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

Artikel: Protective antigens in experimental schistosomiasis
Autor: Capron, A. / Pierce, R. / Balloul, J.M.
DOI: https://doi.org/10.5169/seals-313843

Acta Tropica *44*, Suppl. 12, 63–69 (1987)

[1] Centre d'Immunologie et de Biologie Parasitaire, Institut Pasteur, Lille, France
[2] Transgène S.A., 11 rue de Molsheim, 67000 Strasbourg, France

Protective antigens in experimental schistosomiasis

A. Capron[1], R. Pierce[1], J. M. Balloul[1], J. M. Grzych[1], Colette Dissous[1], P. Sondermeyer[2], J. P. Lecocq[2]

Important progress has been made during the last decade in the identification of cytotoxicity mechanisms in experimental schistosomiasis. Antibody-dependent cellular cytotoxicity (ADCC) now appears in man and in the monkey and rat models as the major immunological component of acquired resistance [1, 2].

Three main cell populations, macrophages, eosinophils and platelets, have been shown to be the active cellular partners, whereas anaphylactic antibodies such as IgE and IgG subclasses appear to be the essential humoral factors.

The construction of appropriate rat hybridomas has allowed the production of monoclonal antibodies of the IgG_{2a} and IgE classes, reproducing in vitro the killing activity of experimental infection sera, and able in vivo by passive transfer to confer a high degree of immunity to reinfection [3]. The use of monoclonal and polyclonal antibody probes has allowed the identification and subsequent purification of several major target antigens from the schistosomulum surface. The purpose of this paper is to describe recent and significant progress made in the molecular cloning and chemical synthesis of two major immunogens of the schistosomulum surface, clearly implied in protective immunity, namely P28 and GP38.

Characterization and molecular cloning of the P28 antigen

The characterization and subsequent molecular cloning of the P28 vaccinating antigen initially derived from studies aimed at developing polyclonal, monospecific antibody probes for screening cDNA expression libraries [4]. The approach involved the separation of *Schistosoma mansoni* adult worm homogenates and preparative SDS-polyacrylamide gels, the excision of stained pro-

Correspondence: Prof. Dr. A. Capron, Centre d'Immunologie et de Biologie Parasitaire, Unité mixte INSERM 167 – CNRS 624, Institut Pasteur, 1, rue du Professeur A. Calmette, B.P. 245, F-59019 Lille Cedex, France

tein bands and the electroelution of the purified proteins. Ten prominent bands were used to immunize Fischer rats and the corresponding antisera were tested by immunoprecipitation of [^{35}S]-methionine metabolically labelled adult worm antigens, in vitro translation products and ^{125}I-labelled schistosomulum surface molecules. Only one serum, produced against a native antigen of 28 kDa, immunoprecipitated molecules in all three cases and always recognized a 28 kDa protein.

The biological relevance of this antigen was suggested by the in vitro cytotoxicity for schistosomula of the anti-28 kDa antiserum in an eosinophil-dependent killing assay, approaching the levels attained with an infection serum [5]. Passive transfer of the anti-28 kDa antiserum protected rats at levels of up to 70% against a challenge infection. More importantly, direct immunization of rats with the purified 28 kDa antigen, in the presence of either complete Freund's adjuvant (CFA) or aluminium hydroxide protected them against a challenge infection by up to 70%. Mice immunized with only 50 ng of the antigen along with CFA were also partially protected (40%) against challenge.

In addition we have developed helper T cell lines specific for the 28 kDa antigen. The transfer i.v. of 1.5×10^7 of these cells to naive rats before a challenge infection with *S. mansoni* cercariae protects them by up to 85%, and this protection is related to an early production of anti-28 kDa antibodies [6].

These encouraging results suggested that P28 antigen was an excellent candidate for molecular cloning, particularly since the molecule is not glycosylated and since the monospecific polyclonal antisera were ideal for the screening of expression libraries.

The cloning strategy involved the synthesis of cDNA from adult worm mRNA, the addition of Eco R1 linkers and cloning first into the Eco R1 site of λ-gt10. This library was amplified, inserts prepared and cloned into the expression vector λ-gt11. Initial screening of this library with the polyclonal rat anti-28 kDa serum yield four clones with inserts ranging from 350 to 650 base pairs. The screening of a subsequent library yielded a further eleven expressing clones. Cross-hybridization of the different clones indicated that they were related and the entire nucleotide sequence for the 28 kDa molecule has subsequently been obtained. This sequence has been confirmed by sequencing CNBr peptide fragments from the native molecule.

