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Vaccinating against zoonotic parasitic diseases: myth or reality?

Dante S. Zarlenga

US Department of Agriculture ARS, ANRI, Immunology and Disease Resistance Laboratory, Beltsville, MD, USA

Abstract

The largely unanticipated difficulties of parasite vaccine development have led us to a renewed awareness of the survival strategies evolutionarily embedded within parasites over hundreds of millions of years. We have grown to appreciate that efforts to disrupt parasite–host relationships are substantially compounded by our incomplete understanding of the complex immune responses that occur in the naturally infected host. Given the inability to transfer laboratory successes to field trials, research is leading us to conclude that genetically defined animal models may not be good predictors of the unique and disparate protective immune responses one can expect from the genetically heterogeneous populations of animals that represent the parasite's natural environment. This is further compounded by the abundance of mechanisms parasites have created for themselves to defend against immune intervention. Thus, in the never-ending saga of vaccine development, it is only appropriate that pitfalls and advancements be critiqued as they apply across parasite groups, with a look towards promising technologies that may propel this field to the level of scientific achievement once envisaged.

Keywords: vaccination; parasitic diseases; zoonoses; recombinant

Introduction

In 1984, two laboratories independently cloned and expressed the circumsporozoite gene, an immunodominant surface antigen from *Plasmodium falciparum* believed to be a key target antigen for vaccine development (Dame *et al*., 1984; Enea *et al*., 1984). Although similar work had been conducted on the monkey parasite *Plasmodium knowlesi* a year earlier (Ellis *et al*., 1983), research performed on *P. falciparum* was of special interest because it was at that time, and remains today, a leading agent of parasitic disease for people living in endemic regions of the world. The scientific community became euphoric over the perceived opportunity to quickly develop a cost-effective, stable vaccine via recombinant DNA technology. Unfortunately, nearly 20 years later, a recombinant vaccine against this deadly parasite still eludes researchers (Graves and Gelband, 2003). The battle to produce functional, genetically engineered vaccines against parasites, however, is not unique to *Plasmodium*. On the contrary, co-evolution at the host–parasite interface has resulted in the selection of reciprocal systems of host adaptation and immune evasion that scientists have only just begun to appreciate. For this reason, failures abound in this field of research to the extent that no commercially available vaccine exists for control or eradication of any human parasitic disease. Of the successful animal parasite vaccines that have been generated, most have been predicated upon fully viable or attenuated organisms rather than recombinant DNA technology. Some would argue this approach to be more of a management tool than a vaccine, in that the parasites are not usually cleared from the host following vaccination. Thus, the

Correspondence: US Department of Agriculture, ARS, ANRI, Immunology and Disease Resistance Laboratory, Bldg 1180 BARC-East, Beltsville, Maryland 20705, USA
E-mail: Zarlenga@anri.barc.usda.gov

ability of parasites to skirt both innate and antigen-specific adaptive immune responses might be better understood by examining this issue from a parasite-centered rather than host-centered point of view, and by reviewing the obstacles that scientists must struggle with in negotiating this elusive problem.

Obstacles to vaccinating against zoonotic parasites

Evolution and evading host immunity

The strongest forces scientists must contend with in developing vaccines against parasitic agents are embedded in evolution and the process of host adaptation. The more time that transpires in the co-evolutionary process between host and parasite, the more difficult it is to disrupt the microenvironment the parasites have created for themselves, and the mechanisms that have developed through selection to obviate and/or manipulate the host protective responses. Direct evidence of the importance of successful parasite adaptation can be seen in the many failed approaches to vaccinations, from injecting cloned DNA and administering cocktails of multiple subunit antigens to treating with antigens in concert with host immune modulators, e.g. cytokines. Additional anecdotal evidence of this phenomenon may be gleaned from successful recombinant vaccine trials against a number of taeniid parasites (Lightowlers *et al*., 1996; Plancarte *et al*., 1999). Although taeniids are by no means a young group of organisms, the correlation between definitive hosts (man) and their intermediate hosts, i.e. bovine (*Taenia saginata*) and swine (*Taenia solium* and *T. asiatica*), coincides with the relatively recent domestication of these animals, and maintenance of these independent but synanthropic host–parasite assemblages over the past 10 000 years (Hoberg *et al*., 2001). A similar argument also may hold true for the non-human parasite *T. ovis* (Lawrence *et al*., 1996). Thus, poor adaptation is a probable impetus for rapid calcification of the muscle cysts following infection in these intermediate hosts, and is thereby a contributor to the extraordinary successes observed in vaccine trials with recombinant antigens. Successful vaccination may also be enhanced, by the localization of the extracellular parasites amidst non-mucosal surfaces. Nonetheless, these tissue cysts define rather unique circumstances that are not often observed with other zoonotic parasites. Consequently, the 'clone it, express it, purify it, inject it' approach to parasite vaccine development has met with lackluster progress.

