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Rational selection of immunodominant and preserved epitope Sm043300e from *Schistosoma mansoni* and design of a chimeric molecule for biotechnological purposes

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ABSTRACT

Human schistosomiasis is a neglected tropical disease of great importance in public health. A large number of people are infected with schistosomiasis, making vaccine development and effective diagnosis important control strategies. A rational epitope prediction workflow using *Schistosoma mansoni* hypothetical proteins was previously presented by our group, and an improvement to that approach is presented here. Briefly, immunodominant epitopes from parasite membrane proteins were predicted by reverse vaccinology strategy with additional *in silico* analysis. Furthermore, epitope recognition was evaluated using sera of individuals infected with *S. mansoni*. The epitope that stood out in both *in silico* and *in vitro* assays was used to compose a rational chimeric molecule to improve immune response activation. Out of 2185 transmembrane proteins, four epitopes with high binding affinities for human and mouse MHCII molecules were selected through computational screening. These epitopes were synthesized to evaluate their ability to induce TCD4+ lymphocyte proliferation in mice. Sm204830e and Sm043300e induced significant TCD4+ proliferation. Both epitopes were submitted to enzyme-linked immunosorbent assay to evaluate their recognition by IgG antibodies from the sera of infected individuals, and epitope Sm043300 was significantly recognized in most sera samples. Epitope Sm043300 also showed good affinity for human MHCII molecules in molecular docking, and its sequence is curiously highly conserved in four *S. mansoni* proteins, all of which are described as G-protein-coupled receptors. In addition, we have demonstrated the feasibility of incorporating this epitope, which showed low similarity to human sequences, into a chimeric molecule. The stability of the molecule was evaluated by molecular modeling aimed at future molecule production for use in diagnosis and vaccination trials.

1. Introduction

The development of an effective vaccine for schistosomiasis has mobilized researchers worldwide. Three antigens are currently under clinical trials: glutathione S-transferase (Sh28GST), tetraspanin surface antigen (Sm-TSP-2) and fatty acid binding protein (Sm14) (Fonseca et al., 2015; Mo and Colley, 2016). However, no vaccine for this disease is available for human use, and a search for new candidates is still needed.

Sh28GST has demonstrated its innocuity and excellent immunogenicity in patas monkeys. Sh28GST with Freund's complete adjuvant elicited specific IgG and IgA responses in all immunized animals, which strongly supports satisfactory antibody coverage in future vaccinated human populations. Glutathione *S*-transferase is preserved in *S. mansoni*, *S. haematobium*, *S. japonicum*, and *S. bovis* and this conservation can be explored in the context of vaccine development, which can simultaneously eliminate different *Schistosoma* species (Boulanger et al., 1999, 1995; Fonseca et al., 2015).

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Another important antigen is the transmembrane protein Sm-TSP-2, which is a potential vaccine candidate that elicits 57–64% protection in mice (Tran et al., 2006). TSP-2 was strongly recognized by IgG1 and IgG3 from naturally resistant individuals, but it was not recognized by IgG from chronically infected or unexposed individuals. Its protection was associated with increased titters for IgG1 and IgG2a antibodies (Fonseca et al., 2015; Tran et al., 2006). Finally, Sm14 has shown promising results in several trials involving animals. The ability of recombinant Sm14 to protect against schistosomiasis was first demonstrated by Tendler and coworkers. These authors demonstrated that Sm14 induces protection levels ranging from 50 to 68% in mice when formulated with Freund's adjuvant (Tendler et al., 1996). This protein has also become a potential target for a fascioliasis vaccine due to the structural similarity of Sm14 and the *Fasciola hepatica* antigen (Mossallam et al., 2015). Interestingly, Tendler has also shown that Sm14 epitopes can induce levels of protection similar to the ones induced by the whole protein (Tendler et al., 2015).

Research advancements towards a successful schistosomiasis vaccine have been a priority for many years. This search is justified by the lack of an effective vaccine that achieve full protection in associated with the wide range of tools and possibilities that have been developed in the field of vaccinology (Bonanni and Santos, n.d.; Leo et al., n.d.; Nascimento and Leite, 2012).

New knowledge in the fields of immunology and molecular biology has also greatly influenced vaccine research, moving it past the production of several types of vaccines involving live or inactivated whole cells, subunits, or recombinant proteins and instead focusing on small portions of antigens, such as peptides (Li et al., 2014; Skwarczynski and Toth, 2016). Synthetic peptide vaccines are intrinsically safer than traditional whole-organism vaccine formulations and are an attractive alternative for inducing immune responses because they avoid allergenic or reactogenic sequences (Skwarczynski and Toth, 2016). Currently, many efforts are focused on vaccine development based on synthetic peptides that encompass B and T cell epitopes, which precisely induce a protective immune response. However, peptide vaccines are not available for human use, and in parasitic diseases, only peptide vaccines against malaria are in clinical trials (Epstein et al., 2007; Li et al., 2014; Reche et al., 2015).

