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LOCALIZATION OF *SCHISTOSOMA MANSONI* PROTECTIVE ANTIGENS BY INDIRECT IMMUNOFLUORESCENCE

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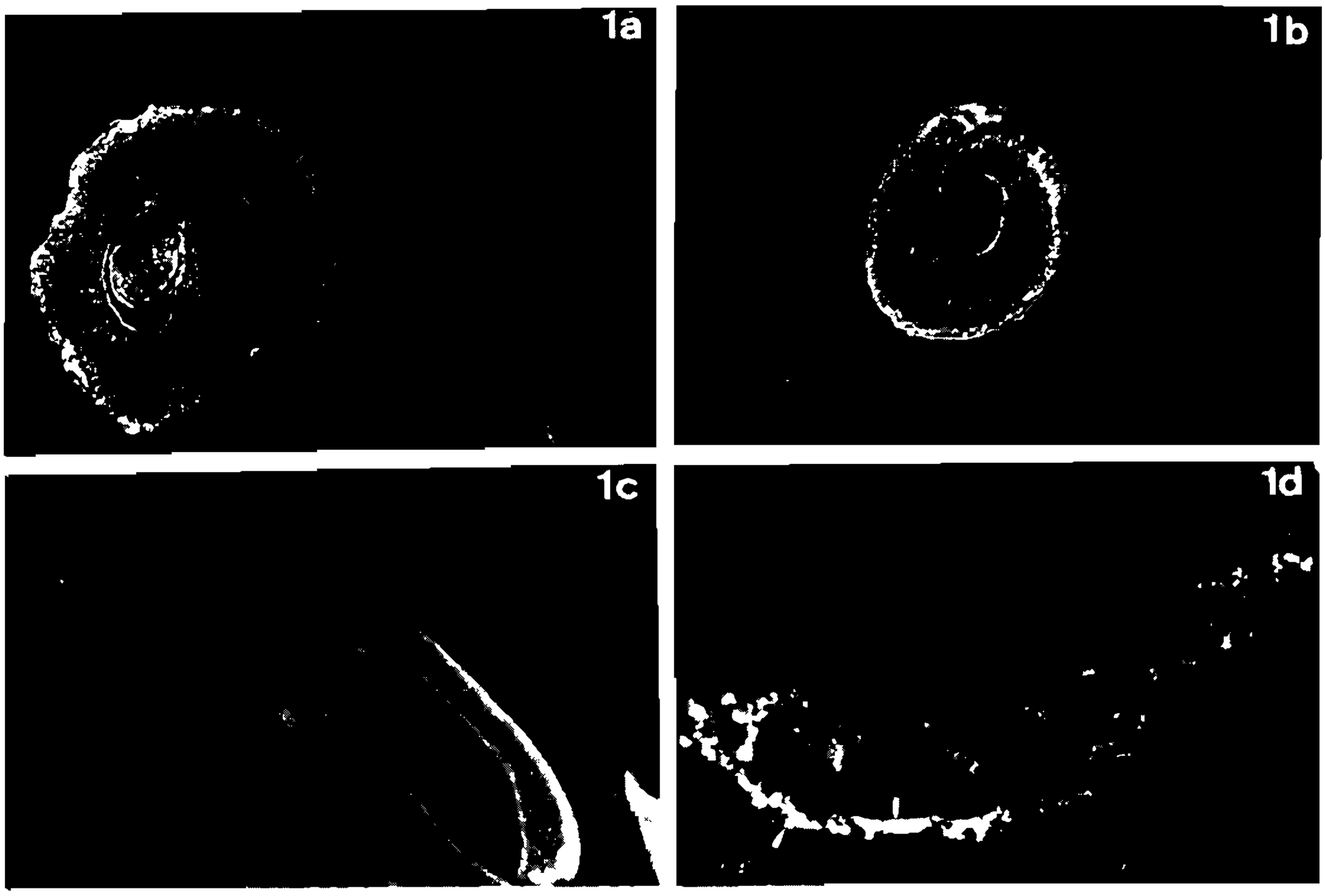
Schistosoma mansoni antigens, released from live adult male and female worms in phosphate buffered saline (called SE, after Saline Extract) were tested in previous studies for protective immunity against cercarial infection. Following the vaccination protocol of subcutaneous/ intradermal administration of SE in Freund's Complete Adjuvant (FCA), we reported on significant rates of worm burden reduction obtained in two animal model systems (SW mice and New Zealand rabbits) (Tendler et al., 1982; Tendler et al., 1986).

The further characterization of SE molecules could have important implications to the study of antigens for protective immunity.

In order to localize these antigens in adult parasites, an indirect immunofluorescence assay was used basically as described by Wilson et al. (1974) with variations as follows: Cryostat sections, 4 μ thick, of *S. mansoni* adult male and female worms obtained by perfusion from portal and mesenteric venous system (Pellegrino & Siqueira, 1956) were incubated with rabbit



Localization of *Schistosoma mansoni* protective antigens by indirect immunofluorescence. Figs. 1a and 1b: adult worm sections treated with anti-SE rabbit sera. A specific fluorescence in the surface and in between the tegumental and muscle layers (100x). Fig. 1c): negative control (160x) pre-immune serum. Fig. 1d: female worm section treated with PBS (negative control). Arrows indicate nonspecific autofluorescence observed in the vitelline glands (160x).

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anti-SE sera, at various dilutions for 30 min. Following several washings in PBS, FITC-conjugated swine anti-rabbit antisera (Dako Corporation) were added for additional 30 min. The sections were then washed and examined under a fluorescence microscope (Zeiss). Normal rabbit sera and PBS treated sections, served as negative controls. Anti-SE sera were obtained from New Zealand rabbits immunized with SE in FCA as previously described (Tendler et al., 1982).

Male and female worm sections treated with anti-SE immune sera (derived from vaccinated rabbits) showed a strong fluorescence in the entire surface and tegumental layers as well as throughout the parasite body as a continuous net between the muscle layers (Figs. 1 a, b).

Controls showed no specific fluorescence (Fig. 1 c) and a yellowish autofluorescence of vitelline glands was seen in female sections (Fig. 1 d).

The described immunofluorescence pattern observed in adult schistosomes with anti-SE sera was similar to that demonstrated for paramyosin (Pearce et al., 1986; Lanar et al., 1986) and compatible with the finding of paramyosin as an SE component (Tendler et al., 1987).

Experiments aiming the localization of SE components in schistosome evolutive forms are presently in progress with monoclonal antibodies raised against the 97/95/78 kD complex (paramyosin) and rabbit anti-SE differential immune sera (from highly and poorly protected individuals).

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