Zeitschrift: Acta Tropica
Herausgeber: Schweizerisches Tropeninstitut (Basel)
Band: 44 (1987)
Heft: (12): Prospects for immunological intervention in human schistosomiasis

Artikel: Characterization and cloning of "Schistosoma mansoni" immunogens recognized by protective antibodies

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DOI: https://doi.org/10.5169/seals-313845

Acta Tropica *44*, Suppl. 12, 75–82 (1987)

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Characterization and cloning of *Schistosoma mansoni* immunogens recognized by protective antibodies

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Summary

In this report we have shown that mice vaccinated twice with radiation-attenuated cercariae elicit a much enhanced or unique response against six adult worm glycoproteins with molecular sizes of 200, 160, 140, 94, 58–56, and 43 kDa. In the case of the schistosomulum, vaccinated mice showed an enhanced or unique response to antigens of 200, 58, 46, 43, 25, and several glycoproteins in the range 65 to 50 kDa. That some or all of these antigens may be important for immunoprophylaxis against schistosomiasis is supported by the observations that 1. polyclonal antiserum (anti-IrV) prepared against these antigens also reacts with the major schistosomular surface antigens, and 2. this antiserum reacts with epitopes exposed on the surface of both newly transformed schistosomula and lung-stage schistosomula.

In this study we also observed that the majority of the surface-iodinated antigens recognized by the anti-IrV serum were also recognized by sera from both vaccinated and patently infected mice. Simpson et al. (1985) have also shown that sera from vaccinated and infected mice recognized the same schistosomular surface antigens. It is possible, however, that the immune response of vaccinated mice is directed against different carbohydrate or peptide epitopes on these molecules, and that recognition of such epitopes is important for immune protection. Towards this goal we have cloned several schistosoma proteins reactive with the anti-IrV serum to identify peptide epitopes relevant for immunoprotection.

Laboratory mice previously exposed to radiation-attenuated cercariae develop resistance to a challenge infection with *Schistosoma mansoni* (Von Lichtenberg, 1985). This resistance is believed to be immunologically specific

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(Sher et al., 1982) and can be transferred between parabiotic partners (Dean et al., 1981). More recently, Mangold and Dean (1986) have shown the first successful homologous transfer of partial protection using sera or purified immunoglobulins from mice vaccinated twice or three times with radiation-attenuated cercariae. We have compared the humoral immune response of mice protected against *S. mansoni* by vaccination with radiation-attenuated cercariae to that of patently infected mice, and have identified antigens which elicit a greater, or unique, immune response in the vaccinated mice. This comparison was carried out using radioimmunoprecipitation and immunodepletion of [^{35}S]methionine-labeled schistosomular and adult worm polypeptides or ^{125}I-labeled egg glycoproteins followed by one- and two-dimensional polyacrylamide gel analyses.

Comparison of the schistosomular and worm glycoproteins recognized by sera from patently infected mice and from mice vaccinated with radiation-attenuated cercariae

The humoral immune responses of patently infected mice and mice vaccinated once were remarkably similar and were directed against schistosome glycoproteins ranging in molecular size >300 to <10 kDa. Exposing mice to a second vaccination resulted in a marked change in the immune response, to one predominantly directed toward high molecular size glycoproteins. This result was observed with both schistosomular and adult worm glycoproteins.

Using the same antibodies previously shown by Mangold and Dean (1986) to protect mice against challenge infection, we demonstrated that a glycoprotein of 38 kDa present in 5-day in vitro-cultured schistosomula and an adult worm glycoprotein of 94 kDa were precipitated by sera from these vaccinated mice but not by sera from patently infected mice (Dalton et al., 1986). Although radiation-attenuated larvae do not reach the adult stage, mice vaccinated with these larvae still elicit a strong immune response against egg glycoproteins. In particular, an egg glycoprotein of 85 to 70 kDa and isoelectric point of 4.8 showed an enhanced reactivity with sera of vaccinated mice in comparison with infected mice (Dalton et al., 1986). The strong immune response of the vaccinated mice against egg glycoproteins supports our previous contention that epitopes are highly conserved through the different stages of development (Strand et al., 1984).

