Review

Infection and treatment immunizations for successful parasite vaccines

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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 measureable 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 (PfSPZ vaccine) 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 naïve vaccine trial participants. For

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Keywords: immunization; treatment; vaccine; malaria; schistosomiasis; parasite.

Table 1. Currently licensed human vaccines[a]

Vaccine	Common name/combination vaccine	Pathogen	Type of vaccine
Anthrax		Bacteria	Subunit[b]
Chicken pox	Varicella	Virus	Live, attenuated[c]
Cholera			Inactivated[d]
Diptheria	DPT	Bacteria	Inactivated toxin
Haemophilus influenza type B	Hib	Virus	Conjugate[e]
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–Géurin (BCG)	bacteria	Live, attenuated
Typhoid		bacteria	Inactivated
Yellow fever		virus	Live, attenuated

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

[b]Subunit 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'.

[c]Live 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.

[d]Inactivated vaccine: a vaccine made by killing the disease-causing microbe with chemicals, heat, or radiation.

[e]Conjugate 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.

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