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GP38, P28-I AND P28-II: CANDIDATES FOR A VACCINE AGAINST SCHISTOSOMIASIS

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Three antigens protective against Schistotoma mansoni *have been extensively characterized. The schistosomulum surface antigen GP38 possesses an immunodominant carbohydrate epitope of which the structure has been defined. Protection can be achieved via the transfer of monoclonal antibodies recognizing the epitope or by immunization with anti-idiotype monoclonal antibodies. The glycan epitope is shared with the intermediate host,* Biomphalaria glabrata *as well as being present on other molluscs, including the Keyhole Limpet. A group of molecules at 28 kDa were initially characterized in adult worms and shown to protect rats and mice against a challenge infection. One of these molecules, P28-I, was cloned and expressed in* E. coli, *yeast and vaccinia virus. The recombinant antigen significantly protected rats, hamsters and baboons against a challenge infection. P28-I is a glutathione-S-transferase and the recombinant antigen produced in yeast exhibits the enzyme activity and has been purified to homogeneity by affinity chromatography. A second P28 antigen, P28-II, has also been cloned, fully sequenced and expressed. This recombinant antigen also protects against* S. mansoni *infection.*

The rapid recent progress that has been made in the molecular cloning of defined schistosome antigens that are potential targets of immunity means that prospects for the obtention of a vaccine against Schistosomiasis have never been brighter. Two main classes of antigens have been chosen; integral surface membrane antigens of schistosomula, and antigens present in excretory-secretory products of both larval and later life-cycle stages.

That the schistosomulum is a priveleged target for immune attack is beyond doubt. Effector mechanisms capable of killing the larvae have been demonstrated both in man and in animal models depending on the participation of macrophages, eosinophils or platelets together with anaphylactic antibody classes, notably IgE (M. Capron & A. Capron, 1986, *Parasitology Today, 2:* 69-75). However, it is becoming clear that "late" resistance also exists (M. Ford et al., 1987, *Parasitology, 94:* 509-522) and that attrition of the infective larvae can occur at the lung or later stages. Therefore excretory-secretory antigens present at the larval stage, but also in lung worms or young adults can form the basis of an immune attrition occuring throughout the maturation process.

This paper discusses recent progress made in respect to two antigen systems, the GP38 schistosomulum surface glycoprotein (C. Dissous et al., 1982, *J. Immunol., 129:* 2232-2234) and the P28 group of antigens present in schistosomulum released products (R. J. Pierce et al., 1985, *Mol. Biochem. Parasitol., 15:* 171-188) and isolated and cloned from adult worms (J. M. Balloul et al., 1987, *Nature, 326:* 149-153).

CHARACTERIZATION OF GP38: ANTI-IDIOTYPE VACCINE STRATEGY

The 38kDa glycoprotein (GP38) was originally detected on the schistosomulum surface using a rat monoclonal IgG_{2a} antibody (IPLSm1). This antibody mediated both eosinophil-dependant cytotoxicity towards schistosomula and protection of rats against a challenge infection by passive transfer (J. M. Grzych et al., 1982, *J. Immunol., 129:* 2739-2743; C. Dissous et al., 1982, *J. Immunol., 129:* 2232-2234). Subsequent investigations showed that the epitope involved in the protective

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response was glycannic in nature (C. Dissous & A. Capron, 1983, *FEBS Lett., 162:* 355-359; C. Dissous et al., 1985, *Mol. Biochem. Parasitol., 16:* 277-288). This, taken together with the fact that the antibody response to GP38 comprised both protective (IgG_{2a}) and blocking (IgG_{2c}) subclasses in rats (J. M. Grzych et al., 1984, *J. Immunol., 133:* 998-1004) represented a major obstacle to its molecular cloning. For this reason an anti-idiotype strategy was developed, based on the IPLSml monoclonal antibody (J. M. Grzych et al., 1985, *Nature, 316:* 74-75). Rats immunized with a monoclonal anti-idiotype antibody (AB2) directed against the antigen-binding site of IPLSm1 produced specific AB3 antibodies that were highly cytotoxic for schistosomula *in vitro* in the presence of eosinophils and protected rats against infection by passive transfer. Equally, immunization with AB2 antibodies protected rats against a challenge infection.

At this stage the apparent difficultues in applying these findings to the problem of vaccinating human populations led us to attempt to characterize the carbohydrate epitope.

DISTRIBUTION AND STRUCTURAL DETERMINATION OF THE CARBOHYDRATE EPITOPE OF GP38

The epitope recognized by the IPLSm1 antibody is not only present on schistosomula, but is carried by a 115kDa adult worm antigen (C. Dissous & A. Capron, 1983, *FEBS Lett., 162:* 355-359) and by a variety of structures ranging from 30kDa to 200kDa on miracidia.