Immunodepletion followed by two-dimensional analysis of adult worm and schistosomular glycoproteins recognized by mice vaccinated once or twice

No qualitative difference was seen in the glycoproteins precipitated by sera from singly vaccinated and patently infected mice, when compared by one-dimensional SDS-PAGE. For this reason, we carried out sequential immunoprecipitations prior to two-dimensional SDS-PAGE analysis, in order to increase our ability to detect minor qualitative and quantitative differences in the spec-

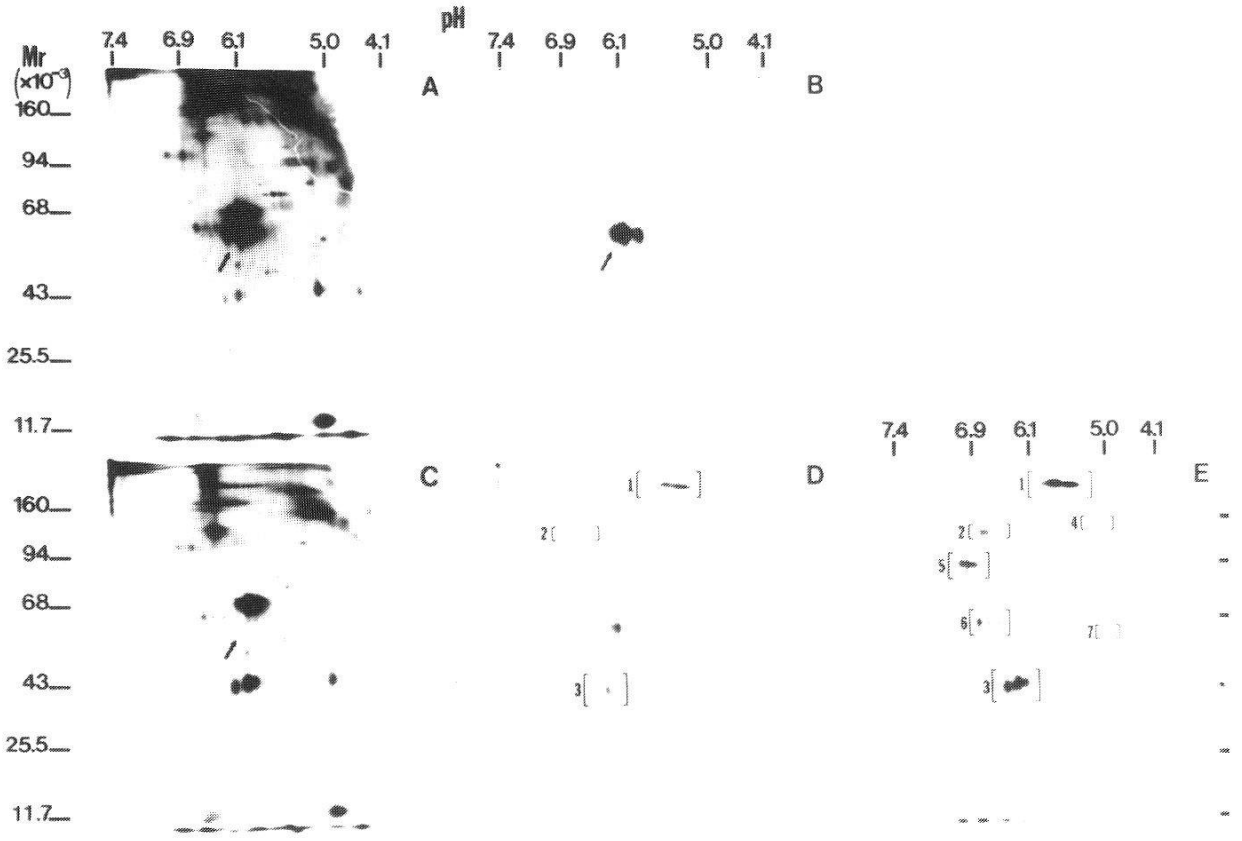

Fig. 1. Immunodepletion: ^{35}S-labeled adult schistosome glycoproteins were immunoprecipitated with sera from (A) mice patently infected; (B) mice patently infected, following immunodepletion of labeled antigens with sera from mice vaccinated once; (C) mice vaccinated once; (D) mice vaccinated once, following immunodepletion of antigens with sera from patently infected mice; and (E) mice vaccinated twice, following immunodepletion of antigens with sera from patently infected mice.

