TEGUMENTAL PROTEINS OF SCHISTOSOMA MANSONI: COMPLEX BIOMOLECULES AND POTENT ANTIGENS

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The passive transfer of monoclonal antibodies, direct vaccination and in vitro assays have all shown that antigens associated with the tegumental membranes of Schistosoma mansoni are capable of mediating protective immune responses against the parasite in animal models. Furthermore, the principal antigens are highly antigenic during natural infection in man and stimulate strong humoral and cellular responses although, at present, their role in mediating protective immune responses in man remains equivocal. This presentation will review the current state of knowledge of the structure and expression of the major antigenic tegumental proteins of the schistosome and will attempt to relate the relevance of their structural features to possible function both in terms of protective immunity and the parasite’s ability to survive within the definitive host. A focus will be the recent advances that have been made in the identification of means of anchoring of the antigenic proteins to the tegumental membrane. In addition, the implications of the structural complexity of the tegumental proteins in terms of their possible utility in vaccination and diagnosis will be considered.

Key words: Schistosoma mansoni – tegumental proteins – antigens

This review is concerned with molecules associated with the membranous structures of the tegument of Schistosoma mansoni that have been defined as antigens using antibodies either from experimental animals or from individuals infected with S. mansoni. Research in this area has been stimulated by the search for molecules that may act as targets of protective immune responses and by the desire to understand the mechanisms whereby the schistosome can evade protective immune responses. The data that has accrued over the last five years, largely from the techniques of gene cloning and protein chemistry, show that the nature of schistosome tegumental antigens is highly varied, that some are quite unexpected in their chemical nature and that some are highly processed and complex molecules. This work has shed light on basic questions of protein membrane interactions, produced important candidates for a schistosome vaccine and may provide the basis of an increased understanding of host immune responses.

Three classes of tegumental antigen will be discussed: (I) soluble proteins associated with the schistosome surface, (II) antigens bearing carbohydrate epitopes and (III) integral membrane glycoproteins.

SOLUBLE PROTEINS ASSOCIATED WITH THE SCHISTOSOME SURFACE

Description of surface associated molecules of the schistosome has been classically centered on the description of molecules on the surface of young schistosomula which is known to be a target of antibody mediated immunity both in vitro and in vivo (McLaren & Smithers, 1987). In three cases, antigens detected on the surface of young schistosomula, by the use of specific antibodies, have been found to be soluble antigens. In each instance, this was discovered by the cloning of the gene for the protein and comparison of the deduced amino acid sequence with previously cloned molecules. In one of these cases, the glutathione-S-transferases (GSTs) the homology between the parasite proteins and those of its host is limited and the majority of the enzyme sequence is quite distinct from that of corresponding host enzymes, the activity of the schistosome enzymes having been confirmed by direct biochemical means (Smith et al., 1986; Balloul et al., 1987a; Taylor et al., 1988). Schistosoma mansoni as well as S. japonicum have at least two GST isoenzymes of M,26 and 28K and cDNA clones for all of these proteins have been isolated (Smith et al., 1986;
Balloul et al., 1987a; Tiu et al., 1988; Henkle et al., 1990; Trotteir et al., 1990). The deduced amino acid sequences give no indication as to why these proteins should be associated with the schistosome surface as they lack both signal sequences that would direct their extrusion into the endoplasmic reticulum (a prerequisite for cell surface expression or secretion) or sequences compatible with direct membrane interaction or the addition of a lipid anchor. Nevertheless, work involving surface iodination, surface immunofluorescence and immunoelectron microscopy has indicated a surface location for both the Mr26 and 28K GSTs (Balloul et al., 1987b; Mitchell et al., 1988; Taylor et al., 1988; Henkle et al., 1990; Trotteir et al., 1990). Extensive investigations have been undertaken concerning the ability of these proteins to stimulate protective immunity. Studies with both native and recombinant Sm28, the Mr28K S. mansoni isoenzyme, have resulted in impressive levels of protection (40-70%) in rats, mice and hamsters (Balloul et al., 1987a, b). This molecule is at present the most advanced candidate for inclusion in a schistosome vaccine. Indeed, since the recombinant antigen is readily synthesized in bacterial systems (allowing large scale production by fermentation), easily purified by affinity chromatography, readily soluble and cross reactive with all the major schistosome species that infect man, if clinical trials prove it to protect man to significant levels this protein may well be used as the first anti-parasite molecular vaccine. In contrast, work with the M26K isoenzyme of S. japonicum (Sj26) has given inconsistent levels of protection although in some experiments resistance of up to 50% has been achieved (Mitchell et al., 1988). It has been suggested by the authors of this work that Sj26 is probably not sufficiently protective alone to act as a vaccine but that it may be a candidate for inclusion in a multi-component vaccine. However, recent studies have failed to demonstrate an additive effect of GST vaccination with other means of stimulating resistance in mice (Mitchell et al., 1990).