The identity of the cloned molecule with the native target antigen at the schistosomulum surface was verified by expressing a 20 kDa cloned fragment in a P_L vector. The protein product was partially purified and injected into rats with CFA and produced an antibody response against the native adult worm molecule. The serum obtained was cytotoxic for schistosomula in the presence of eosinophils.

The 20 kDa fragment has also been cloned into vaccinia virus and the antibody titre obtainable with different viral constructions is under investigation.

The construction of a synthetic vaccine based on the P28 molecule will depend on the fine analysis of both its B and T cell epitopes. To this end we have obtained both monoclonal antibodies and helper T cell clones specific for the 28 kDa protein. The proliferation of the T cell clones obtained against the native antigen is stimulated by the cloned expressed 20 kDa fragment. Using synthetic peptides derived from the sequence information already obtained we hope to define the relevant epitopes and construct a synthetic peptide vaccine.

Characterization of the carbohydrate epitope of GP38

Significance and distribution of the epitope. – Significant progress has also been made in the characterization and chemical structural analysis of the glycan epitope of GB38, described several years ago in our laboratory. After the early demonstration that a protective rat monoclonal antibody of the IgG_{2a} isotype (IPLSml) specifically bound to a 38 kDa antigen on the schistosomulum surface, a variety of experimental evidence demonstrated the glycan nature of the epitope recognized [7, 8].

This observation, together with the demonstration that the GP38 antigen could elicit both a protective (IgG_{2a}) and a blocking (IgG_{2c}) antibody response in rats represented a major obstacle to its molecular cloning and prompted us to use an alternative approach based on the production of anti-idiotype antibodies [9]. In a recent series of experiments we were indeed able to demonstrate that rats immunized with monoclonal anti-idiotype antibodies (AB_2) against the protective AB_1 monoclonal, led to the production of specific and highly cytotoxic antibodies, and more importantly to a high degree of protection to challenge infection in recipient animals.

Although encouraging, these results appeared of limited applicability for vaccination against human disease and encouraged further studies on the chemical characterization of the glycan epitope. We first demonstrated, by the immunoprecipitation of surface radioiodinated *S. mansoni* miracidia, that antibodies produced during the rat infection bound to surface components of molecular weights varying from 30 to 200 kDa. All these antigens contain the epitope defined by the IPLSml protective antibody on the previously characterized P38 schistosomulum surface molecule, confirming that the protective determinant was present in different parasite stages on various molecular structures [10].

The original description in our laboratory some twenty years ago [11] of shared antigens between schistosomes and their intermediate hosts prompted us to study an eventual identity between characterized *S. mansoni* target antigens and *Biomphalaria glabrata* components [12]. Antibodies against mollusc soluble extracts were produced in rabbits and used to immunoprecipitate surface labelled antigens of miracidia and schistosomula. In both parasite stages these antibodies bound to surface molecules and the electrophoretic mobilities of the bound antigens were similar to those obtained with IPLSml. Evidence of

binding to the epitope was given by the ability of antibodies to *B. glabrata* to inhibit (by up to 80%) the recognition by IPLSml of the 38 kDa schistosomulum antigen in a solid phase competition assay. Confirming our previous observation on the glycan nature of the epitope it was shown that rabbit antibodies produced against deglycosylated mollusc extracts failed to recognize surface-labelled parasite antigens and were not able to compete with IPLSml for binding to the GP38 molecule.

The identification of cross-reactive *B. glabrata* components was also confirmed by SDS-PAGE analysis of soluble mollusc extracts followed by Western blotting using the IPLSml antibody. Under these experimental conditions IPLSml was shown to bind preferentially to a 90 kDa *B. glabrata* component and this band was not labelled when a deglycosylated *B. glabrata* extract was analyzed.

The next question to be answered was whether this molecular mimicry was limited to the *S. mansoni-B. glabrata* host-parasite system. Whereas *B. glabrata* extracts strongly inhibited (up to 95%) the immunoprecipitation of labelled 38 kDa antigen by IPLSml we observed that soluble extracts from *Bulinus truncatus* and *Limnaea stagnalis,* which are respectively the intermediate hosts for *Schistosoma haematobium* and *Trichobilharzia ocellata* were also both able to compete with the *S. mansoni* antigen showing respective inhibitions of 80 and 65% for the same maximal quantity of protein added. Moreover, a similar level of inhibition (75%) was observed with a non-schistosome host, *Limnaea limosa* and such results suggested that the presence of the protective epitope in molluscs was not restricted and would be found in a wide variety of snail species.

