Infection and treatment immunizations for successful parasite vaccines

Citation for published version:
Mutapi, F, Billingsley, PF & Secor, WE 2013, 'Infection and treatment immunizations for successful parasite vaccines' Trends in Parasitology. DOI: 10.1016/j.pt.2013.01.003

Digital Object Identifier (DOI):
10.1016/j.pt.2013.01.003

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published In:
Trends in Parasitology

Publisher Rights Statement:
Gold Open Access paid

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
Infection and treatment immunizations for successful parasite vaccines

Francisca Mutapi¹, Peter F. Billingsley², and W. Evan Secor³

¹Institute for Immunology and Infection Research, Centre for Immunity, Infection and Evolution, School of Biological Sciences, University of Edinburgh, Edinburgh, EH9 3JT, UK
²Sanaria Inc., Rockville, MD 20850, USA
³Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Atlanta, GA 30329, USA

Since the advent of techniques for the expression of recombinant peptide antigens, the availability of human vaccines for parasitic diseases has been 'imminent'. Yet vaccines based on recombinant proteins are still largely aspirations, not realities. It is now apparent that vaccine development needs additional knowledge about host protective immune response(s), antigen characteristics, and the delivery required to induce those responses. The most successful immune protection against parasites has been generated by infection and treatment, the induction of protective immunity by truncating the course of an infection with drug treatment. Here, we consider the characteristics of an effective, protective anti-parasite vaccine and propose a conceptual framework to aid parasite vaccine development using malaria and schistosomiasis as examples.

Development of protective immune responses resulting from infections

Exposure to pathogens allows vertebrate hosts to mount pathogen-specific acquired immune responses that sometimes protect against subsequent infection, forming the basis of vaccinology [1]. The original observation that protection often succeeds infection and recovery led to the artificial induction of immunity by infection with attenuated parasites [2,3], which triggered tremendous interest in the nature and development of naturally acquired protective immunity and characterization of measurable markers of immune protection. The broad range of veterinary [3] and human [4] vaccines against bacterial and viral pathogens are predominantly live attenuated or inactivated pathogen formulations (Table 1). Similarly, a significant proportion of protozoan vaccines against economically significant veterinary parasites (e.g., Theileria) of livestock and companion animals are based on inoculation with attenuated or drug treated parasites. In humans, the most widely used 'vaccination' for a parasitic infection is the practice of leishmanization [5], where children are inoculated with parasite-containing exudate from a cutaneous Leishmania sore in a location typically covered by clothing. The resulting, self-limiting lesion provides protection against subsequent infections that might otherwise form a disfiguring ulceration on an exposed area. However, no vaccines against parasitic infections are licensed for human use. This is at least in part attributable to the antigenic complexity of parasites, arising from multiple life cycle stages, immune evasion strategies, and use of intermediate and reservoir hosts. Unfortunately, obtaining adequate numbers of parasites, attenuated or otherwise, of consistent and acceptable quality to use in vaccinations is highly challenging, as demonstrated by recent studies of the Plasmodium falciparum attenuated sporozoite vaccine (PISPS) in humans [6]. Nevertheless, the recent Phase 1 trial demonstrating that injection of cryopreserved P. falciparum sporozoites can be used in controlled human malaria infections will greatly facilitate this research in the future [7].

An alternative to infection with attenuated parasites is the infection and treatment (I&T) approach where immunity is induced by the release of antigens from parasitic infections that are treated or naturally die in the host (Figure 1). One of the most striking examples of the effect of previous infection on subsequent protection is the relative resistance to symptomatic malaria in older children and adults who have grown up in areas endemic for P. falciparum. Recently, an I&T trial for malaria was performed by exposing volunteers who were receiving chloroquine prophylaxis to P. falciparum sporozoites. The chemoprophylaxis with sporozoites (CPS) protocol succeeded in inducing sterile immunity in all immunized participants and was maintained in four of six participants for >2 years [8]. An I&T effect is also observed in schistosome infections as praziquantel treatment of persons infected with Schistosoma haematobium or Schistosoma mansoni can induce partially protective immunity against subsequent infections [9,10].

Another outcome of I&T is that individuals from areas where they are likely to have been exposed to malaria or schistosome antigens early in life tend to have a lower risk of developing severe pathological consequences such as cerebral malaria or hepatosplenic schistosomiasis, respectively. Protective mechanisms against pathology are poorly understood but are hypothesized to involve induction of different regulatory or memory immune responses. In addition to modulation of pathology in subsequent infections, I&T effects on host immune responses are also instructive with respect to development of defined antigen vaccines. Most vaccine recipients in endemic areas are likely to have had some exposure to the parasite, leading to reactions during immunization that may differ from those of parasite naive vaccine trial participants. For
example, the Phase I clinical trial evaluating the vaccine against human hookworm using *Ancylostoma* secreted protein (ASP-2) was discontinued when vaccination induced urticarial reactions in people with pre-existing IgE responses to ASP-2 [9]. No such adverse events have been reported in I&T.

