

Chronic parasite infections cause immune changes that could affect successful vaccination

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Several important issues must be considered when performing any vaccination in areas with high prevalence of geohelminths. Immunization of populations infected with geohelminths could be sub-optimal if the immune background is not taken into consideration. Immune modulation and deworming might be essential for effective protective vaccination. In addition, further animal models and clinical studies addressing these issues are required. Underscoring the importance of these issues, a recent report has highlighted several vaccination studies in which nematode-infected mice or pigs failed to mount efficient protective immune responses.

Geohelminth infections undermine successful vaccination

Vaccination is the most effective and efficient procedure for disease prevention [1]. However, in many parts of the developing world, mass vaccination has failed to confer the desired protection. For example, the century-old anti-tuberculosis vaccine, Bacillus Calmette-Guerin (BCG), has failed to confer protection against tuberculosis in Africa [2,3]. The reasons for the discrepancy between the successes of several vaccines in some areas of the globe versus their failures in others remain unclear. Given the similar geographic distribution of geohelminth infections, Human Immunodeficiency Virus type 1 (HIV-1) and *Mycobacterium tuberculosis* (MTB), and the high prevalence of geohelminth infections in the developing world, the chronic immune activation and T helper cell type 2 (Th2) immune profile caused by geohelminth infections might make the host more susceptible to HIV or MTB infection and less able to cope with it once infected [4–6]. Furthermore, despite some conflicting results [7–10], the cumulative immunological and epidemiological evidence is in favor of deworming as a preventive and possible therapeutic measure with regards to HIV-1 infection [11–13]. Without eradication of helminthic parasites, the efficacy of vaccines could be compromised [14–16]. Although concurrent infection with helminthic parasites might not prevent the development of protective responses following vaccination in all cases [17], a negative correlation between the infectious helminthic dose and the magnitude of the immune response could exist [18,19]. Thus, without eradication of these parasites, HIV and TB vaccines might fail to confer protection in geohelminth-endemic areas, implying that

eradication of these infections, or modulation of the immune changes that they cause, should be instituted before HIV and MTB mass vaccination [2]. Importantly, school- or population-based treatment programs taking place in several regions of the world have reduced infection intensities, considerably reducing the possible detrimental immunological effects that these low-intensity infections could cause. It should also be noted that geohelminthic infections tend not to occur before nine months of age, so because many vaccines are provided to babies shortly after birth, their vaccination might occur before the acquisition of geohelminth infections.

A recent article by Urban *et al.* [20] reviewing several vaccination studies in nematode-infected animals in which the infected animals failed to mount protective immune responses following vaccination (compared with non-infected control animals) gives further support to the notion that the skewed immune profile present in many animal and human populations because of endemic geohelminth infections undermines the efficacy of vaccination (Figure 1). It re-emphasizes the need to take into consideration the immune background of the target population to be vaccinated in the design of the vaccines and the potential need to eradicate the parasitic infection before instituting mass vaccination.

Parasite-infected animal models for testing vaccination modalities

Before potential vaccines can be tested in human trials they are usually tested in animal models, so as to show that they generate the cellular and humoral immune responses required to achieve effective protection in the host. For example, a requisite for vaccines to confer protection against intracellular infections such as HIV or MTB is their capacity to induce Th1-type immune responses. Indeed, many animal models are used to address this particular issue [21,22]. However, there are only a few instances in which animal models were studied to determine vaccine efficacy in the presence of chronic concurrent parasitic infections, or in immune dysregulated and/or chronically activated animals. In their recent manuscript, Urban *et al.* [20] describe parasite–murine and parasite–swine experimental models (mice infected with *Heligmosomoides polygyrus* and pigs infected with *Ascaris suum*) in which the animals' immune profiles are affected by the ongoing concurrent parasitic infection in a way that resembles the systemic and mucosal immune profile observed in humans infected with geohelminths. These

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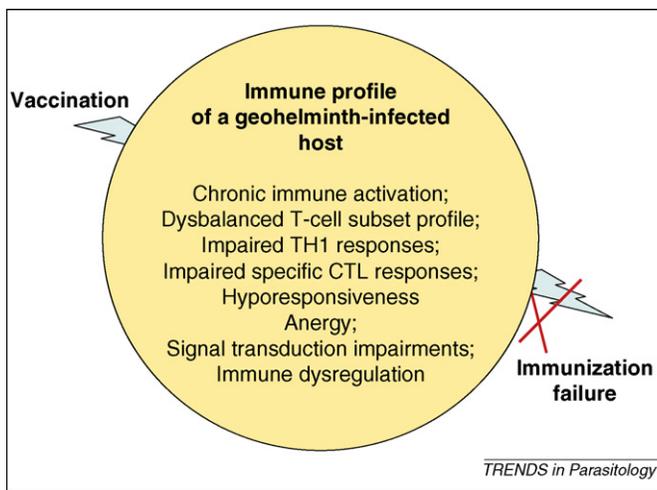


Figure 1. Geohelminthic infections endanger effective vaccination. The immune profiles of individuals chronically infected with helminthic parasites undergo extensive changes (as outlined in this article). These immune changes could compromise the effectiveness of vaccination and lead to sub-optimal immunization.

common and widely spread infections of the animals result in a clear Th2-type cytokine secretion, high TGF- β (transforming growth factor beta) levels, extremely low IFN- γ secretion, primarily IgG1 and IgE production, increased CD4⁺/CD25⁺ T regulatory cell population, eosinophilia, basophilia and mucosal mast cell and goblet cell hyperplasia. Not surprisingly therefore, vaccines administered to these animals failed to elicit protection following challenge by relevant pathogens as opposed to the protection that they conferred to non-parasite-infected matched controls. Thus, a novel ovalbumin (OVA)-expressing oral *Salmonella* vaccine (Salmonella-OVA) failed to elicit the Th1-dominant OVA-specific protective responses in animals infected with *H. polygyrus* [20], and dominant Th2 responses and poor Th1 immune responses were observed in *Schistosoma mansoni*-infected mice immunized with either plasmid DNA encoding β -galactosidase (β -gal) or HIV antigens [23].