The description of shared antigenic determinants between schistosomes and their snail intermediate hosts that was made by A. Capron et al. (1965, *Ann. Inst. Pasteur Paris, 109:* 798-810) prompted an investigation into the possibility that the GP38 immunodominant glycan was one such determinant (C. Dissous et al., 1986, *Nature, 323:* 443-445). Antibodies produced in rabbits against *Biomphalaria glabrata* extracts bound to schistosomulum surface molecules and inhibited the binding of IPLSml to its target in a solid phase competition assay. IPLSm1 antibodies, on the other hand, recognized a 90kDa molecule in *B. glabrata* extracts. Deglycosylation of the antigen preparations abolished this recognition. This molecular mimicry was not limited to the intermediate host of *S. mansoni*, but similar cross-reactivity was found in extracts of several other freshwater snails, including schistosome hosts such as *Bulinus truncatus* and *Limaaea stagnalis*, and non-hosts suc as *Limnaes limosa.*

That we could expect to find this protective carbohydrate epitope in a wide variety of mollusc species was confirmed by the chance observation of its presence in Keyhome Limpet Hemocyanin (KLH). It was in fact observed that when KLH was used as a carrier molecule for the immunization of rats with the anti-idiotype monoclonal antibody, those control animals immunized with KLH alone developed antibodies recognizing the schistosomulum surface (J. M. Grzych et al., 1987, *J. exp. Med., 165:* 865-878). These antibodies immunoprecipitated GP38 from radiolabelled larval surface extracts and IPLSm1 recognized high molecular weight components of KLH (as did sera from infected animals).

Experiments in which either KLH or schistosomulum membrane antigens were deglycosylated, abolishing the cross recognition, clearly demonstrated that KLH also possessed the carbohydrate epitope previously described. In addition, immunization with KLH protected rats against a challenge infection, and anti-KLH sera were cytotoxic for schistosomula in an eosinophil-dependant assay, as well as transferring resistance to recipient animals.

The availability of KLH meant that, for the first time, the problem of the structural determination of the carbohydrate epitope could be approached. Initially, glycopeptide fractions were produced from KLH and their ability to inhibit the binding of IPLSm1 to its target antigen was assayed. Structural analysis of the corresponding fractions has recently allowed the definition of a concensus glycan structure for the epitope (manuscript in preparation).

This finding means that the chemical synthesis of the immunodominant epitope can be envisaged and hence the construction of neoglycoproteins. These structures may facilitate studies on the problem of the generation of blocking antibody subclasses and could also from part of a future vaccine. Whether or not this proves to be the case, the questions of basic biology raised by this common structure are fascinating. Such molecules may be involved in osmotic adaptation (K. J. Miller et al., 1986,

Science, 231: 48-51) and it is tempting to speculate on the role of the GP38 glycan in the rapid adaptation of free-swimming *S. mansoni* larvae to the vertebrate host.

CHARACTERIZATION AND MOLECULAR CLONING OF THE P28 ANTIGENS

The initial work on the group of antigens at 28kDa (P28) arose from an alternative strategy for the characterization of the target molecules of immunity. The first element of this approach was the discovery that the antigens excreted-secreted by schistosomula, termed SRP-A (C. Auriault et al., 1984, *Eur. J. Immunol., 14:* 132-138) induced the production of IgE antibodies that were cytotoxic for schistosomula in the presence of macrophages, eosinophils and platelets and that transferred immunity to naive rats against a challenge infection (M. Damonneville et al., *Clin. exp. Immunol., 65:* 244-252). Direct immunization with SRP-A also protected rats to a large extent against infection. Among the antigens recognized by anti-SRPA sera, a 28kDa molecule was present in the *in vitro* translation products of adult worm mRNA (R. J. Pierce et al., 1985, *Mol. Biochem. Parasitol., 15:* 171-188) and was therefore a potential candidate for molecular cloning.

The definition of P28 as a protective antigen arose from the second element of the approach that aimed at developping polyclonal, mono-specific antibody probes for screening cDNA expression libraries (J. M. Balloul et al., 1985, *Mol. Biochem. Parasitol., 17:* 105-114). Sera raised against the P28 fraction electroeluted from SDS-polyacrylamide gels detected the antigen among the ^{125}I-labelled molecules on the schistosomulum surface. These sera were highly cytotoxic for schistosomula in the presence of eosinophils and protected rats against infection (by up to 70%) by passive transfer. Immunization of both rats and mice with the electroeluted molecule led to protection against challenge infection by 65-70% and 40-43% respectively (J. M. Balloul et al., 1987, *J. Immunol., 138:* 3448-3453).

At this stage helper T-cell lines and clones specific for the P28 fraction were developed (C. Auriault et al., 1987, *Infect. Immun., 55:* 1163-1167). The passive transfer of these cells (1.5×10^7) protected rats against infection (85%) and the protection was related to an early production of anti-P28 antibodies.

