Rethinking Schistosomiasis Vaccine Development: Synthetic Vesicles

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Abstract

There is currently no vaccine against schistosomiasis. With few *Schistosoma* vaccine candidates in clinical trials, unexplored antigens from the vulnerable schistosomulum should be considered as possible vaccine candidates. In addition, we suggest developing synthetic vesicles as a new delivery vehicle and adjuvant for immunoprophylactic schistosomula vaccine candidates.

Keywords: *Schistosoma,* schistosomula, synthetic vesicles, vaccine candidates, delivery, adjuvant

It is Time to Think Elimination

Schistosomiasis is one of the most prevalent parasitic diseases worldwide. Treatment of schistosomiasis in populations at risk with a single dose of praziquantel annually has not prevented transmission of *Schistosoma* and subsequently reinfection is common in endemic areas. The World Health Organisation (WHO) reported that 219 million people worldwide needed preventative treatment against schistosomiasis in 2015. Of those who required treatment, less than one third received it through mass drug administration (MDA) programmes (1). Even more disconcerting is that modelling studies suggest that MDA will only reduce the prevalence of schistosomiasis if more than 70% of communities participate and the MDA is conducted annually (2). A drug-based strategy alone therefore may not move national schistosomiasis programs of low to middle income countries from morbidity control towards elimination (3). Other interventions, working alongside MDA, such as vaccination, could effectively prevent reinfection, and thus eliminate schistosomiasis. Vaccination with radiation-attenuated cercariae protects murine and non-human primate models against challenges with schistosomes. However, using radiation-attenuated cercariae in human trials is impractical because it is difficult to produce under good manufacturing practice (GMP), and delivery of the vaccine under liquid nitrogen presents considerable logistical challenges. As a consequence, recombinant antigens that can be easily produced are being considered as potential subunit vaccine candidates (Reviewed in (4)). Some of these vaccine candidates are efficacious against challenge infection in animal models, but show low immunogenicity as purified single antigens when tested further in human preclinical tests. Therefore, we suggest two approaches to improve the immunogenicity of *Schistosoma* vaccine antigens. Firstly, multiple, and not single, antigens should be used (both EV and non-EV encoded) in the development of schistosomiasis vaccine. Secondly, we consider synthetic vesicles as a proof of concept antigen delivery and adjuvant system. *Schistosoma*-shed vesicles have been recently identified (5, 6), but whether we can design synthetic stimulatory versions of these vesicles to deliver *Schistosoma* vaccine targets is a question yet to be addressed. This forum article examines the potential of using synthetic vesicles as adjuvant and delivery vehicle containing multiple schistosomula vaccine candidates.

Targeting Schistosomula Antigens as Vaccine Candidates

The schistosomulum is the transition phase between a free-living non-feeding cercaria in fresh water and the parasitic blood fluke in the mammalian host. When cercariae penetrate human skin, they transform into the skin-stage schistosomula (Fig. 1). The skin-stage schistosomula up regulate specific genes during transformation to facilitate invasion and to survive the hostile host immune response (7). The schistosomula also develop a new double lipid bilayer outer membrane covering the tegument that facilitates survival within the host. Just as the new coat develops, the early post-penetration schistosomulum is vulnerable to host immune-mediated attack (8). The late phase schistosomulum is less susceptible to both eosinophil and macrophage mediated cytotoxicity when it develops towards adulthood. Early schistosomulum antigens are therefore possible candidates to develop a prophylactic vaccine against human schistosome infections. However, there are few current efforts to identify and prioritise schistosomula antigens for a novel vaccine. One such initiative was TheSchistoVac consortium that targeted antigens highly expressed by the skin schistosomula for vaccine development (http://www.theschistovac.eu/). The work done provides a template for future targeted (stage-specific) vaccine development.

Schistosomes are complex multicellular organisms, and this may partly explain why current vaccines composed of a single antigen are not capable of inducing long-lived protective immunity. We propose multiple antigen preparations to target different aspects of the early stage schistosomula ranging from tegument formation and turnover to metabolite (glucose) uptake. In fact, the multivalent chimeric schistosomiasis vaccine of SmTSP-2 and Sm29 induces more robust immune responses compared to single antigen preparations in mice (9). Although identifying new antigens based on the schistosomulum is a critical step, combining new and existing antigens as a multiple vaccine preparation is, we believe, a necessary step in designing the next generation of vaccine to a complex, multicellular organism. We would suggest both non-EV (to target the schistosomula) and EV encoded (to target secreted EVs) antigens. Finally, the multiple antigen vaccine will require new tools such as synthetic vesicles to be delivered to immune cells.

