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Summary and Conclusions

During the last five years, considerable progress has been made in the field of schistosomiasis immunology. Advances have come from the use of several different experimental systems and approaches and convergent views have emerged confirming the existence of human and animal immunity, the mechanisms and the antigens involved. Furthermore, the reproducible induction of protective immunity with attenuated parasites, and more recently with antigenic preparations, has now led workers in the field to agree that vaccination against schistosomiasis is an achievable goal. The evidence that led to this optimistic position was reviewed at a meeting of the Scientific Working Group on Schistosomiasis, held in Geneva 26–28 May 1986, at which the prospects of immunological intervention in human schistosomiasis were discussed.

1. Evidence for acquired immunity in man

A fundamental issue in evaluating the prospects of immunological intervention in human schistosomiasis is whether or not man acquires an immune-dependent resistance to reinfection. To investigate this, field studies on immunity to schistosomes in man that involve simultaneous estimates of the intensity of reinfection after drug treatment, and of the degree of contact with contaminated water are being carried out for *S. haematobium* in the Gambia and for *S. mansoni* in Kenya. In both studies, although intensity of reinfection correlated with levels of water contact, a marked age-dependent resistance to reinfection, distinct from age-dependent changes in exposure was observed which indeed indicates the development of acquired immunity. Progress towards the identification of the immunological parameters which determine the development of protective immunity in man has been achieved. Thus, in the Gambia, reinfection was significantly lower in individuals with high eosinophil counts and high serum titres of antibodies to adult worm antigens, suggesting a role for eosinophils in resistance to reinfection. In the Kenyan study, a positive correla-

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tion was found between intensity of reinfection after treatment and high levels of antibodies recognizing egg glycoconjugates cross-reacting with schistosomulum surface epitopes. Such antibodies include IgM and IgG isotypes which can block the expression of protective immune responses suggesting that the immune response to eggs may play a modulating role in the development of immunity and that resistance results from a complex interaction between protective and modulating factors. Additional studies are being undertaken in Brazil and Egypt, the latter providing preliminary evidence for a role for cell-mediated responses in conferring protection against reinfection.

2. Nature of acquired immunity in experimental hosts

A more detailed description of immune effector mechanisms involved in the development of resistance to schistosome challenge has come from the use of experimental animals, predominantly rats and mice. Resistance to reinfection in these hosts can be induced either by infection with normal cercariae or immunization with attenuated larvae. Recent experimental evidence suggests that common mechanisms may be involved in resistance induced in these two ways and that inflammatory responses may be instrumental in producing protection. Immunization with irradiated cercariae has been the method of choice in most investigations.

Comprehensive studies on inbred strains of mice indicate that delayed-type hypersensitivity, dependent on activated macrophages, is involved in resistance. On the other hand, passive transfer experiments with sera from both mice and rats as well as the use of μ-suppressed mice demonstrate a role for antibody. In addition, a role for granulocytes, in particular eosinophils, macrophages, and platelets has emerged from experimentation in the rat. Levels of species-specific immunity of up to 80% in both rats and mice can now be achieved consistently with irradiated cercariae. Parasite attrition in this model appears to occur predominantly in the lungs of the animals, but there is also strong evidence for parasite killing in the skin. In the guinea pig model, parasite death is also observed after the schistosomes have reached the liver. Thus the exact site of attrition of a challenge infection varies between experimental hosts but the targets are always immature parasites. Likewise, a variety of immune effectors are involved in an orchestrated and synergistic response which results either in direct antibody dependent cell cytotoxicity in the skin or in an inflammatory reaction which impedes the subsequent normal migration of the parasite. As also suggested from the observations on human immunity, blocking antibodies appear to be a factor in the modulation of immunity in experimental animals. Thus a blocking rat IgG_{2c} monoclonal antibody inhibited the in vivo protective role of both monoclonal and polyclonal IgG_{2a} antibodies and mouse IgM antibodies reduced the in vitro cytotoxicity of immune serum.

Significantly high levels of blocking IgM antibodies were found to be present in serum from chronically infected mice (with which consistent passive

transfer of immunity cannot be achieved), but were present in very low levels in the serum of mice immunized with irradiated cercariae.

The search for antigens which mediate protective immunity has concentrated on the exposed surface of young schistosomula. This structure is rich in carbohydrates which are recognized by antibodies from rats and chronically infected mice. Polypeptide epitopes are also detectable and appear to be preferentially recognized by antibodies from mice immunized with irradiated cercariae. The exact identity of these molecules and direct evidence for their role in immunity has been determined by experiments with monoclonal antibodies. Most of these studies have used only *S. mansoni* and the following section refers, therefore, exclusively to this species.

3. Antigens defined by protective monoclonal antibodies

Monoclonal antibodies have been derived from mice and rats exposed to normal and irradiated cercariae or injected with various schistosome stages and fractions. Several hundreds of different varieties of such antibodies with specificity for schistosome components have been obtained and about a dozen of them have been reported to confer partial resistance when passively administered to experimental animals. Retrospectively, the production of protective monoclonal antibodies can be seen as a crucially important step in the understanding of host immunity to schistosome infection. Not only has it enabled protective antigens to be defined, but the passive transfer of monoclonal reagents preceeded, in the mouse model, the reproducible transfer of protection with intact serum, presumably by the separation of protective antibodies from these with blocking specificities. Thus researchers gained increased confidence that resistance in this model is indeed an immunologically based phenomenon.

