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#### TITLE

**CAN WORM FEEDING BE BLOCKED USING POLYCLONAL ANTIBODIES AGAINST ESOPHAGEAL GLAND EPITOPES FROM SCHISTOSOMA MANSONI?**

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#### ABSTRACT

**Background:** Schistosomiasis is endemic in 78 countries and has the highest morbidity among helminth infections. A vaccine against this disease would therefore be a valuable addition to the toolbox of control measures and elimination strategies. Most vaccines investigated to date have been based on single antigens, none of which have reached the final stage of development. We propose that using multiple immunogenic epitopes associated with protective responses would be a more promising strategy for developing an effective vaccine. Our group previously mapped epitopes associated with protection in the murine model immunized with attenuated cercariae. Based on this study, 86 epitopes, each 10 - 22 aa long, were selected for their protective potential and localization in host-parasite interface tissues (esophageal gland, tegument, and gastrodermis). **Objective:** The primary goal of this study is to assess whether high titers of polyclonal antibodies against a recombinant protein containing 20 previously mapped epitopes from the esophageal gland are effective in blocking the development of schistosomula in vitro. **Methods:** A synthetic gene was designed containing 20 epitopes from Esophageal Gland proteins (19 MEGs and VAL7). The recombinant protein (rESO) was expressed in *E. coli* (BL21 strain) and purified using nickel affinity chromatography. For polyclonal antibody production, BALB/c mice were immunized six times with 10 ug of rESO formulated in Alum adjuvant. Sera were collected to evaluate the IgG1 antibody titer by ELISA. For hybridoma production, splenocytes from immunized mice were collected and fused with myeloma cells (P3X63Ag8.653) at a ratio of 5 x 10<sup>7</sup> myeloma cells per spleen. Following the hybridoma fusion, the polyclonal antibodies were collected, and a dot blot assay was performed to verify reactivity against individual epitopes. Additionally, immunohistochemistry was conducted to assess reactivity against the native proteins in whole-mount worms. **Results:** The rESO protein was purified with a yield of 67 mg/L of culture. The IgG1 titer in the serum of the immunized animals reached a high titer (1/13M). The hybridoma fusion yielded 39 clones, of which 36 were reactive against rESO. Dot blot analysis of antibodies against individual epitopes showed that the hybridoma culture supernatant recognizes 70% of the epitopes present in the protein. The most reactive epitopes were those from the MEG-8.2 and MEG-4.2 proteins. The polyclonal antibodies produced were able to recognize the native proteins of the worm, primarily in the esophageal plates of the anterior esophageal gland. **Conclusion and Perspectives:** It was possible to obtain 36 reactive clones against rESO that recognizes 70% of the epitopes. The polyclonal antibodies could recognize the native proteins of the worm. The next step is to functionally evaluate the capacity of these antibodies to impair or block the development of 3-day-old to 21-day-old-schistosomula in vitro cultures.

#### KEYWORDS

Epitopes; Polyclonal Antibodies; Vaccine; Schistosoma

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