Another protein of M28K has been detected on the schistosomulum surface by means of monoclonal antibody binding and surface iodination (Harn et al., 1985a). Gene cloning and sequencing showed that this protein is distinct from the GST of the same M, and is in fact schistosome triose-phosphate isomerase (TPI) (Gross et al., 1988). The monoclonal antibody that recognizes this protein is protective in animal models and protection has also been achieved by direct vaccination (Harn et al., 1987). The detection of TPI at the parasite surface is perhaps even more unexpected than with GSTs. The enzyme is part of the glycolytic pathway that catalyzes carbohydrates to produce ATP and is normally found in the cell cytoplasm. Nevertheless, independent work has led to the detection of another enzyme of the glycolytic pathway, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) exposed at the surface of schistosomula (Goudet-Crozat et al., 1989). Attention was first drawn to this molecule because it was observed that it is recognized more frequently by antibodies from individuals resistant to S. mansoni infection than by non-resistant individuals (Dessein et al., 1988). In this case, the homology with the host enzyme is extensive and the investigators undertaking this work have pointed out that this greatly reduces the number of epitopes likely to be recognized by the host immune system. It is thus probable that genetic restriction of the immune response will mean that not all individuals will respond to the antigen thus providing a possible molecular basis for the genetic dependence of resistance to reinfection that the same authors have described (Abel et al., 1991).

In all three cases further studies of the mechanisms and molecular basis of the interaction of proteins with the parasite surface will be of great interest. It seems possible that the schistosome surface is adapted for the adsorption of soluble proteins, perhaps as part of its strategy to acquire host antigens that act as an antigenic disguise, and defend the host against immune attack (Smithers, et al., 1969). This may also result in the surface location of enzymes and other proteins released from the parasite. As yet, the chemical nature of the interactions have not been studied but in a separate area, sequence analysis of the gene for an adult schistosome antigen that behaves as an integral membrane protein (Sm15) has shown that it is very highly negatively charged and contains no sequences likely to be involved with membrane anchoring further indicating the propensity of soluble proteins to form stable interactions with schistosome membranes (F. Abath, manuscript in preparation).

PROTEINS WITH CARBOHYDRATE EPITOPES

Analysis of monoclonal antibody binding and chemical treatment of the parasite surface
has shown that antigens of $M_r > 200, 38$ and $17K$ are defined by highly antigenic carbohydrate epitopes and that such epitopes constitute $> 90\%$ of those recognized on the schistosomulum surface by antibody from infected animals (Omer Ali et al., 1986). These epitopes are targets of protective antibodies (Zodka & Phillips, 1982; Grzych et al., 1982; Harn et al., 1984; Tarrab-Hazdai et al., 1985; Dissous et al., 1985; Omer Ali et al., 1988) but also of antibodies that block complement and eosinophil mediated schistosomula killing and that may also have an involvement in the formation of granulomas (Grzych et al., 1984; Khalife et al., 1986; Yi et al., 1986a; Simpson et al., 1990). There is a strong antibody response to these epitopes during schistosome infection in mice but not following vaccination with highly irradiated cercariae (Omer Ali et al., 1986a; Yi et al., 1986b). They are strongly recognized only during the early phases of infection in man and antibodies against them have been proposed to limit the efficacy of protective immune responses and be a factor in the slow development of resistance in naturally infected individuals (Butterworth et al., 1987; Omer Ali et al., 1989). Use has been made of these observations to develop a rapid diagnostic assay for acute schistosomiasis based on the recognition of these carbohydrates expressed on keyhole limpet hemocyanin (Mansour et al., 1989; Alves-Brito et al., 1991).