Similar to inoculation with attenuated parasites, I&T has limitations that may preclude it from being a feasible public health tool; for some parasite species, it may not be possible to generate sufficient quantities of infectious stage parasites to vaccinate the millions of people exposed to these infections. Nevertheless, I&T approaches provide key answers to some fundamental intellectual and practical questions for successful vaccine development. By concentrating on the principles of classical vaccination, we describe how I&T protocols have overcome some of the challenges of using recombinant protein immunizations.

**Desirable I&T characteristics for successful vaccines**

Parasites causing the greatest morbidity and disease typically induce a more or less protective immunity very slowly. Reasons for this include poor immunogenicity of individual antigens, poor protective immunity of major antigens, antigenic variation (protozoa), antigen polymorphism, immune evasion, immunomodulation of effector responses, and/or the requirement for a threshold amount of antigen which is released more easily upon treatment than from natural parasite death [10–14]. I&T approaches have overcome some of these parasite survival strategies. Several important characteristics that underlie their success are discussed below.

**The pathogen must be immunogenic**

Parasites successfully controlled by I&T are immunogenic during natural infections. *Echinococcus granulosus* onchospheres provoke a high degree of protective immunity, which is the basis of a highly effective vaccine in lambs (90% protection [15]) and offering great potential as a human parasite vaccine [16]. By contrast, vaccine development against *Fasciola hepatica* and *Fasciola gigantica* is hampered by their inability to induce immunity in their natural hosts, even after repeated infections, suggesting low immunogenicity of these flukes [2]. Parasites might be immunogenic but still infect the host if the host is unable to recognize the pathogen or mount a protective immune response during the parasite’s immune-susceptible period. For example, infective stages of schistosomes, filarids, and hookworms are susceptible to immune attack but migrate and mature before effective immune responses develop. Subsequent infections may be prevented but only after the initial parasites become established [17]. Furthermore, adult schistosomes avoid the host’s protective immunity

---

**Table 1. Currently licensed human vaccines**

| Vaccine                | Common name/combination vaccine | Pathogen       | Type of vaccine                  |
|------------------------|---------------------------------|----------------|----------------------------------|
| Anthrax                |                                 | Bacteria       | Subunitb                        |
| Chicken pox            | Varicella                       | Virus          | Live, attenuatedd               |
| Cholera                |                                 | Inactivateda    |                                |
| Diptheria              | DPT                             | Bacteria       | Inactivated toxin                |
| Haemophilus influenza type B | Hib                   | Virus          | Conjugatec                      |
| Hepatitis A            |                                 | Virus          | Inactivated                      |
| Hepatitis B            |                                 | Virus          | Subunit                         |
| Human papillomavirus   | HPV                             | Virus          | Subunit                         |
| Influenza vaccine      |                                 | Virus          | Live, attenuated                |
| Japanese encephalitis vaccine |                |                | Inactivated                      |
| Measles                | MMR                             | Virus          | Live, attenuated                |
| Mumps                  | MMR                             | Virus          | Live, attenuated                |
| Rubella                | MMR                             | Virus          | Live, attenuated                |
| Pertusis               | Whooping cough (DPT)            |                | Subunit                         |
| Pneumococcal infections| Meningitis and pneumonia Meningococcus | Bacteria     | Subunit                         |
| Polio                  |                                 | Virus          | Inactivated                      |
| Rabies                 |                                 | Virus          | Inactivated                      |
| Rotavirus              |                                 | Virus          | Live, attenuated                |
| Small pox              |                                 | Virus          | Attenuated (Sabin polio vaccine) |
|                        |                                 |                | Inactivated (Salk polio vaccine) |
| Shingles               | Herpes zooster                  | Virus          | Live, attenuated                |
| Tetanus                | DPT                             | Bacterial toxin| Inactivated toxin               |
| Tuberculosis           | Bacilli Calmette–Gérimin (BCG)  | bacteria       | Live, attenuated                |
| Typhoid                |                                 | bacteria       | Inactivated                      |
| Yellow fever           |                                 | virus          | Live, attenuated                |

*Table adapted from [5] and definitions adapted from [http://www.niaid.nih.gov/topics/vaccines/understanding/pages/typesvaccines.aspx.](http://www.niaid.nih.gov/topics/vaccines/understanding/pages/typesvaccines.aspx)*

bSubunit vaccine: a vaccine made up of only the antigens that best stimulate the immune system. They are made in one of two ways: either by chemical extraction of the native antigen, the whole organism, or as recombinant proteins expressed in other organisms (e.g., bacteria), in which case they would be termed ‘recombinant subunit vaccines’.

cLive attenuated vaccine: a vaccine made from the living microbe that has been weakened in the laboratory so it cannot cause disease but may still be able to replicate in the host.

dInactivated vaccine: a vaccine made by killing the disease-causing microbe with chemicals, heat, or radiation.

eConjugate vaccine: a vaccine created by covalently attaching a poorly immunogenic antigen (e.g., a polysaccharide) to a carrier protein thereby conferring the immunological attributes of the carrier to the attached antigen. This type of vaccine is a special type of subunit vaccine.
through evasive mechanisms such as rapid membrane turnover, host mimicry, and masking themselves with host proteins [18].

