacteria immunisation/vaccine			
<i>Brugia malayi</i>	Gp29 glutathione peroxidase	NT	187
<i>Echinococcus granulosus</i>	Fatty acid-binding protein	NT	186
<i>Schistosoma mansoni</i>	Glutathione S-transferase	56%	185
Recombinant viral (adenovirus) vector			
<i>Taenia ovis</i>	45W	65%	233
DNA immunisation/vaccine			
<i>Schistosoma japonicum</i>	Paramyosin	NT	234
<i>Schistosoma japonicum</i>	Sj22	NT	235
<i>Schistosoma mansoni</i>	Glutathione S-transferase	NT	236
<i>Taenia crassiceps</i>	KETc7	59–100%	190
<i>Taenia ovis</i>	45W	99%	233

NT = Protection not tested

NB: where multiple constructs or vectors of the same parasite antigen have been tested (e.g. *S. mansoni* GST) only the most recent publication is cited.

cumventing issues of recombinant protein expression and purity; their synthesis of antigens with mammalian post-translational modification and conditions favourable for protein folding; and the fact that DNA itself has adjuvant properties, evidently biasing towards the Th1 pathway (188).

DNA vaccines have been shown to be effective at generating protective immune responses against a wide variety of infections (189), but with relatively little attention paid to multicellular parasites. Some antigen genes from *Schistosoma* and *Taenia* species have been found to induce significant humoral immune responses in mice and sheep by DNA immunisation (Table 4). Most studies have simply demonstrated immunogenicity and have not tested for protection, with the exception of DNA vaccination with a proline-rich peptide gene from *Taenia crassiceps*. This yielded 59% protection, similar to the level induced by the protein itself, indicating that DNA vaccination may be a feasible approach for helminth antigens (190).

Co-immunisation strategies

An important approach to immunisation is to potentiate the host response by co-administration of cytokines in protein or DNA form and/or to selectively drive particular subsets, according to the choice of cytokine. For example, as immunity to schistosomes in the mouse model is Th1 dependent, Mountford et al. used IL-12 to convert a non-protective larval extract into an immunogen eliciting 15–50% protection, less than an irradiated vaccine but relatively effective for a non-living schis-

tosome immunisation (191). Equally, the irradiated vaccine itself is boosted from 56% to 87% protection if IL-12 is co-administered with the vaccine (82). The incorporation of cytokine genes as molecular adjuvants has been reported in a number of experimental DNA vaccines (192). Because DNA vaccination generally favours Th1-type responses, it may be desirable to co-deliver DNA expression constructs encoding antigen with the gene plasmids encoding the Th2-type cytokines to provide a more appropriate and balanced response. However, as discussed above, the most appropriate cytokines for resistance to helminth infection have yet to be defined.

In many helminthiases, disease is partly or even mostly the result of immunological reactions. There is therefore due concern that injudicious amplification of responses may exacerbate rather than alleviate disease, as has been recorded for certain viral vaccines (193). This issue is reversed by recent thinking on developing vaccines with an objective purely of blocking parasite-induced pathology and not attacking the parasites themselves. Although this would not lead to eradication, it would greatly alleviate the health consequences of infection. One example of an antipathology vaccine has been suggested in schistosomiasis, in which co-administration of IL-12 with *S. mansoni* eggs reduces the granulomatous liver reactions to those eggs (194, 195). It is entirely possible that selected helminth proteins themselves would be desirable components for co-immunisation, particularly where a type-2 response is desired. Secreted proteins from *Nippostrongylus brasiliensis*, if injected subcutaneously, stimulate a generic rise in total IgE lev-

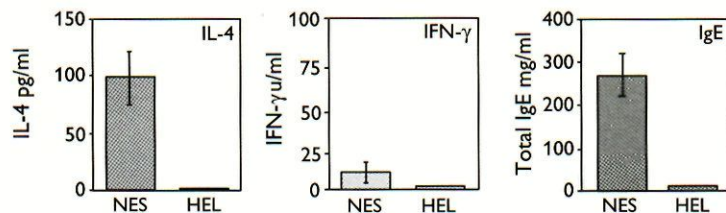


Fig. 7. *Nippostrongylus brasiliensis* ES (NES) provokes Th2 cells and polyclonal IgE production in mice. BALB/c mice were immunised with NES or control protein (HEL) in complete Freund's adjuvant at d0. At d35, they were boosted with NES or HEL in incomplete Freund's adjuvant, and at d42, serum and popliteal lymph nodes were collected for IgE assay and *in vitro* lymphocyte stimulation with the homologous antigen. IL-4 and IFN- γ release was quantified in culture supernatants by enzyme-linked immunosorbent assay (ELISA) (M. J. Holland, R. M. Maizels, manuscript in preparation).

els in mice (Fig. 7) (M. J. Holland, R. M. Maizels, manuscript in preparation).

The striking induction of antigen-specific hyporesponsiveness in helminth-infected patients and animal models, bears some similarity to tumour immune responses. One exciting development in tumour vaccination recently has been that tumour lysate- or peptide-pulsed dendritic cells (DC) induced therapeutic antimelanoma immunity in humans *in vivo* (196). The parallels between tumour and parasite evasion of immune responses suggest that it would be well worth investigating the potential for DC-based vaccines against helminths. In particular, it is now known that DC can be equally potent stimulators of Th2 responses (197, 198), even in the absence of IL-4 (199). This would obviate a direct role of early IL-4 in establishing a primary Th2 bias following infection, and provide a unifying model for Th2 induction irrespective of mucosal, epithelial or deep tissue sites of invasion. Direct experimental evidence of DC polarisation by parasite products is now being sought, and this approach may lead to definition of new pathways for driving Th2 responses. In parallel, vaccine constructs containing determinants which target expressed proteins to DC or other key antigen-presenting cell subsets may allow us to direct the form of immune response elicited by vaccination (200).

Conclusions

There is an enormous need for antihelminth vaccines, as chemotherapy provides only temporary respite from infection in the vast majority of helminth diseases. There is also a consider-

able number of helminth species, both human and animal parasites, for which vaccines would be widely applicable. Taken together, it is perhaps surprising that only a few vaccines have been brought into practical use, and that these are all for veterinary use. However, the immunological context of helminth parasitism is relatively complex, and in most instances reviewed here a simplistic approach to identifying prospective vaccine components has yielded disappointing results. Only once has the holy grail been attained – single, novel parasite protein which, when purified and correctly presented, constitutes a potent vaccine – in the case of *Taenia ovis* in which a recombinant 45W of unknown function behaves in exactly this fashion (201).

So far, the most immunogenic antigens from the perspective of antibody reactivity have been identified, cloned and tested as potential vaccine molecules. In this first phase, a small number of protein classes dominated: paramyosin and tropomyosin, GSTs, and metabolic enzymes such as TPI. These are not necessarily the most suitable vaccine antigens, and some of these dominant antigens may simply be major antibody targets on account of their relative abundance in the parasite, if antibody levels are attuned to antigen concentrations. Rational antigen selection demands an understanding of how the irradiated vaccines work, and an appreciation of the contributions of individual mechanisms rather than T-cell subsets as a whole to protective immunity.