Synthetic Vesicles to Deliver *Schistosoma* Vaccine Candidates

Schistosomes release excreted/secreted (E/S) products to survive the hostile host immune system. Among these products characterised to date are *Schistosoma*-shed vesicles known as extracellular vesicles (EVs) (5, 6), spherical structures encapsulated by a lipid bilayer and shown to be responsible for intercellular communication (10). The major subsets of EVs are exosomes, microvesicles and apoptotic bodies. EVs are classified based on their biogenesis, their size, and what surface markers they express. Of importance is that *Schistosoma* EVs (derived from both schistosomula and adult worms) contain potential vaccine candidates including SmTSP-2 and Sm29 (5, 6).

We suggest packaging schistosome vaccine antigens in synthetic vesicles because naturally occurring *S. mansoni* EVs may contain inhibitory biological material such as miRNAs and tsRNAs (6). Packaging parasite antigens into vesicles will improve their antigenicity compared to using the antigens directly for vaccination (11). Another advantage of utilizing synthetic vesicles is that they are free of host proteins that have been described in EVs of other parasites such as *Echinostoma caproni* and *Fasciola hepatica* (12). The proof-of-concept for manufacturing synthetic vesicles already exists with other lipid molecules such as virus like particles (VLPs) and outer membrane vesicles (OMVs). The pros and cons of antigen delivery using synthetic vesicles are summarised in Table 1. We suggest it is now time to take this technology forward and target the schistosome.

Vaccine candidates within vesicles are also effectively protected from degradation as they move through body fluids, improving their stability within host. For synthetic vesicles to work as adjuvants, additional ligands that target receptors on antigen presenting cells such as pathogen recognition receptors on dendritic cells could be added on the vesicle surface using glycosylphosphatidylinositol (GPI) anchors for robust cellular responses. With appropriate thought given to the incorporation of membrane-embedded glycoprotein ligands or receptors, targeting specific immunological cells could be engineered and achieved. In addition to targeting the actual schistosomula, immune responses induced by synthetic vesicles (to EV encoded antigens) will also target and neutralise *Schistosoma* EVs, decreasing the ability of the schistosomula to dampen immune responses and make the environment less suitable for survival. All in all, immune responses to multiple antigen preparations from the early phase of the schistosomulum packaged in synthetic vesicles may prevent development of the adult schistosomes and subsequently the laying of eggs that cause pathology associated with schistosomiasis.

Conclusion

Although schistosomiasis is treatable, reinfections are common in endemic areas. It is widely acknowledged that a vaccine used alongside chemotherapy would control and possibly eliminate schistosomiasis. We have suggested using synthetic vesicles that are preloaded with multiple schistosomula antigens to elicit protective, skin-stage host responses as a next-generation anti-schistosomal vaccine. As we move towards 2025, the year WHO set to eliminate schistosomiasis globally, these and other novel approaches are required to develop vaccines.

Conflict of Interests

The authors declare that there is no conflict of interest.

