Opportunitites and Constraints in Schistosomiasis Vaccine Development: Infection Characteristics and Industry Realities

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The notes provided in this article relate to two components of the development of vaccines against schistosomiasis: (1) The characteristics of schistosome infections (eg. features of the schistosome life cycle), and the parasite itself, that have implications for vaccination strategies; (2) The characteristics of the biopharmaceutical industry that have implications for product development. As will be seen, these two topic areas are not vastly disparate.

Key words: schistosomiasis - vaccines - life cycle - immunopathology - pharmaceutical industry

Various aspects of the schistosome life cycle (attention here being restricted to *Schistosoma mansoni* and *S. japonicum*) are relevant to the approaches taken to vaccination in mammalian hosts. These include skin penetration by cercariae, migration of schistosomules through the lungs, residency of adult worms in the portal system, and tissue location of maturing eggs and the immunopathologic responses elicited. In addition, much has been written about the fact that worms do not proliferate in their vertebrate hosts and, since the severity of disease is related to worm burden, a schistosomiasis vaccine need not be 100% effective to be a useful adjunct control measure.

Cercarial dermatitis in humans provoked by bird schistosomes raises some concerns that vaccination with cercarial antigens may sensitize for undesirable skin reactions on challenge. Such reactions are not a prominent recorded feature of schistosome-related pathology in endemic areas but, depending on the type and magnitude of responses induced by vaccination and subsequent skin exposure to cercariae, some cutaneous side effects may become apparent.

Studies on life cycle characteristics of Philippines isolates of *S. japonicum* in the mouse have raised the possibility that this schistosome in mammalian hosts may differ from others in transiting the lungs very rapidly (Mitchell et al. 1991). Petechiae are prominent on the lung surface of mice infected a few days previously with cercariae of *S. japonicum* (Philippines) and the number matches the number of adult worms found in the portal system at later times. However, finding schistosomules in lungs is very difficult. Moreover, young schistosomes can be found readily in the liver at one week, compared with two weeks in *S. mansoni* infection. If this is true in human infections with *S. japonicum* (Philippines) then the lung stage of the schistosome life cycle may not be an attractive target for immune intervention. Schistosomules are something of a "moving target" but those of *S. japonicum* (Philippines) may be a "speeding target". Inflammatory responses (including subsequent expulsion into airways) and IFN-γ production by sensitised T cells in lungs have been implicated in expression of resistance to infection with *S. mansoni* in mice exposed previously to irradiated cercariae (see Wilson RA, this Symposium). It is of some interest that no demonstration of resistance to infection in mice has been made using irradiated cercariae of *S. japonicum* (Philippines) [c.f. *S. mansoni* and *S. japonicum* (Chinese)]. Moreover, mice that are exposed to irradiated *S. japonicum* (Chinese) and that are protected against homologous challenge, are not resistant to infection with *S. japonicum* (Philippines) (Moloney et al. 1985)

The mouse model of chronic schistosomiasis has been used to demonstrate unequivocally that portal system changes that follow granuloma formation, fibrosis and subsequent portal hypertension can militate against incoming parasites remaining in the portal system. The collateral blood flow presumably transports them back to the lungs continuously and demonstrations of resistance to reinfection in the mouse can be ascribed readily to these (immuno) pathological events rather than to anti-worm immune responses. Not surprisingly, no candidate vaccine molecules have been identified through analysis of apparent resistance to reinfection in the mouse host. Interestingly, a similar phenomenon of high resistance to first infection in a mouse strain studied at The Walter & Eliza Hall Institute (WEHI) in Melbourne, namely the WEHI 129/J mouse (and to a lesser extent in the related

C57Bl/6 strain), can be ascribed to portal system peculiarities that result in young schistosomes being shunted to the lungs. The basis of this peculiarity may be nutritional, though this has not been demonstrated. Yet through analysis of immune responses differentially expressed in resistant WEHI 129/J mice compared with other susceptible (permissive) mouse strains such a BALB/c, the glutathione S-transferases (GSTs) were first identified as vaccine candidates! (Smith et al. 1986). The 26 kDa GST (Sj26) forms the basis of the useful pGEX expression system developed by Donald Smith at WEHI, but the molecule has proven not to induce consistent host-protective immunity to *S. japonicum* (Philippines) in various mouse strains. [On the question of a nutritional contribution to portal system peculiarities in 129/J ± C57Bl/6 mice on which we have speculated (Mitchell et al. 1990), it is possible that hypervitaminosis A may lead to accumulation of fat droplets in Ito cells in the Space of Dissé (with or without differentiation into myofibroblasts and fibroblasts) and occlusive events in the liver (see Almeida Barbosa, Pfeifer and Andrade this Symposium). The 129/J mouse could be genetically prone to respond to any subsequent changes, such as an increase in portal pressure, by formation of intrahepatic portal shunts. It must be remembered that 129/J mice purchased from the Jackson Laboratories are entirely permissive hosts of schistosomes and their "resistance" develops subsequent to breeding in the WEHI mouse rooms. At the time, mouse foods were being supplemented with a vitamin mix.]