The expansion of parasite genome projects offers an excellent opportunity to focus on 'novel' vaccine antigens with no identifiable homology to previously characterised proteins,

which represent parasite-specific products. Interestingly, the 45W antigen of *T. ovis* falls into this category. Such antigens may well be essential to the parasitic way of life and represent adaptations to the mammalian environment. If so, they are likely to be excellent targets for immune attack, and of course carry minimal risk of stimulating any autoimmune reactions.

Secondly, the window through which we look to select vaccine antigens should be that of the T cell, not just the B cell. The major T-cell antigens of helminth parasites are still undefined, due to current difficulties identifying CD4⁺ T-cell-stimulating antigens (202). Even those T-cell antigens, such as *S. mansoni* calpain, which have been found were first selected through an antibody reactive filter. While the presence of serum antibodies against parasite antigens is certainly proof that the antigens are 'seen' by the host's immune system during

natural infection, the complexity of the serum response is such that it is quite likely that many predominantly T-cell antigens have been missed by antibody screening. Therefore, some new approaches are required to directly screen T-cell antigens, such as those used to isolate Ldp23 from *Leishmania donovani* (203) and *Leishmania* homologue of receptor for activated protein C kinase (LACK) from *Leishmania major* (204).

Armed with a new generation of vaccine antigens, perhaps isolated by their stimulation of T cells or equally derived from a genome-inspired analysis of expression patterns, the substantial advances in understanding the immunology of helminth infections can be exploited to develop new, rational vaccines for the wide range of these highly prevalent and debilitating diseases of the world's human and animal populations.

References

1. Crompton DWT. The prevalence of ascariasis. *Parasitol Today* 1988;**4**:162–169.
2. Allen JE, Maizels RM. Immunology of human helminth infection. *Int Arch Allergy Immunol* 1996;**109**:3–10.
3. Allen JE, Maizels RM. Th1–Th2: reliable paradigm or dangerous dogma? *Immunol Today* 1997;**18**:387–392.
4. Urban JF, et al. The importance of Th2 cytokines in protective immunity to nematodes. *Immunol Rev* 1992;**127**:205–220.
5. Sher AF, Coffman RL. Regulation of immunity to parasites by T cells and T cell-derived cytokines. *Annu Rev Immunol* 1992;**10**:385–409.
6. Maizels RM, Bundy DAP, Selkirk ME, Smith DF, Anderson RM. Immunological modulation and evasion by helminth parasites in human populations. *Nature* 1993;**365**:797–805.
7. Locksley RM. Th2 cells: help for helminths. *J Exp Med* 1994;**179**:1405–1407.
8. Abbas AK, Murphy KM, Sher A. Functional diversity of helper T lymphocytes. *Nature* 1996;**383**:787–793.
9. Del Prete G, et al. Purified protein derivative of *Mycobacterium tuberculosis* and excretory-secretory antigen(s) of *Toxocara canis* expand human T cells with stable and opposite (type 1 T helper or type 2 T helper) profile of cytokine production. *J Clin Invest* 1991;**88**:346–350.
10. Mahanty S, et al. IL-4- and IL-5-secreting lymphocyte populations are preferentially stimulated by parasite-derived antigens in human tissue invasive nematode infections. *J Immunol* 1993;**151**:3704–3711.
11. Maizels RM, Sartono E, Kurniawan A, Selkirk ME, Partono F, Yazdanbakhsh M. T cell activation and the balance of antibody isotypes in human filariasis. *Parasitol Today* 1995;**11**:50–56.
12. Dimock KA, Eberhard ML, Lammie PJ. Th1-like antifilarial immune responses predominate in antigen-negative persons. *Infect Immun* 1996;**64**:2962–2967.
13. De Boer B, Fillié YE, Kruize YCM, Yazdanbakhsh M. Antigen-stimulated IL-4, IL-13 and IFN- γ production by human T cells at a single cell level. *Eur J Immunol* 1998;**28**:3154–3160.
14. Sartono E, Kruize YCM, Kurniawan A, Maizels RM, Yazdanbakhsh M. In Th2 biased lymphatic filarial patients, responses to PPD remain Th1. *Eur J Immunol* 1996;**26**:501–504.
15. Kullberg MC, Pearce EJ, Hieny SE, Sher A, Berzofsky JA. Infection with *Schistosoma mansoni* alters Th1/Th2 cytokine responses to a non-parasite antigen. *J Immunol* 1992;**148**:3264–3270.
16. Brunet LR, Dunne DW, Pearce EJ. Cytokine interaction and immune responses during *Schistosoma mansoni* infection. *Parasitol Today* 1998;**14**:422–427.
17. Doenhoff MJ, et al. Immunological control of hepatotoxicity and parasite egg excretion in *Schistosoma mansoni* infections: stage specificity of the reactivity of immune serum in T-cell deprived mice. *Trans R Soc Trop Med Hyg* 1981;**75**:41–53.
18. Amiri P, et al. Tumour necrosis factor α restores granulomas and induces parasite egg-laying in schistosome-infected SCID mice. *Nature* 1992;**356**:604–607.
19. Chiamonte MG, Schopf LR, Neben TY, Cheever AW, Donaldson DD, Wynn TA. IL-13 is a key regulatory cytokine for Th2 cell-mediated pulmonary granuloma formation and IgE responses induced by *Schistosoma mansoni* eggs. *J Immunol* 1999;**162**:920–930.
20. Stadecker MJ. The shrinking schistosomal egg granuloma: how accessory cells control T cell-mediated pathology. *Exp Parasitol* 1994;**79**:198–201.
21. Stadecker MJ, Flores Villanueva PO. Accessory cell signals regulate Th-cell responses: from basic immunology to a model for helminthic disease. *Immunol Today* 1994;**15**:571–574.
22. Goerdt S, Orfanos CE. Other functions, other genes: alternative activation of antigen-presenting cells. *Immunity* 1999;**10**:137–142.
23. Brunet LR, Finkelman FD, Cheever AW, Kopf MA, Pearce EJ. IL-4 protects against TNF- α -mediated cachexia and death during acute schistosomiasis. *J Immunol* 1997;**159**:777–785.
24. Behnke JM, Barnard CJ, Wakelin D. Understanding chronic nematode infections: evolutionary considerations, current hypotheses and the way forward. *Int J Parasitol* 1992;**22**:861–907.
25. King CL, et al. Cytokine control of parasite-specific anergy in human lymphatic filariasis. Preferential induction of a regulatory T helper type 2 lymphocyte subset. *J Clin Invest* 1993;**92**:1667–1673.

