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Immunoproteomic analysis of *Schistosoma japonicum* schistosomulum proteins recognized by immunoglobulin G in the sera of susceptible and non-susceptible hosts

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ABSTRACT

The aim of this study was to search for immunogenic schistosomula proteins in the hope of identifying novel intervention targets. Schistosomula proteins were analyzed by immunoproteomic which the probes were sera derived from BALB/c mice (susceptible hosts) and *Microtus fortis* (resistant hosts). A total of 116 immunoreactive proteins recognized by 10 days post-infected BALB/c mice, *M. fortis* sera, and uninfected *M. fortis* sera were selected for further analysis. Finally, 95 protein spots were identified by mass spectrometry (MS) analysis. Bioinformatics analysis showed that the differentially identified immunogenic proteins participated mainly in cytoskeleton organization, cell motility, energy metabolism, responses to stimuli, and protein folding. Many of these proteins were the tegument or excretory–secretory products of schistosomes reported in previous studies. Among of them, *Schistosoma japonicum* DnaJ (Hsp40) homologue (SjDnaJ) was successfully expressed and the purified recombinant product was evaluated by immunoprotective experiment. After immunization of BALB/c mice with recombinant SjDnaJ, it could induce 34.5% and 48.9% reductions in the numbers of worms and eggs in the liver. These results contribute to a better understanding of the molecular mechanisms underlying the host–parasite relationship and provide a major dataset to

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facilitate the further development of new vaccine candidates and/or diagnostic markers for schistosomiasis.

Biological significance
Schistosomiasis is caused by parasitic blood-dwelling flukes in tropical and subtropical areas, and it is one of the world's most prevalent tropical diseases. The lack of effective vaccine and reliable diagnostic methods make this disease difficult to control. In China, *S. japonicum* can infect more than 40 different susceptible mammals for this parasite. However, *M. fortis* is the only known mammal where the schistosome cannot develop and it exhibits no significant pathological effects. Many studies' results showed that native antibodies against *S. japonicum* are present in *M. fortis* that may have important anti-schistosomiasis roles during the infection process. The aim of this study was to search for immunogenic schistosomula proteins in the hope of identifying novel intervention targets. We present a comparative immunoproteomics analysis of the proteins recognized by susceptible and resistant host antibodies before and 10-days after infections. The results of this analysis will be helpful for identifying the key molecules required for the survival and development of schistosomes. At the same time, the study contributes to a better understanding of the molecular mechanisms underlying the host–parasite relationship associated with schistosomes and they also provide a major dataset to facilitate the further development of new diagnostic assays and/or vaccine candidates for schistosomiasis.

1. Introduction

Schistosomiasis is caused by parasitic blood-dwelling flukes in tropical and subtropical areas, and it is one of the world's most prevalent tropical diseases. It is estimated that about 200 million individuals are infected in 74 developing countries and territories [1,2]. Millions of people suffer from severe morbidity as a consequence of schistosomiasis and hundreds of thousands of deaths occur each year due to schistosome infections via portal hypertension and kidney failure [3]. In China, *Schistosoma japonicum* is epidemic along the Yangzi River and in lake regions (e.g., Dongting Lake and Poyang Lake regions) where 330,000 people are infected out of an at-risk population of 40 million [4]. Currently, the only strategy for schistosomiasis control aims to reduce morbidity using the drug praziquantel. However, problems are increasingly emerging because chemotherapy does not prevent re-infection and drug-resistant isolates have been reported [5], while some individuals have a high worm burden and irreversible pathology cannot be prevented by chemotherapy in the late stage. Thus, a vaccine or a new drug is required to combat schistosomiasis, although a vaccine used alone or in combination with anthelmintic drugs may be a strategic tool for the long-term sustainable control of this disease [6]. Thus, the screening and identification of vaccine candidates or new drug targets are urgent and important.

In China, *S. japonicum* can infect more than 40 different mammals, which are susceptible hosts for this parasite. Almost 60–70% of cercariae can develop into adult worms in these hosts based on the specific antibodies produced by the host immune system. However, the reed vole or *Microtus fortis*, is the only known mammal where the schistosome cannot develop and it exhibits no significant pathological effects [7–10]. The growth and survival of the worms are extremely poor in *M. fortis* and most of the worms die within 15 days of challenge with cercariae [9]. Different hosts provide a distinct environment for the schistosomes, which affects the survival and development of the parasite [11]. It is possible that *M. fortis* has a stronger immune response to the schistosomes during the early phases of infection, with more severe pathological lesions. Previous studies have also shown that humoral and/or cellular immunity play important roles in the restricted development of *S. japonicum* in *M. fortis* [12]. Normal sera were transferred from *M. fortis* into mice and the growth and development of schistosomes in these mice were affected [13]. When schistosomula were co-cultured with normal sera from *M. fortis* in vitro the schistosomula mortality was significantly higher than that when co-cultured with normal sera from mice [14]. These results showed that native antibodies existed in *M. fortis* sera might be one of the important factors in anti-schistosomiasis roles during the infection process. Immunoglobulin G (IgG) is the most abundant antibody isotype found in the blood, which may be correlated with protection against schistosome infections. Thus, we present a comparative immunoproteomics analysis of the proteins recognized by susceptible and resistant host antibodies before and 10-days after infections. The results of this analysis will be helpful for identifying the key molecules required for the survival and development of schistosomes.

During the past few years, immunoproteomics has become an increasingly popular method that has been used widely for identifying various types of immunoreactive proteins in bacteria and parasites [15–21]. Adult *Schistosoma haematobium* soluble worm antigens were recognized by pooled serum samples from infected Zimbabweans using immunoproteomics [15]. Pretreatment serum samples recognized 59 spots that represented 21 different proteins whereas praziquantel posttreatment serum samples recognized an additional 12 spots that represented eight different proteins. However, only five proteins (calreticulin, tropomyosin 1, tropomyosin 2, paramyosin, and triose phosphate isomerase) were recognized exclusively by posttreatment serum samples. The sera from infected rabbits have been used to identify antigenic proteins from adult *S. japonicum* worms [16]. Four immunoreactive antigens have been identified successfully