In the CPS and PfSPZ-CVac studies, the timing of drug treatment allows full development of the liver stage parasites, thereby increasing the number and diversity of parasite antigens. It also eliminates the parasites before the onset of disease [19] and prevents inhibition of anti-liver stage cellular immunity that would otherwise occur during blood stage infections [20]. Additionally, with the increased complexity of whole parasite antigens against parasite stages for which protective immunity might not otherwise develop, I&T offers great potential for strain-transcending protection.

However, protozoan parasites can vary the antigens seen by host immune systems through mechanisms including transcriptional and epigenetic control (in situ switching, e.g., *P. falciparum* or *Giardia lamblia*) or through gene conversion (unidirectional recombination, e.g., *Trypanosoma brucei* and *Babesia bovis*) [21]. I&T may overcome antigenic variation and immune avoidance by inducing immunity to many antigens of several parasite strains/variants. Such broad coverage is very challenging to achieve with a recombinant vaccine, even if it is multivalent, such as the AMA1 [22] or MSP-1 [23] vaccine candidates. To date, antigenic switching has not been demonstrated in helminths, although a micro-exon mechanism for potentially generating antigenic variation is present in the schistosome genome [24].

**Inducing protective effector, rather than regulatory responses**

Individuals infected naturally with schistosomes or malaria parasites eventually develop effector responses that may confer protection despite also stimulating regulatory responses. Schistosome infection intensities are associated with the balance between protective and regulatory responses, which is affected by host age [25]. An often overlooked aspect of protective immunity is how effector responses surpass regulatory responses during natural infection and progress to long-lived memory B and T cells. Understanding this phenomenon may help unlock the door to successful vaccine design.

Not all immune responses result in parasite killing or resistance to re-infection. Indeed, some parasite evasive mechanisms may divert the immune system to respond against ‘decoy’ antigens. In *Plasmodium*, T cell mimotopes are protein variants of parasite antigens (altered peptide ligands) that prevent development of memory T cell effector functions of cytotoxic lymphocytes [26]. Similarly, carbohydrate epitopes on schistosome cercariae and egg antigens predominantly induce IgM and IgG2 antibodies, which are not as efficacious against schistosomulae as other antibody subclasses [27,28] and skew the immune system towards anergy [29]. Why the host maintains these ineffective responses is unknown but there may be homeostatic reasons for maintaining them [30]. Thus, the value of these responses must be considered before disregarding them entirely. Alternatively, certain responses may be regulatory and suppress protective immunity, such as the AgEm2 carbohydrate in *E. granulosus* that interferes with antigen presentation and cell activation [31].

Amelioration of autoimmunity in rodents by *Plasmodium* suggests that blood stage parasites induce immunosuppression. This was confirmed in humans as blood stages of *P. falciparum* suppress T lymphocyte reactivity to malarial and unrelated antigens [32]. The several immunosuppressive strategies employed by *Plasmodium* parasites include utilization of T cell mimotopes to inhibit T cell activation, induce anergy, or shift the T cell phenotype [33]; alteration of antigen presentation by impairing dendritic cell function and maturation [34]; and induction of regulatory T cells [35]. By contrast, the *P. falciparum* I&T trial in humans resulted in equivalent or better protection than that produced by irradiated sporozoite immunizations [36]. I&T exposes the host to all pre-erythrocytic stages, allowing effector responses to be mounted against a broader range of antigens, while limiting exposure to the pathogenic and immunosuppressive asexual blood stages [37,38]. In natural infections, people are typically infected with *Plasmodium* from a single mosquito bite and treated based on clinical symptoms or diagnosis, at which time the blood stage parasites may be suppressing pre-erythrocytic immunity. In successful *Plasmodium* CPS, the drug was

---

**Figure 1.** Approaches to infection and treatment vaccinations for malaria and schistosomiasis. Vaccination by infection and treatment (I&T) against malaria is achieved by providing chemoprophylaxis to susceptible individuals followed by spaced treatments with sporozoites delivered by mosquito bite or needle and syringe. Under these conditions, parasites develop in the liver but do not go beyond early blood stage infections. After final treatment, the chemoprophylaxis is withdrawn and individuals demonstrate increased immunity against future challenges. Vaccination by I&T against schistosomiasis is based upon infections acquired naturally followed by treatment with praziquantel or natural worm death, resulting in individuals with decreased susceptibility to future exposure.
administered prophylactically, including during inoculation of sporozoites from 15 mosquitoes [39]. This requirement to curtail exposure to blood stage parasites for full protection is supported by development of protective acquired immunity in children receiving treatment that restricts the development of symptomatic malaria [40]. Thus, successful vaccination must overcome the effects of regulatory responses that are stimulated by certain parasite life cycle stages. I&T for Plasmodium has achieved this by minimizing immune exposure to immunosuppressive blood stage parasites. How or even if this is achieved in people who develop natural resistance to malaria is unclear.