26. Yazdanbakhsh M, et al. T cell responsiveness correlates differentially with antibody isotype levels in clinical and asymptomatic filariasis. *J Infect Dis* 1993;**167**:925–931.
27. Colley DG, et al. Immune responses during human schistosomiasis. XII. Differential responsiveness in patients with hepatosplenic disease. *Am J Trop Med Hyg* 1986;**35**:793–802.
28. Grogan JL, Kremsner PG, Deelder AM, Yazdanbakhsh M. Elevated proliferation and interleukin-4 release from CD4⁺ cells after chemotherapy in human *Schistosoma haematobium* infection. *Eur J Immunol* 1996;**26**:1365–1370.
29. King CL, et al. Cytokine control of parasite-specific anergy in human urinary schistosomiasis. IL-10 modulates lymphocyte reactivity. *J Immunol* 1996;**156**:4715–4721.
30. Greene BM, Fanning MM, Ellner JJ. Non-specific suppression of antigen-induced lymphocyte blastogenesis in *Onchocerca volvulus* infection in man. *Clin Exp Immunol* 1983;**52**:259–265.
31. Sartono E, Kruize YCM, Kurniawan-Atmadja A, Maizels RM, Yazdanbakhsh M. Depression of antigen-specific interleukin-5 and interferon- γ responses in human lymphatic filariasis as a function of clinical status and age. *J Infect Dis* 1997;**175**:1276–1280.
32. Yazdanbakhsh M. Common features of T cell reactivity in persistent helminth infections: lymphatic filariasis and schistosomiasis. *Immunol Lett* (In press).
33. Maizels RM, Lawrence RA. Immunological tolerance: the key feature in human filariasis? *Parasitol Today* 1991;**7**:271–276.
34. Ottesen EA. Infection and disease in lymphatic filariasis: an immunological perspective. *Parasitology* 1992;**104**:S71–S79.
35. Sartono E, et al. Elevated cellular responses and interferon- γ release after long-term diethylcarbamazine treatment of patients with human lymphatic filariasis. *J Infect Dis* 1995;**171**:1683–1687.
36. Mahanty S, et al. High levels of spontaneous and parasite antigen-driven interleukin-10 production are associated with antigen-specific hyporesponsiveness in human lymphatic filariasis. *J Infect Dis* 1996;**173**:769–773.
37. Dimock KA, Addiss DG, Eberhard ML, Lammie PJ. Differential proliferative and interleukin-10 responses to fractionated filarial antigens: preferential recognition by patients with chronic lymphatic dysfunction. *J Infect Dis* 1994;**170**:403–412.
38. Sartono E, Kruize YCM, Partono F, Kurniawan A, Maizels RM, Yazdanbakhsh M. Specific T cell unresponsiveness in human filariasis: diversity in underlying mechanisms. *Parasite Immunol* 1995;**17**:587–594.
39. Grogan JL, Kremsner PG, Deelder AM, Yazdanbakhsh M. The effect of anti-IL-10 on proliferation and cytokine production in human schistosomiasis: fresh versus cryopreserved cells. *Parasite Immunol* 1998;**20**:345–349.
40. Oxenius A, Zinkernagel RM, Hengartner H. Comparison of activation versus induction of unresponsiveness of virus-specific CD4⁺ and CD8⁺ T cells upon acute versus persistent viral infection. *Immunity* 1998;**9**:449–457.
41. Chen Y, Kuchroo VK, Inobe J-I, Hafler DA, Weiner HL. Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. *Science* 1994;**265**:1237–1240.
42. Groux H, et al. A CD4⁺ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* 1997;**389**:737–742.
43. MacDonald TT, et al. Histopathology and immunohistochemistry of the caecum in children with *Trichuris* dysentery syndrome. *J Clin Pathol* 1991;**44**:194–199.
44. Ploegh HL. Viral strategies of immune evasion. *Science* 1998;**280**:248–253.
45. Molinari M, et al. Selective inhibition of Ii-dependent antigen presentation by *Helicobacter pylori* toxin VacA. *J Exp Med* 1998;**187**:135–140.
46. Harnett W, Harnett MM. Inhibition of murine B cell proliferation and down-regulation of protein kinase C levels by a phosphorylcholine-containing filarial excretory-secretory product. *J Immunol* 1993;**151**:4829–4837.
47. Auriault C, Ouassii MA, Torpier G, Eisen H, Capron A. Proteolytic cleavage of IgG bound to the Fc receptor of *Schistosoma mansoni* schistosomula. *Parasite Immunol* 1981;**3**:33–41.
48. Ou X, Tang L, McCrossan M, Henkle-Dührsen K, Selkirk ME. *Brugia malayi*: localisation and differential expression of extracellular and cytoplasmic CuZn superoxide dismutases in adults and microfilariae. *Exp Parasitol* 1995;**80**:515–529.
49. Zang XX, Yazdanbakhsh M, Kiang H, Kanost MR, Maizels RM. A novel serpin expressed by the blood-borne microfilariae of the parasitic nematode *Brugia malayi* inhibits human neutrophil serine proteinases. *Blood* (In press).
50. Allen JE, Lawrence RA, Maizels RM. Antigen presenting cells from mice harboring the filarial nematode, *Brugia malayi*, prevent cellular proliferation but not cytokine production. *Int Immunol* 1996;**8**:143–151.
51. MacDonald AS, Maizels RM, Lawrence RA, Dransfield I, Allen JE. Requirement for in vivo production of IL-4, but not IL-10, in the induction of proliferative suppression by filarial parasites. *J Immunol* 1998;**160**:1304–1312.
52. Wilson RA, Coulson PS. Lung-phase immunity to schistosomes: a new perspective to an old problem. *Parasitol Today* 1989;**5**:274–278.
53. Butterworth AE, Sturrock RF, Houba V, Mahmoud AA, Sher A, Rees PH. Eosinophils as mediators of antibody-dependent damage to schistosomula. *Nature* 1975;**256**:727–729.
54. Capron A, Dessaint JP, Capron M, Ouma JH, Butterworth AE. Immunity to schistosomes: progress toward vaccine. *Science* 1987;**238**:1065–1072.
55. James SL. Induction of protective immunity against *Schistosoma mansoni* by a nonliving vaccine. III. Correlation of resistance with induction of activated larvicidal macrophages. *J Immunol* 1986;**136**:3872–3877.
56. Lange AM, Yutanawiboonchai W, Lok JB, Trpis M, Abraham D. Induction of protective immunity against larval *Onchocerca volvulus* in a mouse model. *Am J Trop Med Hyg* 1993;**49**:783–788.
57. Taylor MJ, van Es RP, Shay K, Townson S, Bianco AE. *Acanthocheilonema viteae*: reduction in the expression of protective immunity against infective larvae in the jird as assessed by micropore chamber vs systemic challenge infections. *Exp Parasitol* 1995;**80**:560–562.
58. Lloyd S, Soulsby EJJ. The role of IgA immunoglobulins in the passive transfer of protection to *Taenia taeniaciformis* in the mouse. *Immunology* 1978;**34**:939–945.
59. Mangold BL, Dean DA. Passive transfer with serum and IgG antibodies of irradiated cercaria-induced resistance against *Schistosoma mansoni* in mice. *J Immunol* 1986;**136**:2644–2648.
60. Medeiros F, Baldwin CI, Denham DA. *Brugia pahangi* in cats: the passive transfer of anti-microfilarial immunity from immune to non-immune cats. *Parasite Immunol* 1996;**18**:79–86.
61. Ahmad A, Wang CH, Bell RG. A role for IgE in intestinal immunity. Expression of rapid expulsion of *Trichinella spiralis* in rats transfused with IgE and thoracic duct lymphocytes. *J Immunol* 1992;**146**:3563–3570.

