ANTIGEN FORMULATION AND DELIVERY

Extracellular vaccine candidates need to be expressed in bacteria or eukaryotic expression systems. Many of the selected targets are likely to require processing through the endoplasmic reticulum by virtue of their expression sites in the parasite (i.e., secreted or anchored in the tegument), and this may prove challenging. An additional important consideration is that antigen identification and successful protective results are of little value if GMP cannot be applied for scaling-up of production of any vaccine candidate (18).

The selection of a suitable adjuvant and delivery system to aid in the stimulation of the appropriate immune response are critical steps in the path to the development and employment of successful antischistosome vaccines, and a number of approaches have been tested with some success. Traditional approaches have seen Freund's adjuvants used when antigens are first being assessed as vaccines in the mouse model. It must be remembered, however, that Freund's complete adjuvant, although the mainstay of immunological adjuvants in research for decades, is not suitable for human application as it can produce a number of undesirable side effects that include the formation of local inflammatory lesions at the site of the injection that can be severe and result in chronic granulomas and abscesses. Once efficacy has been proven, other adjuvants, particularly those that are licensed (or have the potential for licensing) for human use, are used to formulate an antigen. Less conventional or less widely used approaches have been explored for adjuvanting schistosome vaccines, including live *Salmonella* (132), tetanus toxin (120), filamentous phage (133), recombinant *Mycobacterium bovis* Bacille Calmette-Guérin (80,134), nanoparticles (135), and various methods of mucosal delivery (136–138).

Before a well-informed decision can be made on adjuvant selection, a comprehensive understanding of the desired immune response (phenotype) is necessary. This in turn implies that the immune parameters required to obtain optimal protection are known. For human schistosomiasis, this is not the case. For example, very few people develop natural resistance to the parasite in the absence of repeated anthelmintic therapy (see earlier section on PR individuals). We advocate the use of such cohorts to guide vaccine development (both antigen discovery and the phenotype of the protective response), but in reality, a schistosome vaccine will be delivered as part of an integrated control package that involves PZQ treatment before vaccination. Therefore, should we look more to the people who develop resistance to reinfection after PZQ therapy (51)? These two groups of individuals make very different immune responses to different antigens on different stages of the parasites (51,53,139). All this information is relevant, albeit complicated, when deciding how best to formulate and deliver a vaccine for human schistosomiasis. If we are to target toll-like receptors (TLRs) on antigen-presenting cells that induce a Th1 response, such as TLR-9, then adjuvants such as un-methylated CpG oligonucleotides are attractive, and although not yet widely used for schistosomiasis vaccinology, this adjuvant is showing promise for experimental vaccines against other pathogens (140). Indeed, the PR individuals identified in Brazil (53), who were utilized to identify two new tegument antigens (56,61), mounted a vigorous Th1 response to schistosomula surface antigens, making CpGs a potentially attractive adjuvant for these vaccines. CpGs are being used in conjunction with more conventional adjuvants such as alum, which induce a more Th2-like immune response. For the diphtheria-tetanus-pertussis (DPT) vaccine, which is currently formulated with alum, the addition of CpGs reduced the total IgE levels and increased anti-PT specific IgG2a in comparison with the ordinary DPT-alum vaccine (141). CpG-7909 has been used to improve the antibody responses generated to licensed vaccines in humans, such as the anthrax vaccine adsorbed and the Engerix-B hepatitis B vaccine (reviewed in Ref. 139). If a mixed Th1/Th2 response is optimal for a schistosomiasis vaccine, combination adjuvants such as alum-CpG seem to be a suitable way forward.

CONCLUDING COMMENTS

Taking the breadth of consolidated, international efforts to generate antischistosome vaccines, there is considerable optimism that these endeavors will prove successful. In our opinion, the most recent quantum leaps forward in schistosomiasis vaccinology have been the integrated genomic and proteomic studies that have now equipped us with all the information (antigen selection at least) we need to choose the best antigens for a schistosomiasis vaccine. The recent landmark publications of the *S. japonicum* and *S. mansoni* genomes provide new avenues for antigen discovery (142,143). Again in our opinion, we emphasize that the apical membrane proteins expressed on the surface of the schistosomulum and adult worm are the logical vaccine targets on which to focus, and recent published data with some of these proteins supports this hypothesis (26,56,61). Moreover, there are mRNAs encoding novel, putatively secreted proteins without known homologues that are lodged in the tegument membrane (70,71), and these have yet to be explored. Indeed, there are very few descriptions of schistosomiasis vaccine trials with proteins that are completely unique to schistosomes and do not share sequence identity with any other proteins.

When developed and employed, antischistosome vaccines will not be a panacea. They need to be regarded as one component, albeit a very important one, of integrated schistosomiasis control programs that complement existing strategies including chemotherapy and health education. Although debatable, PZQ resistance is either here or on the horizon at least, and the need for vaccines is now more pressing than ever.

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