Experimental and natural helminth infections are associated with immunoregulatory responses that polarize CD4+ T cells towards a T helper 2 (Th2) phenotype (production of interleukins 4, 5, and 13, secretion of IgE and IgG4 by plasma cells, and activation of eosinophils and mast cells), and immunosuppress antigen-specific [41] and general [42] immune responses. Helminth parasites modulate both innate and adaptive arms of the immune system, targeting both humoral and cellular responses [43]. Regulatory responses are characterized by suppressive cytokines (interleukin (IL)-10 and transforming growth factor β (TGF-β)) produced by natural and adaptive regulatory T (Treg) cells [44,45] that can block resistance to schistosome reinfection in an animal model [46]. Experimental studies clearly show that whilst Treg cells play an important role in shaping anti-schistosome responses, the ‘regulatory’ arm of the immune axis extends beyond this population [47] including other cells such as Th1 cells and macrophages [48,49] (e.g., alternatively activated macrophages [50]). The influence of these regulatory responses on the development of resistance in human hosts is still under investigation. Cross-inhibition between effector CD4+ T cell subsets (Th1, Th2, and Th17) also means that effector cytokines (interferon (IFN)-γ, IL-4, and IL-21) are required to maintain a balanced acquired immune response [51,52], which is associated with protective immunity against infection/re-infection [53,54]. When schistosome infection is cleared by drug treatment, immune reactivity can increase shortly afterwards, presumably in response to: (i) antigen release from dead parasites [14], (ii) reversal of hyporesponsiveness [55], or (iii) an increased effector T (Teff)/Treg ratio [56]. Thus, success of I&T in human schistosomiasis may result partly from the treatment-induced increase in effector responses relative to regulatory responses.

The effective dose: protective, non-pathological immune responses

Quantitative studies in human schistosomiasis show that immunosuppression alone does not explain the effects of age on infection intensity observed in schistosome endemic areas [57]. Rather, protective immunity develops only after exposure surmounts a threshold of parasite immunogens [14,58]. During natural schistosome infection, where adult worms survive for 3–7 years [59], it may take several years for the threshold to be reached as worm death events are spread out over time, only occasionally exposing the host to adult antigens that induce cross-reactive protection against invading schistosomulae. Similarly, it may take several rounds of Plasmodium infection for sufficient amounts of antigens from different circulating strains of the parasite to stimulate development of partially protective immunity [60]. In areas with higher transmission rates, Plasmodium and schistosome infection prevalence rates peak and decline at an earlier age. This pattern, first observed in Plasmodium in 1949 [61], and later confirmed for other parasitic infections, is termed the ‘peak shift’ and has been attributed to development of acquired immunity [62]. Thus, protective immune responses develop earlier in high rather than low transmission areas [63], possibly due to immune stimulation after death of sufficiently large numbers and/or strains of parasites. The greatest exposure occurs when parasites are killed by treatment, providing in a single event the threshold exposure seen only over time or not at all in naturally resolving infections [64]. Praziquantel treatment for schistosomiasis qualitatively and quantitatively increases the antigens recognized by the host’s immune system, mirroring the natural changes observed with host age [14,64]. The complexity of an immune response depends on the relative frequency of antigen-specific B and T cells, the levels of antigen present, and the period during which the antigen remains available to antigen-presenting cells. Thus, the antigen dose is critical at several stages in the generation of a response.

Immunization with irradiated P. falciparum sporozoites or irradiated cercariae suggest that high antigen doses are required to stimulate protective responses [65]. Furthermore, low-dose stimulation can induce antigen-specific FOXP3+ regulatory responses in humans and promote the development of tolerance [66]. The interaction between the antigen dose/duration of antigen stimulation and the immune system in humans exposed naturally to parasitic diseases is less well studied compared with responses induced in primary responses. An important factor in chronic parasitic infections such as schistosomiasis is the persistence of antigenic stimulation. In general, the immunological outcome of persistent stimulation by low antigen doses differs from intermittent stimulation with high antigen doses and determines whether the outcome is tolerance, pathology, or protection. Chronic immune activation in helminth infections results in impaired signal transduction and anergy [67], contributing to hyporesponsiveness [54]. I&T against schistosomiasis might overcome this hyporesponsiveness by releasing a higher immunizing antigen dose, thus avoiding low-dose anergy, while removing potentially immunosuppressive adult worms. Repeat- ed treatment provides the additional necessary ‘doses’ for improved protection by stimulating development of high-affinity antibodies, such as anti-schistosome IgE [68].

Long-lasting induced immunity

Almost all current vaccines work through induction of serum or mucosal antibodies that block infection or interfere with invasion and proliferation. Although antibody half-life is ~30 days, effective vaccine-induced protection persists beyond the time when antibodies should have disappeared. Possible reasons for this include boosting from natural infection, antigen retention in peripheral lymph nodes by follicular dendritic cells, presence of sequestered memory B cells in bone marrow sanctuaries, or
maintenance of antigen-specific memory B cells by nonspecific B cell activators or idiotypic networks. The importance of natural boosting has been demonstrated in animal I&T protocols for protozoa infections (e.g., *Theileria* [3]) and may explain why protective immunity against parasites can be lost when people migrate from endemic areas and are no longer exposed [69].