Importantly, these animal models can also serve to test the capacity of different adjuvants for modulating the Th2-dominant and immune-activated profile in geohelminth-infected animals, aiming to increase Th1-dependent immune responses required to confer protection. This has been demonstrated previously in *Schistosoma*-infected mice following intradermal immunization with CpG-rich plasmid DNA encoding β -gal or with oligodeoxynucleotides containing CpG immunostimulatory sequences co-administered with gp120-depleted HIV-1 viral particles (HIV immunogen), eliciting potent Th1 type anti- β -gal and anti-HIV-1 immune responses [23].

These parasite-infected animal models can also be used to explore the effect of deworming on the capacity of potential vaccines to elicit the desired immune responses. For example, antihelminthic treatment before immunization against malaria, but not after the immunization, restored the protective immunity to malaria challenge [24]. Similarly, anti-*Schistosoma* treatment of *S. mansoni*-infected mice allowed the generation of cytotoxic T-lymphocyte responses against HIV antigens following immunization with HIV immunogen [23].

It is important to acknowledge that animal models, while being especially useful in investigating mechanisms

of disease or immune responses, do not necessarily represent the 'real-life scenario'. For example, repeated exposures to one or several helminths are common in human populations and fluctuations in infectious doses of helminths can vary significantly. These variables are not taken into consideration in experimental animal models.

Future animal models

Future parasite–animal models used to test vaccinations should aim to include additional common geohelminths or other parasites, such as *Entamoeba histolytica*. These models should enable the study of immune responses elicited during vaccination and immunomodulation of the infected hosts, and they should also enable the testing of the capacity of potential vaccines to confer protection against subsequent challenge. Future models should also directly test the capacity of cells obtained from human subjects suffering from parasitic infections to mount effective immune responses and confer protection to pathogens when implanted in animal hosts. For example in the Trimeria-HIV-1 animal model the human peripheral mononuclear cells that are implanted in mice can generate primary and secondary immune responses [25]. This model can also be used for the purpose of studying the effectiveness of potential vaccines and adjuvant in hosts with pre-existing biased immune profiles. For example, human peripheral blood mononuclear cells from HIV-1 seronegative donors with a dominant pre-existing Th2 immune profile can be transplanted to the Trimeria mice, which will be then used to test the ability of CpG oligonucleotides to overcome the pre-existing dominant Th2 immune background and induce a potent HIV-1-specific Th1 immune response.

Conclusions

The animal models reviewed by Urban *et al.* [20], as well as other vaccination studies using parasite-infected animals, highlight the impact of chronic parasitic infections on the generation of protective immune responses after vaccination. Though huge efforts are currently invested in the development of new vaccines, few are tested in hosts that accurately reflect the natural immune background of the populations to be vaccinated, and particularly those chronically infected with helminths. This underscores the importance of using more relevant animal models that will simulate the immune profile of populations living in parasite-endemic areas, and of performing clinical vaccine trials that consider host immune background and its modulation as an integral part of their design.

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doi:10.1016/j.pt.2008.02.009 Available online 30 April 2008

Letters

Live immunisation against *Theileria parva*: spreading the disease?

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In a recent Research Focus article in this journal [1], Declan McKeever raised some important concerns regarding the method of live immunisation against *Theileria parva*. Although persistence of vaccine strains in ticks and their presence in co-grazing non-vaccinated cattle is well documented, there could be some questions raised regarding the results reported by Geysen [2] of the presence of one or more components of the Muguga cocktail in the Southern province of Zambia after its limited deployment 10 years earlier.

The argument goes that neither the Muguga nor the Serengeti component has given rise to a significant carrier status in animals immunised with the cocktail. This statement is based mainly on the work of Oura [3] in Uganda and is also in agreement with PCR results previously reported by Bishop [4] and Skilton [5].

The results in Zambia are based on the same antigenic loci used by Bishop and Oura, and clearly show that nearly

all field isolates (n = 126) had the Muguga or Serengeti alleles (Muguga is identical to Serengeti for the loci used). Southern blotting on genomic DNA from one of these field isolates confirmed the Muguga/Serengeti genotype [2].

These results seem to contradict the observations in Oura's article [3]. Differences in sensitivity or specificity of the diagnostic methods can be excluded. Both are based on a multilocus semi-nested PCR approach using *T. parva*-specific single-copy markers. An important initial consideration is the lack of information regarding the biology and genetics of the carrier status in *T. parva*. Moreover, *T. parva* Muguga/Serengeti seems to be the only exception as it has a short carrier status [6]. Low and fluctuating parasitaemias have been reported in many *T. parva* isolates and might have been the reason for negative PCR diagnosis in Oura's study [3]. Antigen-detecting tests are done at discrete time points, not necessarily coinciding with parasitaemias above diagnostic threshold level. It could be that even the Muguga/Serengeti type strains never reach true sterile-carrier status, but they might give

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