A number of investigators have shown that vaccination of experimental animals with carbohydrate dependent antigens, either in purified form or in the form of an idiotypic monoclonal antibody, can stimulate protective immunity in experimental animals (Smith & Clegg, 1985; Grzych et al., 1985; Tarrab-Hazdai et al., 1985). Furthermore, vaccination with the readily available KLH also stimulates high levels of resistance in the rat (Grzych et al., 1987). Although the carbohydrate epitopes obviously cannot be produced by gene cloning the availability of KLH in large amounts and in purified form from commercial sources perhaps solves the problem of supply so that it could also be considered as a vaccine candidate. Since the initial demonstration of the protective effect of KLH, further work investigating its ability to protect other animal models has not been reported, those involved in vaccine development preferring to concentrate on protein antigens that can be produced by genetic engineering or peptide synthesis. Clearly the complex nature of the immune response to the carbohydrate antigens suggests that vaccination may not be worth while but given the availability of the molecule, the vast amount of literature available where it has been used in immunological investigations both in experimental animals and man (where vaccination has not been reported to have untoward effects), the further evaluation of the molecule as a vaccine against schistosomiasis would seem to be warranted.

INTEGRAL MEMBRANE PROTEINS

Studies demonstrating the presence of proteins with glycosylphosphatidylinositol (GPI) anchors as well as studies using the detergent triton X-114 have described proteins that are integral to schistosome tegumental membranes. Isolation of tegumental membranes from adult parasites and their use in vaccination of mice has shown that they contain protective antigens (Smithers et al., 1989). In addition, it has been found that the same antigenic preparation contains antigens that are strongly recognized by both antibodies and T-cells during human infection (Bahia-Oliveira et al., manuscript in preparation). The cellular responses to the membrane associated antigens appear to be distinct from those to soluble antigens since the relative levels of proliferation of peripheral blood mononuclear cells to the two antigen fractions alters significantly in patients with different clinical forms of the disease and the membrane antigens stimulate significantly higher levels of gamma-interferon than the soluble antigens (Bahia-Oliveira et al., manuscript in preparation).

Antibodies from mice protectively vaccinated with adult worm tegumental membranes recognize antigens on the surface of young schistosomula showing that there are proteins that are conserved between the two phases of the life cycle (Smithers et al., 1989). Those most strongly recognized are of $M_r 38, 32$ and $20K$. They are also strongly recognized following vaccination with highly irradiated cercariae and during chronic infection in man (Simpson et al., 1985, 1986). Sm32 is recognized by a protective monoclonal antibody (Bickle et al., 1986). It has recently been shown that these molecules are linked to the schistosomulum surface via GPI anchors and released from the parasite surface by treatment with GPI specific enzymes (Pearce & Sher, 1989). In cultured parasites these molecules
are released from the parasite surface in membrane associated vesicles (Simpson et al., 1984; Pearce & Sher, 1989). This observation may explain their strong antigenicity during vaccination with irradiated cercarie. It may be that their shedding is facilitated by their mode of membrane attachment that does not involve interactions with the membrane cytoskeleton.

An interesting observation is that antigens are not released from lung worms and although Sm32 has been unambiguously identified in adult membrane preparations it does not have a GPI anchor susceptible to enzyme cleavage (Pearce & Sher, 1989; Pearce et al., 1991). These data indicate a change in the mode of membrane attachment during maturation that may involve the conversion of the GPI anchor to a resistant form containing an additional bound lipid. Nevertheless, GPI anchored proteins have been demonstrated in the adult tegument the best studied of which has a M, of approximately 200,000 and has been demonstrated to be exposed following praziquantel treatment so that antibodies to it participate in the subsequent immune dependent killing of the parasite (Brindley et al., 1989; Sauma & Strand, 1990; Pearce et al., 1991). Sm20 is also detectable in the adult worm tegument but Sm38 is not despite the strong cross reaction reported above. This is an example of the complex nature of the cross reaction between proteins exposed on the surface of schistosomula and the adult tegument part of which may be explained by the differential processing of precursor gene products so that cross reactive proteins have different M, s as is discussed further below. Nevertheless, there is a general pattern of cross reaction between the adult tegumental membranes and the schistosomulum surface which is independent of the dominant carbohydrate epitopes and involves antigens not shared with the egg (Simpson et al., 1990).

The genes for several of the membrane associated molecules of adult worm have been cloned. Wright et al. (1990) have recently cloned the gene for Sm23. This protein was identified as one of the principal integral membrane glycoproteins of the parasite. Interestingly, it bears considerable homology with a tumor associated antigen which has a possible role in growth regulation. The antigen is recognized by a monoclonal antibody that binds to the parasite surface (Harn et al., 1985b). The hydrophathy profile of the molecule predicts that it probably has four transmembrane domains that serve to anchor it to the membrane. This makes it very unusual amongst parasite antigens in general which are most commonly anchored to the parasite surface via GPI.