Each of the many parasite groups has developed its own defense or escape mechanisms to infect and persist in the host. Among the more common methods are: (i) antigenic variation, whereby the parasite systematically and in a cyclical manner alters surface proteins to remain one step ahead of the host's defense mechanisms; (ii) antigenic masking, whereby the parasite coats itself in host proteins so as not to be detected as foreign; and (iii) immunosuppression, in which the parasite produces factors that either directly suppress the host's immune responses or cause the proliferation of suppressor T cells. Such agents would strongly assist in maintaining chronic infections. 'Mimicking' – the production of proteins that simulate host proteins – can actually fall into many categories, among which is the imitation of host cytokines for modulating or tricking the host immune system. In this regard, research tends to focus on the parasite and not the host as the variable factor. One example is the identification of transforming growth factor-β (TGF-β) homologues within parasites and the assimilation of these with the potential to down-regulate host immune responses. While there are suggestions that this may indeed occur (Gomez-Escobar *et al*., 2000), the preponderance of evidence is more in line with TGF-β and associated homologues functioning to regulate development and cell function within the homologous organism (Gomez-Escobar *et al*., 1998). The mere characterization of classes of TGF-β homologues in the free-living nematode *Caenorhabditis elegans* (Patterson and Padgett, 2000) and the phylogenetic assessment of TGF-β homologue sequences across life forms (Newfeld *et al*., 1999) supports the evolution of this particular 'immune-related' gene predating the host–parasite relationship. Such a pathway would, however, represent an exaptation that was later co-opted in the repertoire associated with some host–parasite interfaces. Though it is clear that mimicry is part of the arsenal of tactics available to parasites to advance infection and persistence, it is also likely that the redundancies in man's immune system are the result of specific responses to the plethora of infectious agents that surround him, including parasites. The overlapping duties of interleukin (IL)-2 and IL-15, IL-4 and IL-13, and IL-12 and IL-18 are among many examples of this redundancy. Consequently, in each instance, the question of who is imitating whom must be posed when broaching issues of mimicry and using this as a foothold for vaccine development.

The simplest and most common method among protozoan parasites to escape host immune responses is their intracellular location, which in turn protects the organism from direct interaction with the host immune system. Because worms do not invade cells, cytotoxic T lymphocyte responses tend not to be protective and humoral responses become the host's main defense mechanism. Encapsulation and immunosuppression become major defense mechanisms of extracellular parasites. In some very rare circumstances, namely infections with parasites of the genus *Trichinella*, helminths are also capable of separating themselves from the host's protective responses via intracellular localization, though this is stage-specific. Not surprisingly, the intracellular location probably contributes to *Trichinella* parasites

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being among the most cosmopolitan and least host-specific of all nematode groups (Pozio, 2001). Though it is usually not viewed as such, one may construe the use of multiple hosts by parasites as yet another, more aberrant method of adaptation and evasion of specific immune responses to enhance fitness.