62. Baldwin CI, de Medeiros F, Denham DA. IgE responses in cats infected with *Brugia pahangi*. *Parasite Immunol* 1993;**15**:291–296.
63. Nyindo M, et al. Role of adult worm antigen-specific immunoglobulin E in acquired immunity to *Schistosoma mansoni* infection in baboons. *Infect Immun* 1999;**67**:636–642.
64. King CL, Xianli J, Malhotra I, Liu S, Mahmoud AAF, Oettgen HC. Mice with a targeted deletion of the IgE gene have increased worm burdens and reduced granulomatous inflammation following primary infection with *Schistosoma mansoni*. *J Immunol* 1997;**158**:294–300.
65. Amiri P, Haak-Frendscho M, Robbins K, McKerrow JH, Stewart T, Jardieu P. Anti-immunoglobulin E treatment decreases worm burden and egg production in *Schistosoma mansoni*-infected normal and interferon- γ knockout mice. *J Exp Med* 1994;**180**:43–51.
66. El Ridi R, Ozaki T, Kamiya H. *Schistosoma mansoni* infection in IgE-producing and IgE-deficient mice. *J Parasitol* 1998;**84**:171–174.
67. Jankovic D, et al. Fc ϵ RI deficient mice infected with *Schistosoma mansoni* mount normal Th2-type responses while displaying enhanced liver pathology. *J Immunol* 1997;**159**:1868–1875.
68. Maizels RM, Holland M. Parasite immunity: pathways for expelling intestinal parasites. *Curr Biol* 1998;**8**: R711–R714.
69. Finkelman FD, et al. Cytokine regulation of host defense against parasitic gastrointestinal nematodes: lessons from studies with rodent models. *Annu Rev Immunol* 1997;**15**:505–533.
70. Urban JF Jr, Maliszewski CR, Madden KB, Katona IM, Finkelman FD. IL-4 treatment can cure established gastrointestinal nematode infections in immunocompetent and immunodeficient mice. *J Immunol* 1995;**154**:4675–4684.
71. Barner M, Welte M, Brombacher F, Kopf M. Differences between IL-4R α and IL4-deficient mice reveal a novel role of IL-13 in the regulation of Th2 responses. *Curr Biol* 1998;**8**:669–672.
72. Urban JF Jr, et al. IL-13, IL-4R α and Stat6 are required for the expulsion of the gastrointestinal nematode parasite *Nippostrongylus brasiliensis*. *Immunity* 1998;**8**:255–264.
73. Bancroft AJ, McKenzie ANJ, Grencis RK. A critical role for IL-13 in resistance to intestinal nematode infection. *J Immunol* 1998;**160**:3453–3461.
74. Else KJ, Finkelman FD, Maliszewski CR, Grencis RK. Cytokine-mediated regulation of chronic intestinal helminth infection. *J Exp Med* 1994;**179**:347–351.
75. Bancroft AJ, Else KJ, Sypek JP, Grencis RK. Interleukin-12 promotes a chronic intestinal nematode infection. *Eur J Immunol* 1997;**27**:866–870.
76. Nawa Y, et al. Selective effector mechanisms for the expulsion of intestinal helminths. *Parasite Immunol* 1994;**16**:333–338.
77. McKenzie GJ, Bancroft A, Grencis RK, McKenzie ANJ. Central role for interleukin-13 in Th2 cell mediated immune responses. *Curr Biol* 1998;**8**:339–342.
78. Vallance BA, Collins SM. The effect of nematode infection upon intestinal smooth muscle function. *Parasite Immunol* 1998;**20**:249–253.
79. Selkirk ME, Maizels RM, Yazdanbakhsh M. Immunity and the prospects for vaccination against filariasis. *Immunobiology* 1992;**184**:263–281.
80. Lawrence RA, Allen JE, Gregory WF, Kopf M, Maizels RM. Infection of IL-4 deficient mice with the parasitic nematode *Brugia malayi* demonstrates that host resistance is not dependent on a Th2 dominated immune response. *J Immunol* 1995;**154**:5995–6001.
81. Maréchal P, et al. Immune response to the filaria *Litomosoides sigmodontis* in susceptible and resistant mice. *Parasite Immunol* 1997;**19**:273–279.
82. Wynn TA, et al. IL-12 enhances vaccine-induced immunity to schistosomes by augmenting both humoral and cell-mediated immune responses against the parasite. *J Immunol* 1996;**157**:4068–4078.
83. Anderson S, Shires VL, Wilson RA, Mountford AP. In the absence of IL-12, the induction of Th1-mediated protective immunity by the attenuated schistosome vaccine is impaired, revealing an alternative pathway with Th2-type characteristics. *Eur J Immunol* 1998;**28**:2827–2838.
84. Caulada-Benedetti Z, Al-Zamel F, Sher A, James S. Comparison of Th1- and Th2-associated immune reactivities stimulated by single versus multiple vaccination of mice with irradiated *Schistosoma mansoni* cercariae. *J Immunol* 1991;**146**:1655–1660.
85. Emery I, Leclerc C, Sengphommachanh K, Vuitton DA, Liance M. In vivo treatment with recombinant IL-12 protects C57BL/6J mice against secondary alveolar echinococcosis. *Parasite Immunol* 1998;**20**:81–91.
86. Terrazas LI, Bojalil R, Govezensky T, Larralde C. Shift from an early protective Th1-type immune response to a late permissive Th2-type response in murine cysticercosis (*Taenia crassiceps*). *J Parasitol* 1998;**84**:74–81.
87. Sabin EA, Pearce EJ. Early IL-4 production by non-CD4⁺ cells at the site of antigen deposition predicts the development of a T helper cell response to *Schistosoma mansoni* eggs. *J Immunol* 1995;**155**:4844–4853.
88. Abe T, Sugaya H, Ishida K, Khan WI, Tasdemir I, Yoshimura K. Intestinal protection against *Strongyloides ratti* and mastocytosis induced by administration of interleukin-3 in mice. *Immunology* 1993;**80**:116–121.
89. Lantz CS, et al. Role for interleukin-3 in mast-cell and basophil development and in immunity to parasites. *Nature* 1998;**392**:90–93.
90. Faulkner H, Humphreys N, Renauld J-C, Van Snick J, Grencis RK. Interleukin-9 is involved in host protective immunity to intestinal nematode infection. *Eur J Immunol* 1997;**27**:2536–2540.
91. Brown TR. Studies on trichinosis, with especial reference to the increase of the eosinophilic cells in the blood and the muscle, the origin of these cells and their diagnostic importance. *J Exp Med* 1898;**3**:315–347.
92. Sasaki O, Sugaya H, Ishida K, Yoshimura K. Ablation of eosinophils with anti-IL-5 antibody enhances the survival of intracranial worms of *Angiostrongylus cantonensis* in the mouse. *Parasite Immunol* 1993;**15**:349–354.
93. Folkard SG, Hogarth PJ, Taylor MJ, Bianco AE. Eosinophils are the major effector cells of immunity to microfilariae in a mouse model of onchocerciasis. *Parasitology* 1996;**112**:323–329.
94. Korenaga M, Hitoshi Y, Yamaguchi N, Sato Y, Takatsu K, Tada I. The role of interleukin-5 in protective immunity to *Strongyloides venezuelensis* infection in mice. *Immunology* 1991;**72**:502–507.
95. Sher A, Coffman RL, Hieny S, Cheever AW. Ablation of eosinophil and IgE responses with anti-IL-5 or anti-IL-4 antibodies fails to affect immunity against *Schistosoma mansoni* in the mouse. *J Immunol* 1990;**145**:3911–3916.
96. Shin E-H, Osada Y, Chai J-Y, Matsumoto N, Takatsu K, Kojima S. Protective roles of eosinophils in *Nippostrongylus brasiliensis* infection. *Int Arch Allerg Appl Immunol* 1997;**114** (Suppl 1):45–50.
97. Dent LA, et al. Interleukin-5 transgenic mice show enhanced resistance to primary infections with *Nippostrongylus brasiliensis* but not primary infections with *Toxocara canis*. *Infect Immun* 1999;**67**:989–993.
98. Hagan P, Ndhlovu PD, Dunne D. Schistosome immunology, more questions than answers. *Parasitol Today* 1998;**14**:407–412.

