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1 Rethinking Schistosomiasis Vaccine Development: Synthetic Vesicles

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14 Abstract

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

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

20 It is Time to Think Elimination

21 Schistosomiasis is one of the most prevalent parasitic diseases worldwide. Treatment of schistosomiasis
22 in populations at risk with a single dose of praziquantel annually has not prevented transmission of
23 *Schistosoma* and subsequently reinfection is common in endemic areas. The World Health Organisation
24 (WHO) reported that 219 million people worldwide needed preventative treatment against
25 schistosomiasis in 2015. Of those who required treatment, less than one third received it through mass
26 drug administration (MDA) programmes (1). Even more disconcerting is that modelling studies suggest
27 that MDA will only reduce the prevalence of schistosomiasis if more than 70% of communities
28 participate and the MDA is conducted annually (2). A drug-based strategy alone therefore may not move
29 national schistosomiasis programs of low to middle income countries from morbidity control towards
30 elimination (3). Other interventions, working alongside MDA, such as vaccination, could effectively

31 prevent reinfection, and thus eliminate schistosomiasis. Vaccination with radiation-attenuated cercariae
32 protects murine and non-human primate models against challenges with schistosomes. However, using
33 radiation-attenuated cercariae in human trials is impractical because it is difficult to produce under good
34 manufacturing practice (GMP), and delivery of the vaccine under liquid nitrogen presents considerable
35 logistical challenges. As a consequence, recombinant antigens that can be easily produced are being
36 considered as potential subunit vaccine candidates (Reviewed in (4)). Some of these vaccine candidates
37 are efficacious against challenge infection in animal models, but show low immunogenicity as purified
38 single antigens when tested further in human preclinical tests. Therefore, we suggest two approaches to
39 improve the immunogenicity of *Schistosoma* vaccine antigens. Firstly, multiple, and not single, antigens
40 should be used (both EV and non-EV encoded) in the development of schistosomiasis vaccine. Secondly,
41 we consider synthetic vesicles as a proof of concept antigen delivery and adjuvant system. *Schistosoma*-
42 shed vesicles have been recently identified (5, 6), but whether we can design synthetic stimulatory
43 versions of these vesicles to deliver *Schistosoma* vaccine targets is a question yet to be addressed. This
44 forum article examines the potential of using synthetic vesicles as adjuvant and delivery vehicle
45 containing multiple schistosomula vaccine candidates.

46 **Targeting Schistosomula Antigens as Vaccine Candidates**

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

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

72 **Synthetic Vesicles to Deliver *Schistosoma* Vaccine Candidates**

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

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

89 Vaccine candidates within vesicles are also effectively protected from degradation as they move through
90 body fluids, improving their stability within host. For synthetic vesicles to work as adjuvants, additional
91 ligands that target receptors on antigen presenting cells such as pathogen recognition receptors on

92 dendritic cells could be added on the vesicle surface using glycosylphosphatidylinositol (GPI) anchors for
93 robust cellular responses. With appropriate thought given to the incorporation of membrane-embedded
94 glycoprotein ligands or receptors, targeting specific immunological cells could be engineered and
95 achieved. In addition to targeting the actual schistosomula, immune responses induced by synthetic
96 vesicles (to EV encoded antigens) will also target and neutralise *Schistosoma* EVs, decreasing the ability
97 of the schistosomula to dampen immune responses and make the environment less suitable for survival.
98 All in all, immune responses to multiple antigen preparations from the early phase of the
99 schistosomulum packaged in synthetic vesicles may prevent development of the adult schistosomes and
100 subsequently the laying of eggs that cause pathology associated with schistosomiasis.

101 **Conclusion**

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

108 **Conflict of Interests**

109 The authors declare that there is no conflict of interest.

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151

152

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

161 migrates to the lungs. 8 Schistosomulum is coated with host proteins as an immune evasion mechanism.

162 The figures were adapted and modified from Servier Medical Art (<http://smart.servier.com/>).

163

164

165

166 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

167

168

Figure 1