Model systems: good for the goose but not for the gander

One cannot dispute the many contributions that small animal modeling has made to our understanding of immunity. The inter-workings of T-helper cells and the mechanisms involved in their differentiation are clear examples of how models have helped dissect the polarization of immune responses to infection. Still, examples persist showing deviations from conventional wisdom, such as the simultaneous elevation of IL-4 and interferon-γ (IFN-γ) gene transcription in virus-infected (Waldvogel *et al*., 2000) or nematode-infected (Canals *et al*., 1997) cows, and the substantially lower levels of IL-12β2 receptor transcription in swine lymphocytes (Solano-Aguilar *et al*., 2002) which may influence the timing and intensity of IL-12-dependent responses. It is the same system of modeling, however, that has blinded many researchers into overlooking its shortcomings and in so doing, the parasite's ability to circumvent its own model teachings. The literature is laden with successful parasite vaccination trials in mice, only to be followed by lackluster results when translocated to their natural hosts. Research has demonstrated that the machinery for antigen recognition is fairly well conserved among humans, large animals and mice; however, the quality of antigen recognition and processing need not be. These issues are compounded by genetic variability among natural host populations, the unlikely assumption that specific parasite immune evasion mechanisms are equally effective across model and natural hosts, the increasing evidence for genetic components to resistance, and the expectation that results obtained with inbred animals are indicative of outbred populations, which are all compelling arguments to question model systems as specific predictors of protection in the natural host. It is regrettable to think that the converse may be true, where many candidate immunization regimes have been discarded over the years because of negative results from model studies.

Where do we go from here?

Before embarking on the arduous task of developing vaccines against any disease agent, it is prudent to ask if such a vaccine is necessary. Billions of dollars have been spent in the development of vaccines against human parasites, including such parasites as *Cryptosporidium* spp., *Toxoplasma* spp., *Trichomonas* spp. and *Giardia* spp., which are prevalent in North America; however, these parasites are ubiquitous in nature and, other than in rare outbreaks, are generally well tolerated by the immunocompetent individual at environmental levels. It is unlikely that the development of a vaccine against these or other similar parasite groups will ultimately result in unilateral recommendation for treatment. On the other hand, diseases such as malaria, which continues as a leading cause of morbidity and mortality in tropical regions and claims over 2.5 million lives annually, demand that investigations on immune interventions continue.

The science of vaccine development has placed substantial emphasis on altering the host's response to infection, somewhat less attention being given to direct perturbation of the parasite mechanisms involved in avoiding and/or manipulating microenvironments. However, packaged within such small organisms are genes conserved among parasites of a similar lineage, and genes which differentiate them from their close relatives and thus are likely to be involved in host recognition and infection. Identifying these genes is no simple task. The application of microarrays, real-time PCR and proteomics has made its mark on identifying and quantifying unique genes and gene products, and in profiling responses to infections. But, as the data acquisition era of genome sequencing slowly winds down, the major challenge facing biologists today is linking genomic and proteomic data to the development and behavior of an organism. These data constitute strategic foundations upon which more targeted methods and better delivery systems for reducing parasite infections can be developed. RNA interference (RNAi) (Fire *et al*., 1998), a modification of first-generation single-stranded, anti-sense RNA studies, is one of the promising technologies that may help bridge this gap. RNAi first surfaced in the late 1990s, but it is only recently that more holistic approaches have been used to link genetic and phenotypic changes (Maeda *et al*., 2001). Kamath *et al*. (2003) successfully identified mutant phenotypes coinciding with 1722 of 19 427 predicted genes in *C. elegans*. In a follow-up study, Ashrafi *et al*. (2003), used RNAi to focus on fat regulatory genes to identify direct correlations between specific genetic alterations and phenotypic changes in the nematode. Similar approaches show promise for identifying genes and gene products important for invasion and the persistence of parasite infections, and thereby provide a more targeted approach to vaccine development (Aboobaker and Blaxter, 2003; McRobert and McConkey, 2002). RNAi may also find utility as a prophylaxis in treating previously diagnosed infections (Caplen, 2003). As with any new technology, there will be new problems to address. One caveat to RNAi is developing simple assays for detecting parasite mutations sufficient to neutralize

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infections. Nonetheless, technologies unfolding today demonstrate a clear recognition of the shortcomings of past approaches to vaccine development. The new-found desire to link genetic, proteomic and phylogenetic data with changes in parasite development and behavior, i.e. the era of functional genomics, will hopefully make vaccine development more targeted than in days gone by and advance this field to the levels of success anticipated nearly 20 years ago.

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