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The cloning of schistosome antigens

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If a vaccine is to be produced for human schistosomiasis it is almost certain that it will be in the form of antigens produced by recombinant micro-organisms cultured in vitro, an infectious recombinant organism containing and expressing schistosome antigen genes or synthetic peptides constructed from knowledge of antigen gene sequences. Thus the cloning of schistosome antigen genes is an integral step in vaccine development. The need for reliance on recombinant DNA technology is essentially twofold; firstly gene cloning will facilitate highly specific and controlled immunological intervention so that only antigens which have a protective value are presented; secondly gene cloning and associated technologies represent the only forseeable means of producing sufficient antigen even for vaccine trials.

Important implications result from a molecular biological approach to vaccine development. For example, as soon as cloned antigen genes are obtained research can rapidly assimilate developments from related disciplines. Thus the progress from cloned gene to recombinant virus and immunogenic peptides can now be very quick. Likewise the techniques for library construction, gene identification and sequencing are becoming rapidly more and more standardized and are now relatively easily undertaken in most laboratories. Thus there will be no shortage of potential antigens generated by gene cloning. Indeed at the recent «Conference of the Molecular Biology of Schistosomes» held under the auspices of the Edna McConnell Clark Foundation in excess of 40 schistosome gene libraries were reported encompassing all the major species that infect man and most life cycle stages [1].

A second important implication of reliance on molecular biology is that, at least in the short term, attention is concentrated on polypeptide antigens and in particular that subset of epitopes defined by a linear sequence of amino acids.

In using gene cloning to investigate schistosome immunology and to develop a vaccine two distinct approaches are being utilized. In the first schistosome antigens are being characterized, the chemical nature of the epitopes ascertained

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and their involvement in immunity established. Thereafter, through the use of specific antisera and amino acid sequence data the cDNA for polypeptide antigens is cloned. In the second approach cDNA clones encoding antigen genes are identified on the basis of their reactivity with broad specificity sera. Using the clone as an immunogen the corresponding mature antigen is identified and its role in immunity investigated. The two approaches are complementary and should eventually provide not only cloned forms of well characterized antigens but may also result in the identification of novel protective immunogens.

In the identification of antigens likely to play a role in protective immunity and thus be useful components of a molecular vaccine most attention has been focused on molecules expressed at and released from the schistosomulum surface. Evidence continues to accumulate that such antigens expressed on newly transformed and developing schistosomula are indeed protective [2, 3]. Analysis of the intact schistosomulum surface by radioimmunoassay using polyclonal and monoclonal antibodies demonstrates two overlapping sets of antigens expressing carbohydrate and polypeptide epitopes [4, 5]. The carbohydrate epitopes predominate and almost certainly play an important role in the development and modulation of immunity since they are recognized by both protective and blocking antibodies. These carbohydrate epitopes all cross react with the schistosome egg and are not species specific. At least some of the epitopes are expressed on glycoproteins of M_r 200, 38 and 17 K which have previously been defined by ^{125}I surface labelling and immunoprecipitation [5–7].

The schistosomulum surface also expresses polypeptide epitopes, however, which although present at much lower densities than the carbohydrate epitopes can also act as targets of protective immune responses [8, 9]. By direct characterization of the schistosomulum surface it has been established that the M_r 38, 32 and 20 K antigens express polypeptide epitopes [4, 5, 10]. These molecules are structurally related [11] and it has been shown that the M_r 38 and 32 K antigens share the same antigenic polypeptide backbone and differ only in two N-linked glycan units [12]. Evidence for linear polypeptide epitopes on these antigens has come from studies with immunization of rabbits with denatured membrane proteins (unpublished data) and more directly from radioimmunoassay using tryptic fragments derived from the M_r 32 and 20 K antigens [13].

In a chronic infection of experimental animals or man, antibody against the polypeptide schistosomulum surface antigens is induced by the adult worm [14, 15]. Indeed the possible importance of the adult worm in inducing protection in human infection is suggested by a positive correlation of anti-adult worm antibody and resistance to reinfection in Gambian patients [16]. The polypeptide antigens appear not to cross react with the egg, however, which is an important consideration for candidate antigens for a vaccine since it avoids any possible potentiation for enhanced pathology [5, 17].