99. Stelma FF, et al. Epidemiology of *Schistosoma mansoni* infection in a recently exposed community in northern Senegal. *Am J Trop Med Hyg* 1993;**49**:701–706.
100. Ouma JH, et al. The development of schistosomiasis mansoni in an immunologically naive immigrant population in Masongalani, Kenya. *Parasitology* 1998;**117**:123–132.
101. Fulford AJC, Webster M, Ouma JH, Kimani G, Dunne DW. Puberty and age-related changes in susceptibility to schistosome infection. *Parasitol Today* 1998;**14**:23–26.
102. Wilson RA, Coulson PS. Why don't we have a schistosomiasis vaccine? *Parasitol Today* 1998;**14**:97–99.
103. Hagan P, Wilkins HA, Blumenthal UJ, Hayes RJ, Greenwood BM. Eosinophilia and resistance to *Schistosoma haematobium* in man. *Parasite Immunol* 1985;**7**:625–632.
104. Hagan P, Blumenthal UJ, Dunn D, Simpson AJG, Wilkins HA. Human IgE, IgG4 and resistance to reinfection with *Schistosoma haematobium*. *Nature* 1991;**349**:243–245.
105. Rihet P, Demeure CE, Bourgois A, Prata A, Dessein AJ. Evidence for an association between human resistance to *Schistosoma mansoni* and high anti-larval IgE levels. *Eur J Immunol* 1991;**27**:2679–2686.
106. Dunne DW, et al. Immunity after treatment of human schistosomiasis: association between IgE antibodies to adult worm antigens and resistance to reinfection. *Eur J Immunol* 1992;**22**:1483–1494.
107. Grogan JL, et al. Antischistosome IgG4 and IgE responses are affected differentially by chemotherapy in children versus adults. *J Infect Dis* 1996;**173**:1242–1247.
108. Zhang Z, et al. Association between IgE antibody against soluble egg antigen and resistance to reinfection with *Schistosoma japonicum*. *Trans R Soc Trop Med Hyg* 1997;**91**:606–608.
109. Pritchard DI, Quinnell RJ, Walsh EA. Immunity in humans to *Necator americanus*: IgE, parasite weight and fecundity. *Parasite Immunol* 1995;**17**:71–75.
110. Roberts M, et al. Immunity after treatment of human schistosomiasis: association between cellular responses and resistance to infection. *Infect Immun* 1993;**61**:4984.
111. Medhat A, et al. Increased interleukin-4 and interleukin-5 production in response to *Schistosoma haematobium* adult worm antigens correlates with lack of reinfection after treatment. *J Infect Dis* 1998;**178**:512–519.
112. Grogan JL, Kremsner PG, Deelder AM, Yazdanbakhsh M. Antigen-specific proliferation and interferon- γ and interleukin-5 production are down-regulated during *Schistosoma haematobium* infection. *J Infect Dis* 1998;**177**:1433–1437.
113. Elson LH, et al. Immunity to onchocerciasis: putative immune persons produce a Th1-like response to *Onchocerca volvulus*. *J Infect Dis* 1995;**171**:652–658.
114. Mwatha JK, et al. High levels of TNF, soluble TNF receptors, soluble ICAM-1, and IFN- γ , but low levels of IL-5, are associated with hepatosplenic disease in human schistosomiasis mansoni. *J Immunol* 1998;**160**:1992–1999.
115. Karanja DM, Colley DG, Nahlen BL, Ouma JH, Secor WE. Studies on schistosomiasis in western Kenya: I. Evidence for immune-facilitated excretion of schistosome eggs from patients with *Schistosoma mansoni* and human immunodeficiency virus coinfections. *Am J Trop Med Hyg* 1997;**56**:515–521.
116. Karanja DM, et al. Studies on schistosomiasis in western Kenya: II. Efficacy of praziquantel for treatment of schistosomiasis in persons coinfecting with human immunodeficiency virus-1. *Am J Trop Med Hyg* 1998;**59**:307–311.
117. Mitchell GE, Garcia EG, Rivera PT, Tiu WU, Davern KM. Evidence for and implications of anti-embryonation immunity in schistosomiasis. *Exp Parasitol* 1994;**79**:546–549.
118. Abel L, Demenais F, Prata A, Souza AE, Dessein A. Evidence for the segregation of a major gene in human susceptibility/resistance to infection by *Schistosoma mansoni*. *Am J Hum Genet* 1991;**48**:959–970.
119. Marquet S, et al. Genetic localization of a locus controlling the intensity of infection by *Schistosoma mansoni* on chromosome 5q31-q33. *Nat Genet* 1996;**14**:181–184.
120. Müller-Myhsok B, et al. Further evidence suggesting the presence of a locus, on human chromosome 5q31-q33, influencing the intensity of infection with *Schistosoma mansoni*. *Am J Hum Genet* 1997;**61**:452–454.
121. Garcia A, et al. Linkage analysis of blood *Plasmodium falciparum* levels: interest of the 5q31-q33 chromosome region. *Am J Trop Med Hyg* 1998;**58**:705–709.
122. Rihet P, Traore Y, Abel L, Aucan C, Traore-Leroux T, Fumoux F. Malaria in humans: *Plasmodium falciparum* blood infection levels are linked to chromosome 5q31-q33. *Am J Hum Genet* 1998;**63**:498–505.
123. Waine GJ, Ross AG, Williams GM, Sleight AC, McManus DP. HLA class II antigens are associated with resistance or susceptibility to hepatosplenic disease in a Chinese population infected with *Schistosoma japonicum*. *Int J Parasitol* 1998;**28**:537–542.
124. Meyer CG, et al. HLA-D alleles associated with generalized disease, localized disease, and putative immunity in *Onchocerca volvulus* infection. *Proc Natl Acad Sci USA* 1994;**91**:7515–7519.
125. Murdoch ME, et al. HLA-DQ alleles associate with cutaneous features of onchocerciasis. *Hum Immunol* 1997;**55**:46–52.
126. Chan SH, et al. HLA and filariasis in Sri Lankans and Indians. *Southeast Asian J Trop Med Public Health* 1984;**15**:281–286.
127. Yazdanbakhsh M, et al. HLA and elephantiasis in lymphatic filariasis. *Hum Immunol* 1995;**44**:58–61.
128. Munn EA. Rational design of nematode vaccines: hidden antigens. *Int J Parasitol* 1997;**27**:359–366.
129. Dunne DW. Schistosome carbohydrates. *Parasitol Today* 1990;**6**:45–48.
130. Goudot-Crozel V, Caillol D, Djabali M, Dessein AJ. The major parasite surface antigen associated with human resistance to schistosomiasis is a 37-kDa glyceraldehyde-3P-dehydrogenase. *J Exp Med* 1989;**170**:2065–2080.
131. El Ridi R, et al. T and B cell reactivity to a 42-kDa protein is associated with human resistance to both schistosomiasis mansoni and haematobium. *J Infect Dis* 1998;**177**:1364–1372.
132. Shoemaker C, Gross A, Gebremichael A, Harn D. cDNA cloning and functional expression of the *Schistosoma mansoni* protective antigen triose-phosphate isomerase. *Proc Natl Acad Sci USA* 1992;**89**:1842–1846.
133. Dunne DW, et al. The isolation of a 22 kDa band after SDS-PAGE of *Schistosoma mansoni* adult worms and its use to demonstrate that IgE responses against the antigen(s) it contains are associated with human resistance to infection. *Parasite Immunol* 1997;**19**:79–89.
134. Webster M, et al. Human immunoglobulin E responses to a recombinant 22.6-kilodalton antigen from *Schistosoma mansoni* adult worms are associated with low intensities of reinfection after treatment. *Infect Immun* 1996;**64**:4042–4046.
135. Hafalla JCR, Alamares JG, II, Acosta LP, Dunne DW, Ramirez BL, Santiago ML. Molecular identification of a 21.7 kDa *Schistosoma japonicum* antigen as a target of the human IgE response. *Mol Biochem Parasitol* 1999;**98**:157–161.
136. Santiago ML, et al. Identification of the *Schistosoma japonicum* 22.6-kDa antigen as a major target of the human IgE response: similarity of IgE-binding epitopes to allergen peptides. *Int Arch Allerg Immunol* 1998;**117**:94–104.