Both antibodies and CD4+ T cells form crucial components of naturally acquired protective immunity against blood stage malaria [70]. The longevity of these responses is a subject of much debate, with the majority of earlier immunoepidemiological studies showing them to be short-lived, especially in children [71–73], and characterized by a decline in antibody titers in the absence of parasitemia [71,74,75]. However, even in low transmission areas, *Plasmodium falciparum* and *Plasmodium vivax* can induce long-term protection, despite the fact that effector inflammatory responses are short-lived [76,77]. Serum antibody titers in low malaria transmission areas varied in breadth and magnitude, confirming that several malaria infections are required to induce long-lived effector responses that even then are only partially protective. The implications of this for vaccine development, especially for people resident in areas of high malaria transmission, remain to be investigated, and the mechanisms and generation of long-lived responses in humans also require further investigation. Malaria I&T provides clinical and parasitological protection against malaria for at least 2.5 years [8], compared with the short-lived, partially protective immunity in natural infections. Furthermore, malaria I&T provided longer lived responses than the subunit based RTS,S/AS01 for which protection did not last beyond 12 months, particularly for severe malaria [78]. Mechanistic studies of the protective immune responses resulting from I&T will be very informative for designing long-lasting vaccines.

Understanding the longevity of antibody responses in schistosomiasis is confounded by the effects of repeated infections. Long-lasting responses follow curative treatment of schistosomiasis [79], which may be stimulated by prolonged excretion of antigens from live and dead eggs trapped in host tissues [80]. Similarly, European travelers infected with schistosomes at a single time point had schistosome-specific cytokine responses 8 years after treatment [81]. The possibility of low-grade infection in these participants or cross-reactivity might also explain these observations [82]. However, it is difficult to extrapolate these findings to endemic populations where chronic antigen stimulation may result in different B and T cell dynamics [83].

**Long-term induced immunological memory**

The generation and persistence of immunological memory after an initial encounter with a pathogen provides the basis for subsequent protection. However, little is known about memory responses from repeated or chronic antigen exposure. How each round of infection affects the generation and maintenance of the memory T cell pool is poorly understood. Similar to the protective immunity in the *P. falciparum* I&T trial, effector memory T cells associated with protection against re-infection were maintained throughout the 2.5-year follow-up period [8,19]. By contrast, memory effector cell responses in natural infections decline after 12 months [75]. Thus, I&T might promote the longevity of effector memory responses.

Although activation and differentiation of CD8+ and CD4+ cells are broadly similar, vaccination strategies designed to induce and boost different T cell memory subsets have important distinctions. CD4+ memory T cells require a higher antigen threshold and prolonged stimulation for activation than CD8+ memory cells [84]. Also, persistence of T cell memory after vaccination with small viral fragments is much shorter than that achieved with attenuated whole virus [85]. This may reflect differential temporal dynamics of memory responses and may explain the improved performance of whole parasite vaccines or I&T compared with recombinant vaccines. Furthermore, attenuated sporozoite immunization may stimulate different T cell memory generation pathways in novel sites (i.e., the liver) compared with natural infections, resulting in longer lasting vaccine-induced T cell memory [86].

Long-term antigen persistence and exposure in chronic helminth infections affects the development of immunological memory [86]. Recent work in human schistosomiasis showed that although there were no differences in CD8+ T cells in schistosome-infected versus uninfected people, CD4+ T cell proportions were significantly lower in individuals with schistosomiasis [87]. The reduced memory CD4+ cells during chronic infection may result from the Hayflick limit (the number of times a cell will divide [88]), which can significantly affect the generation of immunological memory under persistent antigenic stimulation [89]. In human schistosomiasis, although praziquantel treatment leads to a significant decline in CD4+ memory T cell proportions, there is a pronounced increase in CD4+ memory cell replication [87]. This suggests that the nature of immunological memory following I&T may differ from what occurs naturally.

**Concluding remarks**

The biological hurdles to successful vaccine development are clear but I&T successfully addresses many of them. Schistosome vaccine development is hindered by factors which include: (i) lack of vaccine candidates providing reproducible protection in experimental models and humans; (ii) limitations in current understanding of the nature, development, and maintenance of protective immune responses, particularly in people already exposed to schistosomes; and (iii) how to induce protective immune responses better and faster than what occurs naturally while at the same time avoiding pathology or tolerance [9]. Similar hurdles face malaria vaccine development. It will be almost 40 years from initial studies to a commercially available formulation of RTS,S/AS01. During this time, the scientific challenges have involved antigen identification, identification of suitable vaccine vehicle and adjuvants, optimization of dosage and boosting schedules, and definition of immune correlates [90]. The highly successful I&T study with *P. falciparum* has provided answers to some of these questions and the tools to answer more. The exciting developments in I&T will hopefully provide the springboard for a better understanding of induced rather than acquired immunity against parasites and offer a new
platform for development of truly effective anti-parasite vaccines.

Acknowledgments
We thanks Drs. S. Chakravarty and S.L. Hoffman for critical reading of the manuscript during its preparation. FM acknowledges support from the World Health Organisation (www.who.org); the Wellcome Trust (http://www.wellcome.ac.uk/) [grant number WT082828MA] and the Thrasher Foundation (http://www.thrasherresearch.org/).