Within the adult schistosome worm the components that induce anti-schistosomulum surface antibody are membrane antigens concentrated in the

tegumental outer membrane [18] but which are probably present at lower densities in plasma membranes throughout the parasite (C. K., unpublished observations). We have investigated the feasibility of vaccination with adult worm antigens by undertaking experiments in mice. For the most part unfractionated antigen has been used but has resulted in a surprisingly limited antibody response as demonstrated by the immunoprecipitation of ^{125}I-labelled surface antigens and cell-free translation products. Nevertheless the polypeptide schistosomulum surface antigens are strongly recognized [17]. Immunization of CBA mice (which exhibit 40–60% immunity when immunized with irradiated cercariae) with whole adult worm or pelleted membrane antigen by subcutaneous injection in the presence of saponin has achieved protection of up to 37% (average 21%) and statistically significant levels of immunity in 11 out of 17 experiments. These immunizations result in a polypeptide dependent antibody recognition of schistosomulum surface as well as delayed and immediate hypersensitivity responses to a cercarial challenge. Importantly neither the recognition of surface antigens nor protection results from immunization with soluble antigen. At least a part of the protective effect of immunization with adult antigens can be attributed to M_r 32 and 20 K polypeptides exposed on schistosomula but the contribution of other polypeptides cannot be excluded. Indeed antigenic analysis of the adult surface membrane reveals 3 major polypeptide antigens of M_r 32, 25 and 20 K together with some other antigens of lower relative molecular mass [18]. The M_r 32 and 20 K antigens appear to be the same as those expressed on schistosomula. The M_r 25 K antigen, however, is not detectable on the exposed surface of 3 h schistosomula but can be labelled weakly on lung stage parasites and 3-week worms. It appears nevertheless as the predominant antigenic component of adult membranes and may play a role in immunity against older forms of the parasite. The three major antigens of M_r 32, 25 and 20 K therefore represent the focus of antigen gene cloning experiments.

In attempting to clone the genes for the major membrane antigens of *Schistosoma mansoni* two approaches have been used. In the first a lambda-gt11 expression library constructed with adult worm DNA was screened with a variety of sera with varying specificities [19]. Monoclonal antibodies raised against the M_r 32 and 20 K antigens as well as a polyclonal antibody raised against the purified 32 K antigen have so far failed to bind to any clones. Several positive recombinants have been identified, however, using rabbit anti-surface membrane antibodies. A set of 12 such clones also bound antibodies from chronically infected mice and mice immunized with highly irradiated cercariae. Cross hybridization demonstrated that these clones contain the same short cDNA insert. This was confirmed by sequencing two clones. The nucleotide sequence did not correspond to amino acid sequence derived from the M_r 32 and 20 K antigens; therefore, to identify the mature antigen to which the cDNA corresponds, lysogenic forms of several of the clones were constructed and used to immunize mice. The immunogenicity of the fusion protein was initially

demonstrated by ELISA against parasite antigen preparations. Immunofluorescence studies of adult worm sections demonstrated that the antigen is concentrated in the tegument. Direct immunoprecipitation of purified surface membrane antigens demonstrated that the epitope contained within the cloned gene was in fact expressed in two antigens the major M_r 25 K polypeptide and a lower M_r antigen of approximately 11 K. The 11 K antigen is recognized by antibodies from both chronically infected mice and mice immunized with irradiated cercariae, whereas the 25 K antigen is only recognized during a chronic infection. The potential for this highly immunogenic peptide to induce protective immunity is currently being investigated in serveal animal models. In addition the clone is being used as a model system for expressing short cDNA fragments as beta-galactosidase fusion proteins in Vaccinia virus in collaboration with Dr. Geoff Smith (Cambridge) as described below.

As well as this now well defined cDNA several other distinct clones expressing membrane associated peptides are being studied but as yet their identity has not been ascertained. In addition further screening of cDNA libraries is currently being undertaken with antibody raised against denatured membrane proteins as well as a set of 3 oligonucleotides constructed from the amino acid sequence derived from the M_r 20 K surface antigen.