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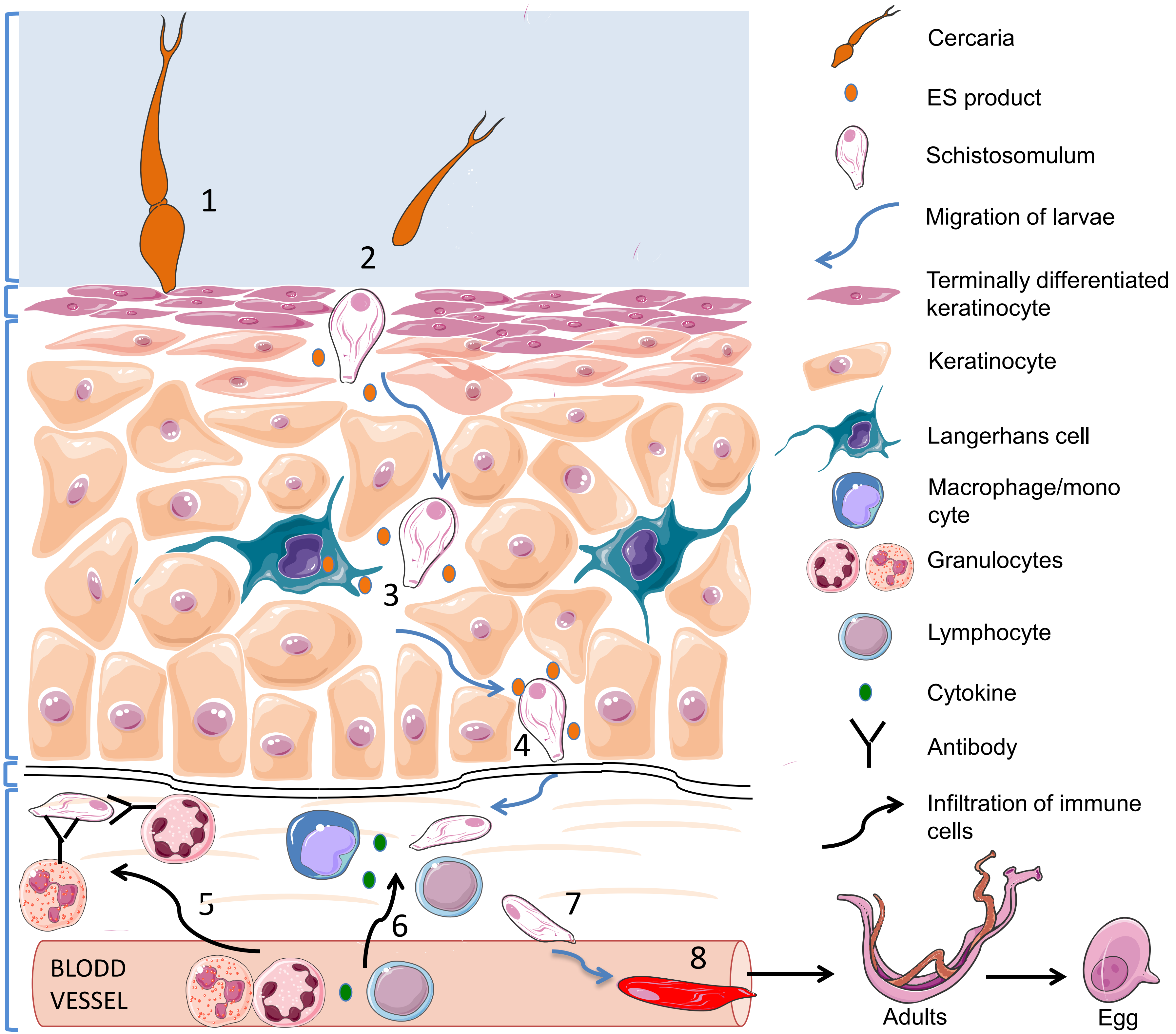
Figure 1. Migration of schistosomula through the host skin. 1. Cercaria is attracted to human skin. 2 Cercaria burrows through the skin and detaches the bifurcated tail to form a schistosomulum. 3 Schistosomulum release excretion/secretion (ES) products including extracellular vesicles (EVs) that interact with resident Langerhans cells, which migrate to skin draining lymph nodes to initiate adaptive immune responses. 4 The schistosomulum moves towards the basement membrane that temporarily halts their migration. 5 Once in the dermis, the schistosomulum is vulnerable to antibody-mediated killing by granulocytes. 6 Schistosome-induced cytokines activate more phagocytes and polarize the immune response towards inflammatory responses. 7 Schistosomulum penetrates dermal veins and

migrates to the lungs. 8 Schistosomulum is coated with host proteins as an immune evasion mechanism. The figures were adapted and modified from Servier Medical Art (http://smart.servier.com/).

Table 1. Pros and cons of antigen delivery via synthetic vesicles

Advantages	EV and non-EV antigens target both schistosomula and EVs increasing immunogenicity
	Antigen presenting cells can be targeted by displaying specific ligands on outer surface of synthetic vesicles using GPI anchors
	Adding molecules that activate antigen presenting cells means that synthetic vesicles are not just antigen delivery vehicles, but also an adjuvant as well
	Synthetic vesicles exhibit natural EV properties such as stability and resistance to enzymatic degradation in body fluids
	Naturally occurring EV cargo, such as miRNA, with inhibitory properties are avoided in synthetic vesicles
	Synthetic vesicles lack host proteins, a potential mechanism for decreasing immunogenicity
Disadvantages	Expensive to manufacture
	Risk of reactogenicity associated with synthetic materials may lead to adverse effects in humans
	Extensive regulatory requirements are expected for human use license

Figure 1


WATER
STRATUM CORNEUM
HOST EPIDERMIS
BASEMENT MEMBRANE
HOST DERMIS
BLODD VESSEL
1
2
3
4
5
6
7
8
Cercaria
ES product
Schistosomulum
Migration of larvae
Terminally differentiated keratinocyte
Keratinocyte
Langerhans cell
Macrophage/mono cyte
Granulocytes
Lymphocyte
Cytokine
Antibody
Infiltration of immune cells
Adults
Egg