Disclaimer statement
The conclusions in this paper are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

References
1 Pichichero, M.E. (2009) Booster vaccinations: can immunologic memory outpace disease pathogenesis? Pediatrics 124, 1633–1641
2 Meeuse, E.N. and Piedra, D. (2003) Exploiting natural immunity to helminth parasites for the development of veterinary vaccines. Int. J. Parasitol. 33, 1285–1290
3 Meeuse, E.N. et al. (2007) Current status of veterinary vaccines. Clin. Microbiol. Rev. 20, 489–510
4 Plotkin, S.A. (2008) Vaccines: correlates of vaccine-induced immunity. Clin. Infect. Dis. 47, 401–409
5 Nadim, A. et al. (1983) Effectiveness of leishmanization in the control of cutaneous leishmaniasis. Bull. Soc. Pathol. Exot. Filiales 76, 377–383
6 Hoffman, S.L. et al. (2010) Development of a metabolically active, non-replicating sporozoite vaccine to prevent Plasmodium falciparum malaria. Hum. Vaccin. 6, 97–106
7 Roestenberg, M. et al. (2013) Controlled human malaria infections by intradermal injection of cryopreserved Plasmodium falciparum sporozoites. Am. J. Trop. Med. Hyg. 88, 5–13
8 Roestenberg, M. et al. (2011) Long-term protection against malaria after experimental sporozoite inoculation: an open-label follow-up study. Lancet 377, 1770–1776
9 Bethony, J.M. et al. (2011) Vaccines to combat the neglected tropical diseases. Immunol. Rev. 239, 237–270
10 Woolhouse, M.E.J. and Hagan, P. (1999) Seeking the ghost of worms past. Nat. Med. 5, 1225–1227
11 Gupta, S. et al. (1999) Acquired immunity and postnatal clinical protection in childhood cerebral malaria. Proc. Biol. Sci. 266, 33–38
12 Good, M.F. (2005) Vaccine-induced immunity to malaria parasites and the need for novel strategies. Trends Parasitol. 21, 29–34
13 Yazdanbakhsh, M. and Sacks, D.L. (2010) Why does immunity to parasites take so long to develop? Nat. Rev. Immunol. 10, 80–81
14 Mutapi, F. et al. (2008) Age-related and infection intensity-related shifts in antibody recognition of defined protein antigens in a schistosome-exposed population. J. Infect. Dis. 198, 167–175
15 Hashemitabar, G.R. et al. (2005) Trials to induce protective immunity in mice and sheep by application of protocolex and hydatid fluid antigen or whole body antigen of Echinococcus granulosus. J. Vet. Med. B: Infect. Dis. Vet. Public Health 52, 243–245
16 Lightowlers, M.W. (2002) Vaccination against hydatid disease. Dev. Biol. (Basel) 110, 81–87
17 Wilson, R.A. and Coulson, S.R. (1999) Strategies for a schistosome vaccine: can we manipulate the immune response effectively? Microbes Infect. 1, 535–543
18 Wilson, R.A. (2012) Virulence factors of schistosomes. Microbes Infect. 14, 1442–1450
19 Roestenberg, M. et al. (2009) Protection against a malaria challenge by sporozoite inoculation. N. Engl. J. Med. 361, 468–477
20 Mota, M.M. et al. (2001) Migration of Plasmodium sporozoites through cells before infection. Science 291, 141–144
21 Craig, A. (2003) Antigenic Variation, Elsevier
22 Kus, K.A. et al. (2009) Humoral immune response to mixed PfAMA1 alleles; multivalent PfAMA1 vaccines induce broad specificity. PLoS ONE 4, e8110
23 Cowan, G.J. et al. (2011) A malaria vaccine based on the polymorphic block 2 region of MSP-1 that elicits a broad serotype-spanning immune response. PLoS ONE 6, e26616
24 DeMarco, R. et al. (2010) Protein variation in blood-dwelling schistosome worms generated by differential splicing of micro-exon transcripts. Genome Res. 20, 1112–1121
25 Nausch, N. et al. (2011) Regulatory and activated T cells in human Schistosoma haematobium infections. PLoS ONE 6, e16860
26 Plebanski, M. et al. (1997) Immune evasion in malaria: altered peptide ligands of the circumsporozoite protein. Parasitology 115 (Suppl.), S55–S66
27 Butterworth, A.E. et al. (1988) Immunity in human Schistosomiasis mansoni: cross reactive IgM and IgG2 anti-carbohydrate antibodies block the expression of immunity. Biochimie 70, 1053–1063
28 Capron, A. et al. (1994) Development of a vaccine strategy against human and bovine schistosomiasis. Background and update. Trop. Geogr. Med. 46, 242–246
29 Everts, B. et al. (2012) Schistosome-derived omega-1 drives Th2 polarization by suppressing protein synthesis following internalization by the mannose receptor. J. Exp. Med. 209, 1753–1767
30 Raberg, L. et al. (1998) On the adaptive significance of stress-induced immunosuppression. Proc. Biol. Sci. 265, 1637–1641
31 Zhang, W. et al. (2008) Mechanisms of immunity in hydatid disease: implications for vaccine development. J. Immunol. 181, 6679–6685