137. Francis P, Bickle Q. Cloning of a 21.7-kDa vaccine-dominant antigen gene of *Schistosoma mansoni* reveals an ED hand-like motif. *Mol Biochem Parasitol* 1992;**50**:215–224.
138. Freedman DO, Nutman TB, Ottesen EA. Protective immunity in Bancroftian filariasis. Selective recognition of a 43-kD larval stage antigen by infection-free individuals in an endemic area. *J Clin Invest* 1989;**83**:14–22.
139. Raghavan N, Freedman N, Freedman DO, Fitzgerald PC, Ottesen EA, Nutman TB. Cloning and characterization of a potentially protective chitinase-like recombinant antigen from *Wuchereria bancrofti*. *Infect Immun* 1994;**62**:1901–1908.
140. Bandi C, Anderson TJC, Genchi C, Blaxter ML. Phylogeny of *Wolbachia*-like bacteria in filarial nematodes. *Proc R Soc Lond B Biol Sci* 1998;**265**:2407–2413.
141. Lloyd S. Progress in immunization against parasitic helminths. *Parasitology* 1981;**83**:225–242.
142. Maizels RM, Selkirk ME. Immunobiology of nematode antigens. In: Englund PT, Sher A, eds. *The biology of parasitism*. New York: Alan R. Liss Inc.; 1988. p. 285–308.
143. Lightowlers MW, Rickard MD. Excretory-secretory products of helminth parasites: effects on host immune responses. *Parasitology* 1988;**96**:S123–S166.
144. Campbell CH. The antigenic role of the excretions and secretions of *Trichinella spiralis* in the production of immunity in mice. *J Parasitol* 1955;**41**:483–491.
145. Jarrett WFH, Jennings FW, McIntyre WIM, Mulligan W, Urquhart GM. Irradiated helminth larvae in vaccination. *Proc R Soc Med* 1958;**51**:743–744.
146. Miller TA. Vaccination against the canine hookworm diseases. *Adv Parasitol* 1971;**9**:153–183.
147. Klei TR. Development of vaccines against equine helminths. *Parasitol Today* 1986;**2**:80–81.
148. Richter D, Harn DA, Matuschka F-R. The irradiated cercariae vaccine model: looking on the bright side of radiation. *Parasitol Today* 1995;**11**:288–293.
149. Coulson PS. The radiation-attenuated vaccine against schistosomes in animal models: paradigm for a human vaccine. *Adv Parasitol* 1997;**39**:271–336.
150. James SL, Pearce EJ, Sher A. Induction of protective immunity against *Schistosoma mansoni* by a non-living vaccine. I. Partial characterization of antigens recognized by antibodies from mice immunized with soluble schistosome extracts. *J Immunol* 1985;**164**:3432–3438.
151. Mountford AP, Pearlman E. Interleukin-12 and the host response to parasitic helminths; the paradoxical effect on protective immunity and immunopathology. *Parasite Immunol* 1998;**20**:509–517.
152. Wilson RA, Coulson PS, Betts C, Dowling M-A, Smythies LE. Impaired immunity and altered pulmonary responses in mice with a disrupted interferon- γ receptor gene exposed to the irradiated *Schistosoma mansoni* vaccine. *Immunology* 1996;**87**:275–282.
153. King CL, Malhotra I, Xia X. *Schistosoma mansoni*: protective immunity in IL-4-deficient mice. *Exp Parasitol* 1996;**84**:245–252.
154. Pearce EJ, et al. Immunochemical characterization and purification of Sm-97, a *Schistosoma mansoni* antigen monospecifically recognized by antibodies from mice protectively immunized with a nonliving vaccine. *J Immunol* 1986;**137**:3593–3600.
155. Taylor JB, et al. The glutathione transferase activity and tissue distribution of a cloned M_r 28K protective antigen of *Schistosoma mansoni*. *EMBO J* 1988;**7**:465–472.
156. Soisson LMA, Masterson CP, Tom TD, McNally MT, Lowell GH, Strand M. Induction of protective immunity in mice using a 62-kDa recombinant fragment of a *Schistosoma mansoni* surface antigen. *J Immunol* 1992;**149**:3612–3620.
157. Reynolds SR, Shoemaker CB, Harn DA. T and B cell epitope mapping of SM23, an integral membrane protein of *Schistosoma mansoni*. *J Immunol* 1992;**149**:3995–4001.
158. Tendler M, et al. A *Schistosoma mansoni* fatty acid-binding protein, Sm14, is the potential basis of a dual-purpose anti-helminth vaccine. *Proc Natl Acad Sci USA* 1996;**93**:269–273.
159. Bergquist NR, Colley DG. Schistosomiasis vaccines: research to development. *Parasitol Today* 1998;**14**:99–104.
160. Doenhoff MJ. A vaccine for schistosomiasis: alternative approaches. *Parasitol Today* 1998;**14**:105–109.
161. Jankovic D, et al. Calpain is the target antigen of a Th1 clone that transfers protective immunity against *Schistosoma mansoni*. *J Immunol* 1996;**157**:806–814.
162. Couissinier-Paris P, et al. Identification of a major T cell immunogen in the anti-schistosome response of adult residents in an area endemic for *Schistosoma mansoni*. *Eur J Immunol* 1995;**25**:903–910.
163. Kohlstädt S, et al. Characterization of a schistosome T cell-stimulating antigen (Sm10) associated with protective immunity in humans. *Mol Biochem Parasitol* 1997;**84**:155–165.
164. Lok JB, Abraham D. Animal models for the study of immunity in human filariasis. *Parasitol Today* 1992;**8**:168–171.
165. Lucius R, Textor G, Kern A, Kirsten C. *Acanthocheilonema viteae*: vaccination of jirds with irradiation-attenuated stage-3 larvae and with exported larval antigens. *Exp Parasitol* 1991;**73**:184–196.
166. Eisenbeiss WF, Apfel H, Meyer TF. Protective immunity linked with a distinct developmental stage of a filarial parasite. *J Immunol* 1994;**152**:735–742.
167. Adam R, Kaltmann B, Rudin W, Friedrich T, Marti T, Lucius R. Identification of chitinase as the immunodominant filarial antigen recognized by sera of vaccinated rodents. *J Biol Chem* 1996;**271**:1441–1447.
168. Hartmann S, Adam R, Marti T, Kirsten C, Seidinger S, Lucius R. A 41-kDa antigen of the rodent filaria *Acanthocheilonema viteae* with homologies to tropomyosin induces host protective immune responses. *Parasitol Res* 1997;**83**:390–393.
169. Li B-W, Chandrasekar R, Alvarez RM, Liftis F, Weil GJ. Identification of paramyosin as a potential protective antigen against *Brugia malayi* infection in jirds. *Mol Biochem Parasitol* 1991;**49**:315–324.
170. Li B-W, Chandrasekar R, Weil GJ. Vaccination with recombinant filarial paramyosin induces partial immunity to *Brugia malayi* infection in jirds. *J Immunol* 1993;**150**:1881–1885.
171. Gregory WF, Blaxter ML, Maizels RM. Differentially expressed, abundant trans-spliced cDNAs from larval *Brugia malayi*. *Mol Biochem Parasitol* 1997;**87**:85–95.
172. Joseph GT, Huima T, Lustigman S. Characterization of an *Onchocerca volvulus* L3-specific larval antigen, Ov-ALT-1. *Mol Biochem Parasitol* 1998;**96**:177–183.
173. Fuhrman JA. Filarial chitinases. *Parasitol Today* 1995;**11**:259–261.
174. Wang SH, et al. Evaluation of recombinant chitinase and SXP1 antigens as antimicrofilarial vaccines. *Am J Trop Med Hyg* 1997;**56**:474–481.
175. Taylor MJ, Jenkins RE, Bianco AE. Protective immunity induced by vaccination with *Onchocerca volvulus* tropomyosin in rodents. *Parasite Immunol* 1996;**18**:219–225.
176. Jenkins RE, Taylor MJ, Gilvary NJ, Bianco AE. Tropomyosin implicated in host protective responses to microfilariae in onchocerciasis. *Proc Natl Acad Sci USA* 1998;**95**:7550–7555.
177. Newton SE, Munn EA. The development of vaccines against gastrointestinal nematode parasites, particularly *Haemonchus contortus*. *Parasitol Today* 1999;**15**:116–122.
178. Tavernor AS, Smith TS, Langford CF, Munn EA, Graham M. Vaccination of young Dorset lambs against haemonchosis. *Parasite Immunol* 1992;**14**:645–655.
179. Strauss EJ, Falkow S. Microbial pathogenesis: genomics and beyond. *Science* 1997;**276**:707–712.