ificity of these antibodies. Immunodepletion experiments identified five major schistosomula antigens of 200, 58, 46, 43, and 25 kDa and three adult worm antigens of 200, 140, and 43 kDa, which had a greater or unique immunogenicity in mice vaccinated once. Mice vaccinated twice had increased antibody titers against these antigens and also recognized four additional worm antigens of 160, 94, 58, and 56 kDa (Fig. 1). Each of these antigens were concanavalin A-binding glycoproteins. Protective antibodies in the serum of vaccinated mice may be directed towards one or more of these glycoproteins.

We therefore isolated the adult worm glycoproteins that were uniquely recognized by sera of vaccinated mice and prepared antiserum against them in a rabbit. This antiserum immunoprecipitated the surface-iodinated antigens of newly transformed schistosomula, specifically the surface-labeled polypeptides of >300, 200, a doublet of 150, and 32 to 38 kDa (Fig. 2). The pattern of polypeptides precipitated with the rabbit serum (anti-IrV) made against the adult worm antigens uniquely recognized by the protective antibody appeared to encompass not only those polypeptides recognized by the sera of vaccinated mice but also those recognized by patently infected mice.

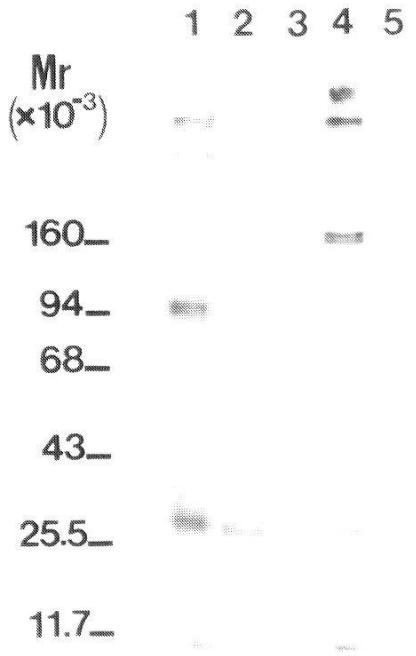

Fig. 2. Surface-iodinated newly transformed schistosomular polypeptides immunoprecipitated with sera from (1) chronically infected mice; (2) mice vaccinated twice; (3) non-infected mice; (4) rabbit anti-IrV; (5) non-infected rabbit.