32 Ho, M. et al. (1986) Antigen-specific immunosuppression in human malaria due to Plasmodium falciparum. J. Infect. Dis. 153, 763–771
33 Casares, S. and Richie, T.L. (2009) Immune evasion by malaria parasites: a challenge for vaccine development. Curr. Opin. Immunol. 21, 321–330
34 Oreno, J.M. et al. (2008) A Plasmodium yoelii soluble factor inhibits the phenotypic maturation of dendritic cells. Malar. J. 7, 254
35 Jangwatapongka, K. et al. (2008) Plasmodium vivax parasites alter the balance of myeloid and plasmacytoid dendritic cells and the induction of regulatory T cells. Eur. J. Immunol. 38, 2697–2705
36 Mellouk, S. et al. (1990) Protection against malaria induced by irradiated sporozoites. Lancet 335, 721
37 Yaron, A. et al. (1983) Stage-dependent effects of chloroquine on Plasmodium falciparum in vitro. J. Protozool. 30, 642–647
38 Bejon, P. et al. (2007) The induction and persistence of T cell IFN-y responses after vaccination or natural exposure is suppressed by Plasmodium falciparum. J. Immunol. 179, 4193–4201
39 Beloue, E. et al. (2004) Protective T cell immunity against malaria liver stage after vaccination with live sporozoites under chloroquine treatment. J. Immunol. 172, 2487–2495
40 Sutherland, C.J. et al. (2007) How is childhood development of immunity to Plasmodium falciparum enhanced by certain antimalarial interventions? Malar. J. 6, 161
41 Gopinath, R. et al. (1999) Long-term persistence of cellular hyporesponsiveness to filarial antigens after clearance of microfilaremia. Am. J. Trop. Med. Hyg. 60, 848–853
42 Maizels, R.M. et al. (2004) Helminth parasites – masters of regulation. Immunol. Rev. 201, 89–116
43 Siracusano, A. et al. (2008) Immunomodulatory mechanisms during Echinococcus granulosus infection. Exp. Parasitol. 119, 483–489
44 Taylor, M.D. et al. (2005) Removal of regulatory T cell activity reverses hyporesponsiveness and leads to filarial parasite clearance in vivo. J. Immunol. 174, 4924–4933
45 Wilson, M.S. et al. (2005) Suppression of allergic airway inflammation by helminth-induced regulatory T cells. J. Exp. Med. 202, 1199–1212
46 Wilson, M.S. et al. (2011) IL-10 blocks the development of resistance to re-infection with Schistosoma mansoni. PLoS Pathog. 7, e1002171
47 Diaz, A. and Allen, J.E. (2007) Mapping immune response profiles: the emerging scenario from helminth immunology. Eur. J. Immunol. 37, 3319–3326
48 Jankovic, D. et al. (2007) Conventional T-bet “Foxp3” Th1 cells are the major source of host-protective regulatory IL-10 during intracacellular protozoan infection. J. Exp. Med. 204, 273–283
49 Loke, P. et al. (2002) IL-4 dependent alternatively-activated macrophages have a distinctive in vivo gene expression phenotype. BMC Immunol. 3, 7
50 Jenkins, S.J. and Allen, J.E. (2010) Similarity and diversity in macrophage activation by nematodes, trematodes, and cestodes. J. Biomed. Biotechnol. 2010, 262899
51 Wilson, M.S. et al. (2008) Suppression of murine allergic airway disease by IL-2-anti IL-2 monoclonal antibody-induced regulatory T cells. J. Immunol. 181, 6942–6954
52 Babu, S. et al. (2009) Alternatively activated and immunoregulatory monocytes in human filarial infections. J. Infect. Dis. 199, 1827–1837
53 Lyke, K.E. et al. (2005) HLA-A2 supertype-restricted cell-mediated immunity by peripheral mononuclear cells derived from Malian children with severe or uncomplicated Plasmodium falciparum malaria and healthy controls. Infect. Immun. 73, 5799–5808
54 Allen, J.E. and Maizels, R.M. (2011) Diversity and dialogue in immunity to helminths. Nat. Rev. Immunol. 11, 375–388
55 Grogan, J.L. et al. (1996) Elevated proliferation and interleukin-4 release from CD4+ cells after chemotherapy in human Schistosoma haematobium infection. Eur. J. Immunol. 26, 1365–1370
56 Watanabe, K. et al. (2007) T regulatory cell levels decrease in people infected with Schistosoma mansoni on effective treatment. Am. J. Trop. Med. Hyg. 77, 676–682
57 Mitchell, K.M. et al. (2008) The predicted impact of immunosuppression upon population age-intensity profiles for schistosomiasis. Parasite Immunol. 30, 462–470
58 Mitchell, K.M. et al. (2012) Protective immunity to Schistosoma haematobium infection is primarily an anti-fecundity response stimulated by the death of adult worms. Proc. Natl. Acad. Sci. U.S.A. 109, 13347–13352
59 Fulford, A.C.J. et al. (1995) A statistical approach to schistosome population dynamics and estimation of the life-span of Schistosoma mansoni in man. Parasitology 110, 307–316
60 Bull, P.C. and Marsh, K. (2002) The role of antibodies to Plasmodium falciparum-infected erythrocyte surface antigens in naturally acquired immunity to malaria. Trends Microbiol. 10, 55–58