180. The Filarial Genome Project. Deep within the filarial genome: an update on progress in the Filarial Genome Project. *Parasitol Today* (In press).
181. Johnston DA, Blaxter ML, Degraeve WM, Foster J, Ivens AC, Melvill SE. Genomics and the biology of parasites. *Bioessays* 1999;**21**:131–147.
182. Fan J, Minchella DJ, Day SR, McManus DP, Tiu WU, Brindley PJ. Generation, identification, and evaluation of expressed sequence tags from different developmental stages of the Asian blood fluke *Schistosoma japonicum*. *Biochem Biophys Res Commun* 1998;**252**:348–356.
183. Hoffman SL, Rogers WO, Carucci DJ, Venter JC. From genomics to vaccines: malaria as a model system. *Nat Med* 1998;**4**:1351–1353.
184. Barry MA, Lai WC, Johnston SA. Protection against mycoplasma infection using expression-library immunization. *Nature* 1995;**377**:632–635.
185. Mielcarek N, Riveau G, Remoué F, Antoine R, Capron A, Locht C. Homologous and heterologous protection after single intranasal administration of live attenuated recombinant *Bordetella pertussis*. *Nat Biotech* 1998;**16**:454–457.
186. Chabalgoity JA, et al. Expression and immunogenicity of an *Echinococcus granulosus* fatty acid-binding protein in live attenuated *Salmonella* vaccine strains. *Infect Immun* 1997;**65**:2402–2412.
187. Chacón MR, Londoño P, Dougan G, Selkirk ME. Heterologous expression of the cuticular glutathione peroxidase of lymphatic filariae in an attenuated vaccine strain of *Salmonella typhimurium* abrogates H-2 restriction of specific antibody responses. *Parasite Immunol* 1996;**18**:307–316.
188. Roman M, et al. Immunostimulatory DNA sequences function as T helper-1-promoting adjuvants. *Nat Med* 1997;**3**:849–854.
189. Donnelly JJ, Ulmer JB, Shiver JW, Liu MA. DNA vaccines. *Annu Rev Immunol* 1997;**15**:617–648.
190. Rosas G, et al. *Taenia crassiceps* cysticercosis: humoral immune response and protection elicited by DNA immunization. *J Parasitol* 1998;**84**:516–523.
191. Mountford AP, Anderson S, Wilson RA. Induction of Th1 cell-mediated protective immunity to *Schistosoma mansoni* by co-administration of larval antigens and IL-12 as an adjuvant. *J Immunol* 1996;**156**:4739–4745.
192. Kim JJ, et al. Engineering of in vivo immune responses to DNA immunization via codelivery of costimulatory molecule genes. *Nat Biotech* 1997;**15**:641–646.
193. Oehnen S, Hengartner H, Zinkernagel RM. Vaccination for disease. *Science* 1991;**251**:195–198.
194. Wynn TA, et al. An IL-12-based vaccination method for preventing fibrosis induced by schistosome infection. *Nature* 1995;**376**:594–596.
195. Sher A, Jankovic D, Cheever A, Wynn T. An IL-12-based vaccine approach for preventing immunopathology in schistosomiasis. *Ann N Y Acad Sci* 1996;**795**:202–207.
196. Nestle FO, et al. Vaccination of melanoma patients with peptide- or tumour lysate-pulsed dendritic cells. *Nat Med* 1998;**4**:328–332.
197. Stumbles PA, et al. Resting respiratory tract dendritic cells preferentially stimulate T helper cell type 2 (Th2) responses and require obligatory cytokine signals for induction of Th1 immunity. *J Exp Med* 1998;**188**:2019–2031.
198. Toellner KM, et al. T helper 1 (Th1) and Th2 characteristics start to develop during T cell priming and are associated with an immediate ability to induce immunoglobulin class switching. *J Exp Med* 1998;**187**:1193–1204.
199. Rissoan MC, et al. Reciprocal control of T helper cell and dendritic cell differentiation. *Science* 1999;**283**:1183–1186.
200. Boyle JS, Brady JL, Lew AM. Enhanced responses to a DNA vaccine encoding a fusion antigen that is directed to sites of immune induction. *Nature* 1998;**392**:408–411.
201. Johnson KS, et al. Vaccination against ovine cysticercosis using a defined recombinant antigen. *Nature* 1989;**338**:585–587.
202. Shastri N. Needles in haystacks: identifying specific peptide antigens for T cells. *Curr Opin Immunol* 1996;**8**:271–277.
203. Campos-Neto A, et al. Cloning and expression of a *Leishmania donovani* gene instructed by a peptide isolated from major histocompatibility complex class II molecules of infected macrophages. *J Exp Med* 1995;**182**:1423–1433.
204. Mougneau E, et al. Expression cloning of a protective *Leishmania* antigen. *Science* 1995;**268**:563–566.
205. Aguinaldo AMA, et al. Evidence for a clade of nematodes, arthropods and other moulting animals. *Nature* 1997;**387**:489–493.
206. Conway Morris S. The fossil record and the early evolution of the Metazoa. *Nature* 1993;**361**:219–225.
207. Gottstein B, et al. Specific cellular and humoral immune responses in patients with different long-term courses of alveolar echinococcosis (infection with *Echinococcus multilocularis*). *Am J Trop Med Hyg* 1991;**45**:734–742.
208. Nelson FK, Greiner DL, Schultz LD, Rajan TV. The immunodeficient scid mouse as a model for human lymphatic filariasis. *J Exp Med* 1991;**173**:659–663.
209. Rajan TV, et al. CD4⁺ T-lymphocytes are not required for murine resistance to the human filarial parasite, *Brugia malayi*. *Exp Parasitol* 1994;**78**:352–360.
210. Ovington KS, Behm CA. The enigmatic eosinophil: investigation of the biological role of eosinophils in parasitic helminth infection. *Mem Inst Oswaldo Cruz* 1997;**92** (Suppl 2):93–104.
211. Lawrence RA, Gray C, Osborne J, Maizels RM. *Nippostrongylus brasiliensis*: cytokine responses and nematode expulsion in normal and IL4-deficient mice. *Exp Parasitol* 1996;**84**:65–73.
212. Ferru I, Roye O, Delacré M, Auriault C, Wolowczuk I. Infection of B-cell-deficient mice by the parasite *Schistosoma mansoni*: demonstration of the participation of B cells in granuloma modulation. *Scand J Immunol* 1998;**48**:233–240.
213. Jankovic D, et al. Optimal vaccination against *Schistosoma mansoni* requires the induction of both B cell- and IFN- γ -dependent effector mechanisms. *J Immunol* 1999;**162**:345–351.
214. Rotman HL, et al. *Strongyloides stercoralis*: complete life cycle in SCID mice. *Exp Parasitol* 1995;**81**:136–139.
215. Ovington KS, McKie K, Matthaei KI, Young IG, Behm CA. Regulation of primary *Strongyloides ratti* infections in mice: a role for interleukin-5. *Immunology* 1998;**95**:488–493.
216. Else K, Grecis RK. Antibody-independent effector mechanisms in resistance to the intestinal nematode parasite *Trichuris muris*. *Infect Immun* 1996;**64**:2950–2954.
217. Chenthamarakshan V, Reddy MVR, Harinath BC. Immunoprophylactic potential of a 120 kDa *Brugia malayi* adult antigen fraction, BmA-2, in lymphatic filariasis. *Parasite Immunol* 1995;**17**:277–285.
218. Nanduri J, Kazura JW. Paramyosin-enhanced clearance of *Brugia malayi* microfilaremia in mice. *J Immunol* 1989;**143**:3359–3363.
219. Sexton JL, et al. Glutathione S-transferase. Novel vaccine against *Fasciola hepatica* infection in sheep. *J Immunol* 1990;**145**:3905–3910.