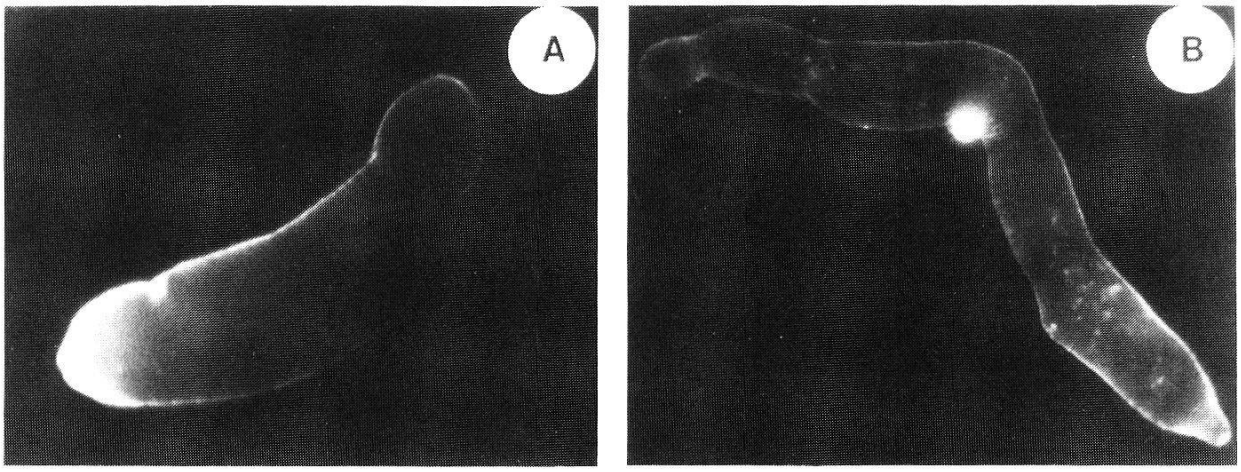

Fig. 3. Surface immunofluorescence of (A) newly transformed schistosomulum and (B) lung-stage schistosomulum, using rabbit anti-IrV.

Immunofluorescence analysis of newly transformed schistosomula and lung-stage schistosomula

Immunofluorescence analysis was used to ascertain whether this rabbit antiserum recognized epitopes exposed on the surface of newly transformed and lung-stage schistosomula. These experiments demonstrated that the antiserum recognized epitopes exposed on the surface of newly transformed schistosomula and also on in vivo lung-stage organisms (Fig. 3). This finding is important since it has been demonstrated that all sera that can transfer resistance at a time

coincident with schistosomulum residence in the lungs have been shown to bind to lung-stage schistosomula in addition to skin-stage larvae (Bickle and Ford, 1982; Ford et al., 1984; Mangold, 1980).

Characterization of a recombinant cDNA expression clone reactive with anti-IrV serum

In order to obtain sufficient quantities of schistosome antigens for use in active protection experiments, we (like many other groups) have turned toward recombinant DNA techniques. An adult worm cDNA expression library has been constructed in λ-gt11 and has been screened with the anti-IrV serum. Several clones reactive with this serum were identified. The properties of one of these, IrV-5, will be described.

Purification and subcloning of IrV-5

IrV-5 was plaque purified and phage DNA isolated. This clone contained an EcoRI insert of approximately 1.5 Kb. The insert was subcloned into a recombinant plasmid containing both the SP6 and T7 promoters flanking a multiple cloning region. Single stranded RNA transcripts from either strand were then produced in vitro using Sp6 polymerase or T7 polymerase. These transcripts were used as probes for Northern and Southern blot analysis, or for translation in a cell-free rabbit reticulocyte system.

Southern, Northern, and coding strand analyses of IrV-5

High molecular weight DNA from adult worms was digested with XbaI, EcoRI and Hind III, electrophoresed and transferred to a nylon membrane. Probing with a radiolabeled RNA transcript of IrV-5 revealed a single band of approximately 7.8 Kb in the EcoRI digest and 4.1 Kb in the Hind III digest. XbaI digestion produced fragments of 6 Kb, 4 Kb, and 3.1 Kb which hybridized to the probe.

In order to determine which strand of IrV-5 cDNA coded for messenger RNA, radiolabeled RNA transcripts were synthesized from both strands of the recombinant IrV-5 plasmid in separate reactions utilizing Sp6 and T7 polymerases. Each transcript was then used to probe duplicate blots of poly(A)$^+$ RNA isolated from adult worm pairs, and electrophoresed in 0.8 percent agarose. Only the probe synthesized using the T7 polymerase hybridized with an RNA species of approximately 5.2 Kb.

Cell-free translation and immunoprecipitation of synthetic IrV-5 RNA transcripts

RNA transcripts from the coding strand of IrV-5 plasmid were synthesized using Sp6 polymerase. The synthetic RNA was translated in vitro using a rabbit reticulocyte lysate system and [^{35}S]methionine. Several major polypeptides ranging in size from 28 kDa to approximately 64 kDa were produced. The synthesis of polypeptides of varying molecular weight is apparently due to