The most obvious feature of schistosomiasis that should be amenable to vaccine-induced modification is the immunopathologic response to eggs that underlies the severe pathology of chronic infection. Clear demonstrations of granuloma modulation ("endogenous desensitization") following prolonged exposure to egg antigens (eg. chronic infection) sparked a flurry of activity throughout the 70s and 80s on immunomodulation and the search for immune mediators and cells responsible for this apparent down-regulation phenomenon. During this period, an alternative explanation - that of antibody-mediated anti-embryonation immunity and accelerated destruction of immature eggs - was propounded to account for the phenomenon in *S. japonicum* (Philippines) - infected mice. This is discussed in a separate presentation at this conference. Essentially, the hypothesis is that destructive immune responses to antigens of maturing eggs destroy the eggs before the miracidium matures. These responses thus reduce production of immunopathologic antigens by the egg. Presensitization with defined antigen (i.e. use of a molecular vaccine) should inhibit the severe patho-

TABLE I

Schistosomiasis vaccines: infection, parasite and epidemiological characteristics that provide

Opportunities	Difficulties and Uncertainties
Antigenically different life cycle stages ∴ multiple sites for immune attack with multiple immune effector mechanisms to be exploited.	Complex organism - moving target for immune responses; antigenic repertoire and polymorphisms ill-defined; multiple immune evasion mechanisms; potential for repair of immune-mediated damage unknown.
Non-proliferating parasite in vertebrate hosts ∴ population antigenic changes in the host unlikely.	
Disease severity related to number of worm pairs ∴ partially effective vaccine still useful in endemic areas.	Immunopathology - chronic schisto is a classical immunopathologic disease; cutaneous hypersensitivity in vaccinees possible ("cercarial dermatitis"); many protective antigens related to self molecules - autoimmune possibilities.
Disease abatement in chronic infection is immunologically based ∴ vaccination against disease is feasible.	
Good epidemiological evidence for age-related resistance to infection as well as disease.	Uncertainties about relevance of the immunology of infection in animal models - what is basis of resistance in humans; relevant model critical for defining a product to be developed (c.f. testing a product concept in early clinical trials). Resistance in some instances known not to be immunologically based - anatomical peculiarities, hormones, etc.
Clear demonstrations of immune-facilitated drug action ∴ vaccine component to increase drug potency or useful life time a possibility.	
Identified as a priority disease for vaccine development by WHO-TDR and other AID agencies.	Reinfection common after drug cure - what will be the duration of memory in vaccinees constantly exposed or not?

logic consequences of infection. No molecules (or immune mechanisms) have yet been identified that would form the basis of such an anti-disease vaccine (see Mitchell et al., this Symposium).

Another means to reduce the overall intensity of disease processes in schistosomiasis would be to induce anti-fecundity responses resulting in reduced egg production by the female worms. Fewer eggs in tissues should lead to reduced pathology and reduced clinical manifestations (see Capron, this Symposium).

Clearly, the complexity of the schistosome life cycle in mammalian hosts provides difficulties as well as additional opportunities for the vaccine developer (Table I). Whilst similarities in the life cycle are obvious between the various schistosomes, differences can be pronounced particularly in infection of mice with *S. japonicum* (Philippines) (see Mitchell et al., this Symposium).

The second topic area embraces issues far more relevant to parasite vaccine development than parasitological issues.

An opportunity for new product development will be evaluated by a potential manufacturer and commercializing organization according to technical feasibility (ie risk) and a combination of commercial attractiveness of the eventual product and strategic fit of the project to corporate objectives (ie value). This risk-value analysis can be quantitated and plotted and will take into account the difference between an idea with limited supporting data, a product concept, and a clearly-defined prototype product, the latter obviously being the most attractive. The intellectual property position (ie. patents and licences but also proprietary information, secret know-how, etc) and market size influence commercial attractiveness with strategic fit embracing opportunities for co-development and joint ventures, introduction of new technologies into the in-house R&D department, and establishment of strategic alliances and long-term linkages with particular research groups. The reality is that any attractions of human parasite vaccines are virtually confined to opportunities for alliances with high-tech research groups or, equally importantly, those able to undertake clinical studies. Another reality - the extent and quality of the documentation required for biologicals taken through the regulatory and registration process - is quite extraordinary and difficult for an academic, laboratory-based scientist to comprehend. The under-resourced or faint-hearted will not embark on this process! In Table II are listed some deterrents to a vaccine developer, manufacturer and marketer taking up a schisto vaccine opportunity.