Expression by KLH of the carbohydrate epitope of P38. – We next made a crucial observation which in fact was more due to serendipity than to a logical progression of experimental analysis. We indeed showed during the course of the immunization of rats with anti-idiotype antibodies conjugated to Keyhole Limpet Haemocyanin (KLH) that sera collected from the control group of rats immunized with KLH alone could bind strongly to the schistosomulum surface, and more significantly inhibit the binding of ^{125}I-labelled IPLSml to GP38 in exactly the same range of inhibition as serum from rats immunized with AB_2-KLH [13].

This preliminary observation suggesting the existence of a common epitope between the haemocyanin of *Megathura crenulata,* an ancestral marine mollusc, and the GP38 antigen was placed on a molecular basis by immunoprecipitation techniques.We indeed showed that sera from KLH-immunized rats were capable of binding to GP38 on the schistosomulum surface. The antigenic similarity betweeen GP38 and KLH was also confirmed by the analysis of a purified fraction of KLH by SDS-PAGE and Western blotting. These experiments showed that molecules of about 150 to 200 kDa were recognized by IPLSml, and by sera from animals infected with *S. mansoni.*

The glycan nature of the epitope of GP38 together with the documented existence of carbohydrate moieties in KLH prompted us to study the potential importance of KLH oligosaccharides in this surprising cross-reactivitiy. A series of inhibition experiments were performed either with purified native KLH, KLH deglycosylated with TFMS (trifluoromethanesulphonic acid) or with sera collected from rats immunized with KLH or deglycosylated KLH. In such conditions it was shown that various concentrations of native KLH, but not of deglycosylated KLH, were able to inhibit strongly the binding of IPLSml to GP38. Antibodies obtained from KLH-immunized rats induced the same level of inhibition as that observed for infected rat sera, IPLSml or purified KLH. We could also inhibit the binding of labelled IPLSml to KLH by various concentrations of unlabelled IPLSml or sera from infected animals. In all these experiments deglycosylation of KLH ablated the effects of the native molecule. More recently, similar inhibition experiments performed with the purified carbohydrate moiety of KLH clearly confirmed the total identity between the glycan epitope of GP38 and KLH oligosaccharides.

The essential role of anti-GP38 antibodies in eosinophil-dependent cytotoxicity for schistosomula naturally led to the investigation of such cytotoxic antibodies in sera form KLH-immunized rats. Significant levels of cytotoxicity (46–94%) comparable to those obtained when using infected rat sera were observed. The antibodies produced by immunizing rats with KLH were also found to be protective when passively transferred to recipient rats to a degree (48%) very close to that previously observed for the IPLSml protective antibody. In addition, KLH immunized rats were shown to be highly protected against a challenge infection.

Since it was previously shown in human infected populations that antibodies to GP38 could be detected in 97% of patients [14] we explored the possibility of using KLH for the detection of anti-schistosome antibodies in humans. Preliminary studies using a competition assay with human sera have indicated the potential use of KLH as a diagnostic reagent.

Work at present in progress in collaboration with J. Montreuil and his coworkers in Lille now indicates the feasibility of defining the chemical structure of KLH oligosaccharides and their possible synthesis.

Taken together, these observations appear to have several main consequences. They illustrate the expression on the surface of a human parasite of a major immunogen not only present in its snail intermediate host, but of which the origins are found in a marine mollusc that has been in existence for the last several hundred million years. The evolutionary conservation of the structure and the similarities of the expression in a human parasite pose a fascinating problem of phylogeny and adaptation. It has been recently reported in various biological systems that membrane oligosaccharides play an important role in osmotic adaptation and osmolarity [15]. It is tempting to speculate that important changes affecting schistosomes in the osmolarity of their cellular environ-

ment during the rapid adaptation of the free larval form to its vertebrate host might rely on this highly preserved structure.

The fact that almost all subjects infected by *Schistosoma mansoni* produce antibodies against the glycanic epitope of the 38 kDa antigen also present in KLH opens an entirely new path towards the development of a simple, easily standardized and cheap reagent for the sero-epidemiology of human schistomiasis. The protective properties linked to this particular epitope confers on KLH and particularly on its glycanic moiety an exceptional potential in the vaccine strategy against schistosomes. Work presently in progress using mass spectrometry analysis of purified KLH oligosaccharides makes feasible in the near future the access to a well defined structure.

Finally it will be certainly of interest to immunologists that have used KLH for 20 years as a carrier for human or animal immunization [16, 17] to know that such immunization can lead to the production of anti-*Schistosoma mansoni* antibodies.