61 Boyd, M.F. (1949) Malariaology, Saunder
62 Woolhouse, M.E. (1994) Immunoepidemiology of human schistosomes: taking the theory into the field. Parasitol. Today 10, 196–202
63 Mutapi, F. et al. (1997) Comparison of humoral responses to Schistosoma haematobium in areas with high and low levels of infection. Parasite Immunol. 19, 255–263
64 Mutapi, F. et al. (2005) Praziquantel treatment of individuals exposed to Schistosoma haematobium enhances serological recognition of defined parasite antigens. J. Infect. Dis. 192, 1108–1118
65 Hsu, S.Y. et al. (1981) Schistosoma mansoni: vaccination of mice with highly x-irradiated cercariae. Exp. Parasitol. 52, 91–104
66 Long, S.A. et al. (2011) Low-dose antigen promotes induction of FOXP3 in human CD4+ T cells. J. Immunol. 187, 3511–3520
67 Berkow, G. et al. (2000) Chronic immune activation associated with intestinal helmint infections results in impaired signal transduction and anergy. J. Clin. Invest. 106, 1053–1060
68 Griffith, Q.K. et al. (2011) CD23-bound IgE augments and dominates recall responses through human naïve B cells. J. Immunol. 186, 1060–1067
69 Struik, S.S. and Riley, E.M. (2004) Does malaria suffer from lack of memory? Immunol. Rev. 201, 268–290
70 Langhorne, J. et al. (2008) Immunity to malaria: more questions than answers. Nat. Immunol. 9, 725–732
71 Cavanagh, D.R. et al. (1998) A longitudinal study of type-specific antibody responses to Plasmodium falciparum merozoite surface protein-1 in an area of unstable malaria in Sudan. J. Immunol. 161, 347–359
72 Dorfman, J.R. et al. (2005) B cell memory to 3 Plasmodium falciparum blood-stage antigens in a malaria-endemic area. J. Infect. Dis. 191, 1623–1630
73 Akpogheneta, O.J. et al. (2008) Duration of naturally acquired antibody responses to blood-stage Plasmodium falciparum is age dependent and antigen specific. Infect. Immun. 76, 1748–1755
74 Polley, S.D. et al. (2006) High levels of serum antibodies to merozoite surface protein 2 of Plasmodium falciparum are associated with reduced risk of clinical malaria in coastal Kenya. Vaccine 24, 4233–4246
75 Osier, F.H. et al. (2008) Breadth and magnitude of antibody responses to multiple Plasmodium falciparum merozoite antigens are associated with protection from clinical malaria. Infect. Immun. 76, 2240–2248
76 Wipasa, J. et al. (2011) Short-lived IFN-γ effector responses, but long-lived IL-10 memory responses, to malaria in an area of low malaria endemicity. PLoS Pathog. 7, e1001281
77 Wipasa, J. et al. (2010) Long-lived antibody and B cell memory responses to the human malaria parasites, Plasmodium falciparum and Plasmodium vivax. PLoS Pathog. 6, e1000770
78 Agnandji, S.T. et al. (2011) First results of phase 3 trial of RTS.S/A01 malaria vaccine in African children. N. Engl. J. Med. 365, 1863–1875
79 Whitty, C.J. et al. (2000) Presentation and outcome of 1107 cases of schistosomiasis from Africa diagnosed in a non- endemic country. Trans. R. Soc. Trop. Med. Hyg. 94, 531–534
80 Tchuente, L.A. et al. (2004) Efficacy of praziquantel against Schistosoma haematobium infection in children. Am. J. Trop. Med. Hyg. 71, 778–782
81 Soonawala, D. et al. (2011) The immune response to schistosome antigens in formerly infected travelers. Am. J. Trop. Med. Hyg. 84, 43–47
82 Van der Kleij, D. et al. (2002) Triggering of innate immune responses by schistosome egg glycolipids and their carbohydrate epitope GalNacβ1–4(Fucα1–2Fucα1–3GlcNAc. J. Infect. Dis. 185, 531–539
83 Swain, S. (2010) Grand challenges in immunological memory. Front. Immunol. 1, 103
84 Rakhov, E.V. and Williams, M.A. (2009) The magnitude of CD4+ T cell recall responses is controlled by the duration of the secondary stimulus. J. Immunol. 183, 2382–2389
85 Pichichero, M.E. (2011) Bacterial conjunctivitis in children: antibacterial treatment options in an era of increasing drug resistance. Clin. Pediatr. (Phila) 50, 7–13
86 Brake, D.A. (2003) Parasites and immune responses: memory illusion? DNA Cell Biol. 22, 405–419
87 Naush, N. et al. (2012) Proportions of CD4+ memory T cells are altered in individuals chronically infected with Schistosoma haematobium. Sci. Rep. 2, 472
88 Hayflick, L. and Moorhead, P.S. (1961) The serial cultivation of human diploid cell strains. Exp. Cell Res. 25, 585–621
89 Pilyugin, S. et al. (1997) Modeling T-cell proliferation: an investigation of the consequences of the Hayflick limit. J. Theor. Biol. 186, 117–129
90 Ballou, W.R. (2009) The development of the RTS,S malaria vaccine candidate: challenges and lessons. Parasite Immunol. 31, 492–500