Studies in Australia by Spithill and colleagues (Sexton et al. 1991), and Hillyer and Tendler et al. (this Symposium) offer what might be an attractive approach to schistosomiasis vaccines through a veterinary pathway. A trematode molecule (eg GST or FABP) that induces a high level of protective immunity against both *Fasciola hepatica* and *Schistosoma* spp. could be developed as a (profitable) fascioliasis vaccine in the first instance for use in sheep and cattle. There should be little difficulty in attracting the necessary development dollars for this product. In a sense, the schisto vaccine could emerge as a "spin off" with comprehensive investigations on the use of the vaccine in a variety of hosts being important information for further development of the product for use in humans as a schisto vaccine.

The issue of autoimmunity and immunopathology is a major one in schisto vaccine development. A disease that is known to be associated with immunopathology -"schisto is a classical immunopathologic disease" - and parasite vaccine candidates that are related to host molecules (closely in some cases) are definite negatives. Despite painstaking efforts to exclude self-like epitopes and to

TABLE II

Deterrents to schistosomiasis vaccine developer

1.	Immunopathology/autoimmunity/hypersensitivities
2.	Expectations on efficacy - especially travellers (+ endemic populations in some instances) (Will an anti-disease or partially-effective vaccine actually be acceptable?)
3.	Profitability and value of the product (technical feasibility [risk] versus commercial attractiveness + strategic fit [value])
4.	The costs and logistics of clinical trials for a prophylactic in endemic countries (attractiveness of therapeutic vaccines in clinical trials)
5.	Regulatory requirements and ethical considerations (Is the vaccine likely to be better and/or cheaper than "the best current therapy?")
6.	Vaccination risks in previously exposed individuals in endemic countries
7.	The adjuvant dilemma

"Most attractive to the vaccine developer are new, patent-protected products for the treatment of illnesses that affect large numbers of affluent people and for which current therapies are inadequate". N.B. Global markets for several veterinary parasite vaccines are also very attractive.

exclude immunopathologic responses induced to eggs through laboratory-based studies, the relevance of autoimmunity and immunopathology is likely to only become evident in late-stage, comprehensive and thus expensive phase III and IV trials. The costs and effort to even get to that stage and having the program clouded by the ogre of immunopathology and autoimmunity (regardless of how difficult it is to induce untoward autoimmune reactions in short-lived laboratory animals) will be enough to dissuade even an organization with an impeccable record in global health endeavours and an altruistic component to its corporate objectives.

The options available in parasite vaccine development are decreasing rather than increasing even when viewed through the eyes of an optimist and long-term advocate of the human parasite vaccine objective. There are definite requirements for full-blooded involvement by tropical countries themselves which will have to take vaccine candidates through the D phase of R&D and into manufacturing and clinical trials. The role of local manufacturers (eg. FIOCRUZ in Brazil) becomes critical with a facilitating role played by WHO and AID agencies of the industrially-developed countries. Most important will be a transfer of knowledge on process development as well as clinical and regulatory affairs from the Western biopharmaceutical industry to counterparts in the developing world with due recognition of infrastructural deficiencies, cultural differences and technological limitations. The need is for "assisted initiative" on the part of endemic countries if the quest for new control measures for parasitic diseases including vaccines (and the quest for improved global health, that must be relentless) are to be successful.

REFERENCES

Mitchell GF, Tiu WU, Garcia EG 1991. Infection characteristics of *Schistosoma japonicum* in mice and relevance to the assessment of schistosome vaccines. *Adv Parasitol 30*: 167-200.

Mitchell GF, Wright MD, Wood SM, Tiu WU 1990. Further studies on variable resistance of 129/J and C57BL/6 mice to infection with *Schistosoma japonicum* and *Schistosoma mansoni*. *Parasite Immunol 12*: 559-567.

Moloney NA, Garcia EG, Webbe G 1985. The strain specificity of vaccination with ultra violet attenuated cercariae of the Chinese strain of *Schistosoma japonicum*. *Trans R Soc Trop Hyg 79*: 245-247.

Sexton JL, Milner AR, Panaccio M, Waddington J, Wijffels G, Chandler D, Thompson C, Wilson L, Spithill TW, Mitchell GF, Campbell NJ 1991. Glutathione S-transferases: novel vaccine against *Fasciola hepatica* infection in sheep. *J Immunol 145*: 3905-3910.

Smith DB, Davern KM, Board PG, Tiu WU, Garcia EG, Mitchell GF 1986. Mr26,000 antigen of *Schistosoma japonicum* recognised by resistant WEHI 129/J mice is a parasite glutathione S-transferase. *Proc Natl Acad Sci USA 83*: 8703-8707.