Conclusion

It appears therefore that major progress has been made in the isolation and possible synthesis of protective antigens against schistosomiasis. The access which we now have not only to important functional structures of a protein, but also to carbohydrate epitopes opens up exciting and promising prospects of constructing synthetic neoglycoproteins that can be used as entirely synthetic vaccines. It remains, however, as previously emphasized that whatever the progress in molecular biology or biochemistry may be, one of the major constraints for immunoparasitologists will be the precise analysis of the T and B cell epitopes of the synthetic molecules and the appropriate antigen presentation for optimal immunisation.

Acknowledgments

This work was partially supported by a grant from the Edna McConnel Clark Foundation (EMCF N°07585) and by the INSERM (U167) and by the CNRS (624).

1 Capron A., Dessaint J. P.: Effector and regulatory mechanisms in immunity to schistosomes: a heuristic view. Ann. Rev. Immunol. *3,* 455 (1985).
2 Capron M., Capron A.: Rats, mice and men – models for immune effector mechanisms against schistosomiasis. Parasit. Today *2,* 69 (1986).
3 Grzych J. M., Capron M., Bazin H., Capron A.: In vitro and in vivo effector function of rat IgG_{2a} monoclonal anti-*S. mansoni* antibodies. J. Immunol. *129,* 2739 (1982).
4 Balloul J. M., Pierce R. J., Grzych J. M., Capron A.: In vitro synthesis of a 28 kilodalton antigen present on the surface of the schistosomulum of *Schistosoma mansoni.* Molec. biochem. Parasit. *7,* 293 (1985).

5 Balloul J. M., Grzych J. M., Pierce R. J., Capron A.: A purified 28 kDa protein from *Schistosoma mansoni* adult worms protects rats and mice against experimental schistosomiasis. J. Immunol., in press (1987).
6 Auriault C., Balloul J. M., Pierce R. J., Damonneville M., Sondermeijer P., Capron A.: Helper T cells induced by a purified 28 kDa antigen of *Schistosoma mansoni* protect rats against infection. Infect. Immun., in press (1987).
7 Dissous C., Grzych J. M., Capron A.: *Schistosoma mansoni* surface antigen defined by a rat protective monoclonal IgG_{2a}. J. Immunol. *129,* 2232 (1982).
8 Dissous C., Grzych J. M., Capron A.: Biochemical studies on the 30–40 kDa *Schistosoma mansoni* surface antigens. Molec. biochem. Parasit. *16,* 277 (1985).
9 Grzych J. M., Capron M., Lambert P. H., Dissous C., Torres S., Capron A.: An anti-idiotype vaccine against experimental schistosomiasis. Nature (Lond.) *316,* 74 (1985).
10 Dissous C., Capron A.: *Schistosoma mansoni:* antigenic community between schistosomulum surface and adult worm incubation products as a support for concomitant immunity. FEBS Lett. *162,* 355 (1983).
11 Capron A., Biguet J., Rose F., Vernes A.: Les antigènes de *Schistosoma mansoni.* II. Etude immunoélectrophorétique comparée de divers stades larvaires et des adultes des deux sexes. Aspects immunologiques des relations hôte-parasite de la cercaire et de l'adulte. Ann. Inst. Past. Paris *109,* 788 (1965).
12 Dissous C., Grzych J. M., Capron A.: *Schistosoma mansoni* and its intermediate host share a protective carbohydrate epitope (1986).
13 Grzych J. M., Capron A., Dissous C., Capron M., Lambert P. H.: A protective carbohydrate epitope of *Schistosoma mansoni* major immunogen is expressed by Keyhole Limpet Hemocyanin (KLH). J. exp. Med. *165,* 865 (1987).
14 Dissous C., Prata A., Capron A.: Human antibody responses to *Schistosoma mansoni* surface antigens defined by protective monoclonal antibodies. J. infect. Dis. *149,* 227 (1984).
15 Miller K. J., Kennedy E. P., Reinhold V. N.: Osmotic adaptation by gram-negative bacteria: possible role for periplasmic oligosaccharides. Science *231,* 48 (1986).
16 Dixon F. J., Jacot-Guillormod H., McConahey P. J.: The antibody responses of rabbits and rats to hemocyanin. J. Immunol. *97,* 350 (1966).
17 Swanson M. A., Schwartz R. S.: Immunosuppressive therapy: the relation between clinical response and immunologic competence. New Engl. J. Med. *277,* 163 (1967).