The first component of the P28 fraction, P28-I, was cloned from a lambda gt_{11} cDNA expression library (J. M. Balloul et al., 1987, *Nature, 326:* 149-153). Three independant candidate clones were initially obtained and sequenced. The full length sequence was obtained by re-screening the library with an oligonucleotide probe derived from the 5' end of the longest insert. Two more candidates were obtained and all five clones contained overlapping sequences corresponding to a 28 kDa protein of 211 amino acids. The sequence also corresponded to the sequences of two tryptic peptides obtained from the native protein.

Major B-cell epitopes seemed to be located between amino acid residues 91 and 200 and the fusion protein expressed by a clone corresponding to this region also restimulated the proliferation of P28-specific T-cell lines and clones. A recombinant antigen comprising 172 amino acids from the C-terminal region of the molecule was expressed in *E. coli* as a cII fusion protein. This protein provoked an intense antibody response when injected into rats. The sera recognized the native protein and were cytotoxic for schistosomula in the presence of eosinophils. The cytotoxicity obtained was mainly IgE-dependant. Immunization of rats and hamsters with this recombinant antigen protected them by up to 65%. Other constructs, involving expression in yeast and in vaccinia virus also led to the production of immunogenic recombinant antigen that induced protection in rats.

Preliminary experiments carried out in baboons using P28-I produced in *E. coli* also indicated that recombinant P28-I protected against a challenge infection, worm burdens being reduced compared to control animals injected with *E. coli* lysate, but with wide individual variations in response. It was notable, however, that immunized animals had both reduced densities of granulomas in the liver, and that the mean granuloma size was reduced, indicating a possible reduction in egg-induced pathology.

LOCATION AND DISTRIBUTION OF P28-I

The study of the location of P28-I using electron microscopy and immunogold detection, indicates that P28-I is not an integral membrane

protein. In both adult worms and schistosomula the antigen appears to be preferentially cytoplasmic but associated with structures, such as the head gland of schistosomula, that could account for its presence in excreted-secreted material. The initial observation of P28 among the surface-labelled products of the larvae may have been due to a process of secretion and readsorption.

The sequence of P28-I did not contain any overall homology to known proteins. However, it was noted that two restricted regions of the molecule presented striking sequence homology with rat glutathione-S-transferase subunits. The first consequence of this finding was that the recombinant P28-I expressed in *E. coli* and in yeast was found to express the enzyme activity and could be affinity purified on glutathione agarose columns. Sequencing of the purified native *S. mansoni* enzyme confirmed that it was identical to the cloned P28-I. A potentially negative aspect of this finding was a possoble cross-reactivity between the parasite and the mammalian enzymes. No evidence for this was found, however, and anti-P28-I antibodies failed to recognize either the rat or the human enzyme.

Antibodies directed against the recombinant P28-I did, however, cross react with molecules present in both *S. bovis* and *S. japonicum*. This result considerably widens the interest of the molecule, and a program of immunization of cattle in the Sudan has been initiated. Preliminary results show that P28-I is highly immunogenic, and experiments are in progress to test the capacity of the antigen to vaccinate calves against a natural infection.

Another approach towards the development of a synthetic vaccine has concerned the analysis of the P28-I sequence and the synthesis of peptides potentially corresponding to T-cell epitopes. Three such peptides are being analyzed both for their capacity to induce a cellular and humoral response against the native P28, and their capacity to restimulate the proliferation of P28 specific T-cells. One such peptide, corresponding to amino acids 24-43 induces an antibody response that recognizes the native P28 and is cytotoxic for schistosomula in the presence of eosinophils. The peptides are also being used in the mouse model to test the possible MHC-restriction of P28 epitope recognition.

P28-II: A SECOND CANDIDATE

The initial cloning of P28-I also turned up clones that were detected by the sera raised against electroeluted P28, but that did not cross hybridize with the P28-I clones. They have since been shown to code for another 28kDa protein, P28-II, quite different from P28-I and that has been expressed in *E. coli*. This recombinant protein also induces a strong humoral response and protects rats against a challenge infection. P28-II thus provides a second recombinant vaccine candidate.

CONCLUSION

The definition of molecules that protect experimental animals against schistosomiasis indicates that the obtention of a vaccine has become a distinct possibility. Both P28-I and P28-II are efficiently expressed in *E. coli* and the discovery of the enzyme activity of P28-I has enabled its purification to homogeneity after expression in yeast. The synthesis of immunogenic peptides that correspond to T-cell epitopes also indicates that a wholly synthetic vaccine may be obtained. In addition the eventual synthesis of the immunodominant glycan epitope of GP38 will enable the construction of neoglucoproteins.

A number of problems remain to be resolved, however, including the individual variation in response towards these molecules that will undoubtedly be encountered in human populations, particularly in view of the encouraging, but variable results obtained with baboons. In the case of the glycan epitope, means have to be developed to avoid the generation of a blocking antibody response. Since immunity to schistosomes in multifactorial, the use of cocktails of different antigens and epitopes, both derived from the P28 antigens as well as from other molecules, will certainly have to be envisaged if an acceptable level of protection is to be obtained in human populations.