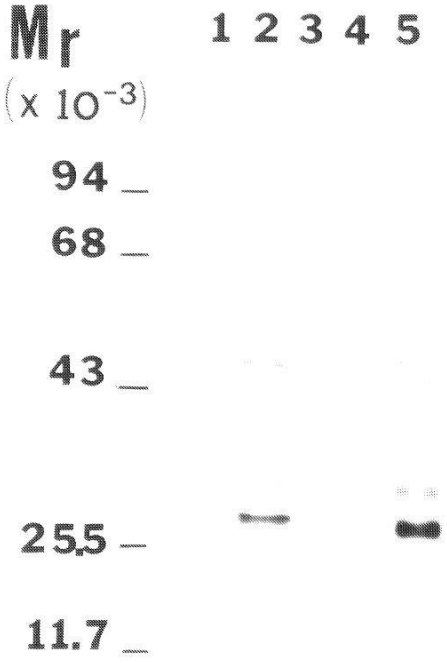

Fig. 4. In vitro translation and immunoprecipitation of SP6 polymerase produced IrV-5 transcripts. (1) Control translation, no messenger RNA added. (2) IrV-5 transcript total translation products. IrV-5 translation products were immunoprecipitated with (3) mouse control sera; (4) mouse control antisera raised against λ-gt11 infected bacterial lysates; (5) mouse antisera raised against IrV-5 recombinant phage infected bacterial lysates.

initiation of translation at different methionine codons in the RNA transcript, as was also observed in the first report utilizing this technique (Lynch et al., 1985). The majority of translation products, namely the polypeptides of approximately 28, 32, 48, and 50 kDa, were immunoprecipitated with antisera (anti-IrV-5) raised in four mice against lysates of bacteria infected with IrV-5 recombinant phage (Fig. 4). The same polypeptides were precipitated by the rabbit anti-IrV serum (not shown). No translation products were immunoprecipitated by mouse antisera raised against lysates of bacteria infected with parental λ-gt11 phage.

Immunoprecipitation of surface-iodinated polypeptides of newly transformed schistosomula by mouse anti-IrV-5

Finally, serum from each of four mice immunized with IrV-5 infected bacterial lysates precipitated a single polypeptide of 38 kDa from surface-iodinated newly transformed schistosomula. A polypeptide of the same apparent molecular weight was also recognized by sera of patently infected or vaccinated mice, although in the latter case only weakly. Antisera prepared in

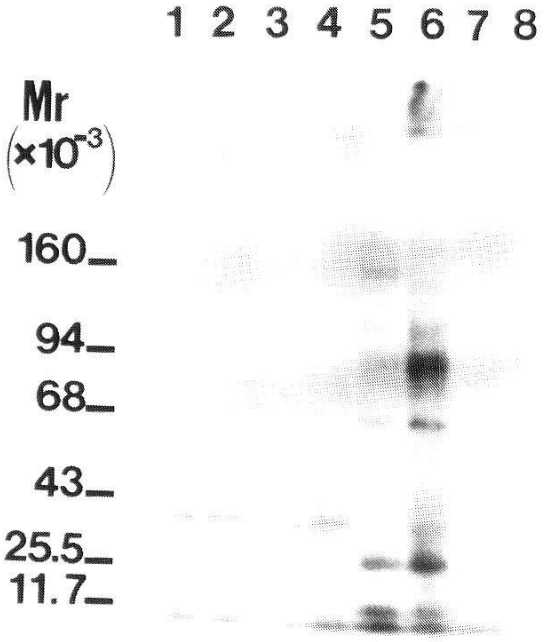

Fig. 5. Surface-iodinated newly transformed schistosomular polypeptides immunoprecipitated with; (1–4) sera from four individual mice immunized against IrV-5 fusion protein; (5) sera from mice vaccinated twice; (6) sera from patently infected mice; and (7 and 8) sera from two individual mice immunized against λ-gt11 and control lysates.

mice against parental λ-gt11 infected bacterial lysates did not precipitate any of the surface iodinated polypeptides (Fig. 5).

Acknowledgments

The excellent technical assistance of Alvin George and Larry Baker and secretarial assistance of Dana Lawrence is greatly appreciated. We would also like to thank Dr. Deborah McClellan for editorial assistance.

This work was supported by Grant AI-19217 from the National Institutes of Health and by Grant 12185 from the Edna McConnell Clark Foundation. JPD was supported by the John D. and Catherine T. MacArthur Foundation. TDT was supported by NIH training Grant 5 T32 GM 07626-08.

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