The dominant antigenic component of the adult tegumental membranes is Sm25 which has been shown to be an integral membrane glycoprotein by partitioning in TX-114 (Omer Ali et al., 1991). Smithers and colleagues showed that when tegumental membranes are used to vaccinate CBA/Ca mice protection correlates with the level of IgG antibody to this antigen suggesting that it may be reasonable for the protection stimulated by this method of vaccination (Smithers et al., 1989). It has also been demonstrated that Sm25 is preferentially recognized by the sera from protected but not unprotected rat sera (El-Sherbini et al., 1990). However, unequivocal proof of the protective antigenicity of Sm25 has not yet been produced. The gene for this molecule has also been cloned and has stimulated a fairly detailed analysis of the antigen which is not present in schistosomula but can be identified on the surface of lung stage parasites (Payares et al., 1985; Omer Ali et al., 1591). It has been shown to be a classical membrane protein in that the sequence contains a signal sequence, the cleavage of which is consistent with the final molecular weight of the molecule. In addition, it is demonstrably glycosylated indicating that it is at least topographically external. The molecule bears no homologies with proteins currently in the various data bases. The hydrophathy plot of the molecule reveals a hydrophobic domain at the C-terminal. However, close examination of the sequence of this part of the molecule suggests that it is neither long enough or sufficiently hydrophobic to securely anchor the molecule to the membrane. Work by Pearce and colleagues (Pearce et al., 1991) has shown that a palmitic acid is bound to Sm25 via a thioester bond to a unique cystein residue in the C-terminal hydrophobic domain. This covalent modification is required for stable interaction with the membrane and represents a novel form of post-translational modification for topographically external integral membrane glycoproteins. The significance of this modifications has yet to be ascertained but is of interest that a further integral membrane protein of the tegument, Sm13, although quite distinct from Sm25, has a considerable degree of homology at the C-terminal suggesting that it is also bound to the membrane via a hydropho-
bic region modified by palmitate (R. Allen & A. Simpson, unpublished observations).

In addition to Sm13, the antigenic profile of the adult worm membrane reveals another prominent low Mr antigen of 15K. It has been found that this antigen is the highly processed product of a protein of approximate Mr 200K (Abath et al., manuscript in preparation). Interestingly, although Sm15 is securely anchored to the membrane and behaves as an integral membrane protein, it is a highly negatively charged molecule which contains neither a signal sequence nor a hydrophobic region capable of anchoring it to the membrane. Furthermore, there is no evidence either from biochemical analysis or from the sequence that it has a GPI anchor. Although Sm15 is only detectable from the lung stage onwards the protein expressed from the cloned gene is also recognized by antibodies from mice vaccinated with highly irradiated cercariae as well as antibodies raised against the schistosomula surface where epitopes cross reacting with Sm15 are apparently exposed (Abath et al., manuscript in preparation; Simpson et al., 1990). The solution to this apparent anomaly has come from a series of experiments using antibodies selected against the fusion protein expressed from a partial cDNA of the precursor. Antibodies selected from an anti-adult membrane serum recognize Sm15 as well as a Mr23K molecule which may be soluble. These antigens are detected in worms of more than two weeks of age but are not detectable in schistosomula. Antibodies selected from an anti-schistosomulum surface serum, on the other hand, recognize principally a Mr 18K antigen that is present in cercariae and schistosomula but not adult worms. Thus, the precursor is processed differently in the two stages of the parasite life cycle to produce distinct antigens which exhibit a complex inter relationship and cross reactivity (Abath et al., manuscript in preparation). It may be that a similar situation will account for the cross reaction of Sm38 between the schistosomulum surface and the adult worm tegument where it cannot be detected.

It is clear that the integral membrane proteins are complex molecules that are providing new insights into the complexity of the molecular structure of the schistosome tegument and subtlety of post-translational modification that is involved in the changing antigenic profile of the schistosome surface. The very complexity of these molecules, however, may limit their use in a synthetic vaccine unless simpler analogs are found to stimulate the immune responses that normally result from the exposure of the immune system to the mature proteins.

In summary, the study of antigens associated with the schistosome tegument has produced many surprises, the immunodominance of carbohydrate epitopes and their cross reaction with KLH, the presence of soluble enzymes, a new form of membrane anchor and a developmentally regulated system of differential processing of a high Mr precursor. Such work has also produced some of the major anti-schistosome vaccine candidates as well as a potentially important new diagnostic reagent, KLH. A major focus of future work is likely to be the use of the recombinant molecules to better understand the molecular basis of the parasites ability to dwell within the host’s blood, a more detailed description of the molecular architecture of the parasite tegument as well as the role that surface components play in the complex interaction between the parasite and its natural host, man.

REFERENCES


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