220. Dalton JP, McGonigle S, Roplh TP, Andrews SJ. Induction of protective immunity in cattle against infection with *Fasciola hepatica* by vaccination with cathepsin L proteinases and with hemoglobin. *Infect Immun* 1996;**64**:5066–5074.
221. Jasmer DP, Perryman LE, Conder GA, Crow S, McGuire T. Protective immunity to *Haemonchus contortus* induced by immunoaffinity isolated antigens that share a phylogenetically conserved carbohydrate gut surface epitope. *J Immunol* 1993;**151**:5450–5460.
222. Balloul JM, Grzych JM, Pierce RJ, Capron A. A purified 28,000 dalton protein from *Schistosoma mansoni* adult worms protects rats and mice against experimental schistosomiasis. *J Immunol* 1987;**138**:3448–3453.
223. Pearce EJ, James SL, Hieny S, Lanar DE, Sher A. Induction of protective immunity against *Schistosoma mansoni* by vaccination with schistosome paramyosin (Sm97), a nonsurface parasite antigen. *Proc Natl Acad Sci USA* 1988;**85**:5678–5682.
224. Silberstein DS, Despommier DD. Antigens from *Trichinella spiralis* that induce a protective response in the mouse. *J Immunol* 1984;**132**:898–904.
225. Frenkel MJ, Dopheide TAA, Wagland BM, Ward CW. The isolation, characterization and cloning of a globin-like, host-protective antigen from the excretory-secretory products of *Trichostrongylus colubriformis*. *Mol Biochem Parasitol* 1992;**50**:27–36.
226. Harn DA, Gu W, Oligino LD, Mitsuyama M, Gebremichael A, Richter D. A protective monoclonal-antibody specifically recognizes and alters the catalytic activity of schistosome triose-phosphate isomerase. *J Immunol* 1992;**148**:562–567.
227. Lightowlers MW, et al. Vaccination against hydatidosis using a defined recombinant antigen. *Parasite Immunol* 1996;**18**:457–462.
228. Manoutcharian K, et al. Cysticercosis: identification and cloning of protective recombinant antigens. *J Parasitol* 1996;**82**:250–254.
229. Lightowlers MW, Rolfe R, Gauci CG. *Taenia saginata*: vaccination against cysticercosis in cattle with recombinant onosphere antigens. *Exp Parasitol* 1996;**84**:330–338.
230. Ghosh K, Hawdon J, Hotez P. Vaccination with alum-precipitated recombinant *Ancylostoma*-secreted protein 1 protects mice against challenge infections with infective hookworm (*Ancylostoma caninum*) larvae. *J Infect Dis* 1996;**174**:1380–1383.
231. Robinson K, Bellaby T, Chan WC, Wakelin D. High levels of protection induced by a 40-mer synthetic peptide vaccine against the intestinal nematode parasite *Trichinella spiralis*. *Immunology* 1995;**86**:495–498.
232. Balloul JM, et al. Molecular cloning of a protective antigen of schistosomes. *Nature* 1987;**326**:149–153.
233. Rothel JS, et al. Sequential nucleic acid and recombinant adenovirus vaccination induces host-protective immune responses against *Taenia ovis* infection in sheep. *Parasite Immunol* 1997;**19**:221–227.
234. Yang W, Waite GJ, McManus DP. Antibodies to *Schistosoma japonicum* (Asian bloodfluke) paramyosin induced by nucleic acid vaccination. *Biochem Biophys Res Commun* 1995;**212**:1029–1039.
235. Waite GJ, Mazzer DR, McManus DP. DNA immunization by intramuscular injection or gene gun induces specific IgG antibodies against a *Schistosoma japonicum* 22 kDa antigen, Sj22, when fused to the murine Ig K-chain secretory leader sequence. *Parasite Immunol* 1999;**21**:53–56.
236. Kayes SG, Shaneyfelt RC, Monteiro C, O'Brien JJ. Overproduction of SM28GST in a baculovirus expression vector and its use to evaluate the in vivo immune responses of mice vaccinated against *Schistosoma mansoni* with naked DNA encoding the SM28GST gene. *J Parasitol* 1998;**84**:764–770.

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