In other laboratories similar success has been achieved with the cloning of schistosome antigens and the cloning of surface associated or released antigens has been reported by the groups working in Cleveland, Cambridge, Washington and Lille. As a result cloned forms of antigens thought to have a protective capacity are becoming available.

As discussed above, as well as screening libraries to identify genes encoding defined antigens thought to have a role in immunity, a second possible way of utilizing cloned cDNA as an approach to vaccine production is to screen cDNA libraries for clones which are strongly antigenic with broad specificity sera and to use these as a starting point for investigations. Thus any clone expressing a fusion protein recognized by antibodies from mice immunized with irradiated cercariae, for instance, could be used to raise antibodies and be evaluated as a protective antigen by passive transfer as well as direct immunization experiments. The cDNA library is thus used as a source of "monoclonal antigens" [20] which have the important property of being antigenic in an unprocessed polypeptide form and if found to possess useful immunological properties are immediately available in the form of cloned cDNA for further manipulation. Given the variety of animal models available which appear to have the ability to kill schistosomes at different developmental stages, as well as evidence that both surface and internal antigens can be protective, it is quite possible that this rather empirical protocol may yield important new lines of research and broaden the general approach to immunoprophylaxis.

We are currently pursuing such an approach for *S. mansoni, S. haematobium* (in collaboration with Paul Hagan and Andrew Wilkins, MRC, The Gam-

bia) and *S. japonicum* (in collaboration with Quentin Bickle and Ann Moloney, LSHTM). From some 100 clones originally identified as expressing antigens using both human and animal infection sera, following analysis of antigenicity and cross hybridization 20 are currently thought to encode distinct antigens and are being processed to lysogens, subcloned in other expression and sequencing vectors, where appropriate, and the resulting fusion proteins being utilized for immunization. Thus a variety of cDNA clones are being pursued which have been identified on the basis of their antigenicity but none of which, so far, has been found to encode entire proteins. Since the goal of the work is to investigate the potential of the antigens for protective immunization it would be extremely useful to be able to express the clones in another infectious agent such as Vaccinia. This generally requires the full length gene. A possible solution to this problem is the expression of the cDNA as another fusion protein in the virus. As a first approach to this we are investigating the immunogenic properties of the schistosome cDNA encoding the immunoreactive peptide from the M_r 25 K surface membrane protein cloned into the homologous EcoRI site in a beta-galactosidase gene, since the plasmid suitable for this cloning and subsequent integration into vaccinia was already available [21]. If this construct proves to be immunogenic the way is then open to express any cDNA clone in vaccinia which expresses in lambda-gt11 or the EcoRI site of Pex 2. Future developments in this area will involve the use of other fusion proteins which will direct the surface expression of secretion of the immunogen.

A further extension of the use of cDNA expression libraries is to investigate normally non-antigenic proteins. In this regard we are particularly interested in major schistosomulum surface proteins which are not recognized during chronic infection or following immunization with irradiated cercariae [22, 23]. In addition the possibility of the interaction of the immune responses with parasite development and sexual maturation are being pursued. In this regard the gene for the predominant female specific-polypeptide has been cloned and sequenced [24, 25] and is being used in the study of the control of schistosome maturation in vitro and in vivo. In addition a number of apparently sex-linked polypeptide antigens have been identified.

In summary it can be stated whith some confidence that gene cloning will have an ever increasing role to play in the development of a schistosome vaccine. It is very encouraging that polypeptide antigens have been shown to be protective and some have the important attribute of not cross-reacting with the egg. A variety of cloned and expressed polypeptide antigens are now becoming available from several laboratories, some of which correspond to well defined antigens while others are simply defined as antigenic peptides and may lead to the identification of novel protective antigens. The increasing use of molecular biology merges schistosome research with that into many other areas of infectious disease and vaccine development and already the possibilities of collaborative research involving the production of hybrid vaccines in vaccinia virus, for

example, are becoming widely appreciated. Future reserach will see the ever closer integration of these new, powerful approaches with the many long-term parasitological and clinical investigations being pursued in many laboratories and will result in a very dynamic and exciting period, the beginnings of which we are now experiencing.

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