Peptide-based vaccines have been rationally selected through reverse vaccinology (RV), an approach that predicts immunodominant epitopes that can induce an immunoprotective response. Rappuoli proposed this term because the vaccine discovery process started *in silico* using genetic information, not the pathogen itself (Rappuoli, 2000). This strategy is still under development and is mainly composed of a rational workflow that accounts for the desired type of immune response and the binding affinities of different human leukocyte antigens (Rappuoli, 2000; Soria-Guerra et al., 2015). The growing search for protective antigens using bioinformatic tools has provided molecular and immunological clues to drive the discovery of new vaccines; however, few studies available in the literature have focused on RV for *Schistosoma mansoni* (Rappuoli et al., 2016).

In addition to the vaccinology field, peptides have also been the focus of studies in the diagnosis field, and many efforts have focused on creating a quick, easy and effective diagnostic test against several diseases (Oliveira et al., 2008). Schistosomiasis diagnosis is based on the identification of eggs in feces, but this approach has its limitations, as the elimination of eggs in feces is intermittent and decreases in parasitic load promote sensitivity loss in parasitological tests (Weerakoon et al., 2015). In addition to direct parasitological examination, there are different forms of schistosomiasis diagnosis, including intradermal tests, antigen detection tests and antibody detection tests, such as the circumoval precipitin test (COPT), indirect hemagglutination test (IHT), indirect immunofluorescence assay (IFA), and enzyme-linked immunosorbent assay (ELISA) (Noya et al., 2003; Weerakoon et al., 2015).

The advantage of using synthetic peptide-based diagnosis is that they are structurally simple and easily engineered; additionally, their use minimizes cross-reactivity (Oliveira et al., 2008). Moreover, similar to efforts in vaccinology, a rational workflow to select peptide targets for use in diagnosis has been developed with the support of bioinformatic tools (Bosze and Hudecz, 2016). Immune diagnosis of parasitic diseases with synthetic peptides has been used in the detection of malaria, leishmaniasis, trypanosomiasis, toxoplasmosis, hydatidosis, schistosomiasis, fasciolosis and cysticercosis (Noya et al., 2003; Weerakoon et al., 2015). Oliveira et al. (2008) have shown that the use of peptides in ELISA had good sensitivity and specificity in acute and chronic *S. mansoni* infections; however, its sensitivity was lower and specificity was higher than the sensitivity and specificity of ELISA using full antigens.

In view of the wide range of possibilities in the peptide field associated with the need to improve immune responses to vaccines and increase sensitivity in diagnostic assays, a next generation of rational molecules has been proposed (Nezafat et al., 2014; Pearson et al., 2012). The peptides selected by computational tools are used to compose multi-antigen molecules, which are more potent than antigenic peptides alone (Humphreys et al., 2000). These chimeric molecules can contain antigenic determinant candidates designed to induce cellular, humoral and innate immune responses (Hajighahramani et al., 2017; Ribeiro et al., 2010) against one or more parasites simultaneously. Moreover, strategies have been adopted to design multiepitope immunogens with improved epitope presentation ability by using enhancer motifs, which are spacers that induce Th lymphocyte responses by polypeptide immunization, linkers or *in tandem* epitope repetition (Humphreys et al., 2000; Livingston et al., 2002; Nezafat et al., 2014). The design of chimeric molecules containing these spacers or junctional epitopes must be carefully studied because the chimeric molecules can create undesired immunodominance effects, redirecting the immune response to irrelevant epitopes or suppressing the induction of responses to the desired epitopes (Livingston et al., 2002). Because chimeric molecules do not exist in nature, peptide positions and the stability of the 3-D structure can be evaluated by molecular modeling (Hajighahramani et al., 2017).

Here, through a workflow of immunodominant epitope prediction, we identified and synthesized promiscuous epitopes from *S. mansoni* and evaluated their ability to induce TCD4+ lymphocyte proliferation in mice. The epitopes were used in serological diagnosis to evaluate their sensitivity and specificity and validate the strategy of rational epitope selection. Finally, we used the epitope that stood out in all analyses to compose a chimeric molecule for biotechnological purposes.

2. Methods

2.1. Selection of S. mansoni transmembrane proteins

Sequences of transmembrane proteins were obtained in GeneDB by searching for *S. mansoni* organism and transmembrane helices (Logan-Klumpler et al., 2012). As in our previous work, the sequences of the proteins were analyzed to confirm their cellular locations (Lopes et al., 2017). Here, only proteins predicted as localized in the plasma membrane were selected.

Extracellular regions of these proteins that contained 35 amino acids or more and coincided in three programs (NetMHCII, SYFPEITHI and RANKPEP) were selected for the next analysis (Lopes et al., 2017).

2.2. Promiscuous epitope prediction

Epitopes with high binding affinity for each HLA molecule were selected and subsequently aligned with the extracellular region of the protein. Epitopes were differentially identified by program to specify which one made the prediction. Epitope predictions were sequentially numbered by algorithm as follows: NetMHCII program, 01–100; SYFPEITHI program, 101–200; and RANKPEP program, 201–300. Subsequent to the alignment, epitopes with 15 amino acids that