Antigens recognized by protective monoclonal antibodies, ranging in size between a molecular ratio of 16 kilodaltons (M_r 16 K) and M_r >200 K have been the object of detailed analyses. Carbohydrate-specific antibodies often recognize more than a single molecular species, thus suggesting the sharing of common glycan epitopes by different worm glycoproteins. Monoclonal antibodies of IgM and IgG isotypes in the mouse, and IgE and IgG isotypes in the rat, are capable of inducing protection upon passive transfer. This demonstrates that protection and blocking activity do not exclusively depend on isotype but are presumably also a reflection of the precise epitope being recognized and possibly also of antibody affinity.

The passive transfer of 30–45% resistance has been achieved in the mouse model by monoclonal antibodies which recognize surface antigens of M_r 16 K, 22 K, 28 K, 30 K, 32 K, 38 K, 45 K and >200 K. In the rat, resistance as high as 70% has been reported with antibodies recognizing antigens of M_r 22 K, 26 K, 28 K, 38 K and >200 K. In addition, the passive transfer of specific anti-M_r 28 K T-helper cell lines has led to a high degree (85%) of protection. All the antigens recognized by protective monoclonal antibodies can be shown to be present on

the surface of early schistosomula, irrespective of the method used for the production of the antibody. Some of these antigens recognized by protective monoclonal antibodies developed in different laboratories have been shown to be identical, in particular the M_r 28 K, 38 K and >200 K antigens, and the same specificity has been found to be involved in the protection of mice and rats. Protective monoclonal antibodies have been used as tools for the isolation of "protective antigens". Mice, rats and monkeys injected with these protective antigens have shown various degrees of resistance against a challenge infection with cercariae as described below.

4. Biochemical analysis of protective antigens

In addition to the protective antigens originally defined by monoclonal antibodies and which are expressed on the surface of young schistosomula, other soluble or released antigens coming from the developing schistosomula are also protective. Most of these antigens have, as yet, received little biochemical analysis except in terms of apparent molecular ratio as defined by SDS-PAGE. An exception, however, is a soluble M_r 97 K polypeptide which is capable of inducing high levels of protection in mice when administered together with BCG (see below). The gene for this antigen has been cloned and from the derived sequence it has been deduced that the molecule corresponds to schistosome paramyosin. This is the first occasion that a molecule has been defined by both its contribution to the immunology of the disease and its physiological function and represents an important step in the use of molecular biology in research on schistosomes.

Much effort has been put into the molecular analysis of protective schistosomulum surface antigens and it has been shown that it is a complex structure, exhibiting at least 15 different identified proteins and glycoproteins. Characterization of the intact surface has demonstrated that at least 90% of the exposed epitopes, recognized by antibodies from chronically infected mice and rats, are carbohydrate in nature and the antibodies cross-react with antigens of the schistosome egg. There are nevertheless, also exposed polypeptide epitopes which are preferentially recognized by antibodies from mice immunized with highly irradiated cercariae. These antibodies, in general, do not cross-react with the egg but instead with shared antigens of the surface membrane of the adult worm.

The highly abundant schistosomulum carbohydrates represent important targets of protective responses as demonstrated by the specificities of protective monoclonal antibodies. A number of such antibodies, produced in several laboratories and which are effective in vivo in both rats and mice, all recognize epitopes which are expressed on glycoconjugates of very high molecular ratio (>200 K) and also on a M_r 38 K molecule. It remains to define the epitopes of these related monoclonal antibodies but the available evidence suggests a variety of oligosaccharide side chains. In one case, however, the structure of the

glycan unit recognized has been determined and has been shown to be common not only to other species of schistosomes and other stages of the life cycle but also the the snail host and other aquatic molluscs. It was suggested that the carbohydrate structure plays a role in facilitating aquatic existence and is indeed expressed at very high levels on both the cercarial and miracidial stages of the schistosome life cycle.

Molecules of M_r 20 K, 22 K, 28 K and 32 K have been shown to include protective polypeptide epitopes. The M_r 20 K, 28 K and 32 K antigens are shared with the adult worm whereas the M_r 22 K antigen is specific to the early stages of the life cycle. Amino acid sequence data have been derived from tryptic or CNBr fragments of the M_r 20 K, 28 K and 32 K antigens and antigenic peptides derived from the M_r 20 K and 32 K antigens have been identified. The complete amino acid sequence for one such peptide is available and a corresponding synthetic 13 amino acid peptide was shown to be antigenic and exposed on the schistosomulum surface by selective antibody absorption studies. The gene for the M_r 28 K antigen has been cloned and the full length sequence obtained. This is an important achievement and facilitates the production of large amounts of the protein for vaccination trials and also enables recombinant infectious organisms to be constructed which could potentially be used for a live vaccine. The gene for the M_r 28 K antigen has actually been cloned into *Vaccinia* virus and ways of optimizing the immunogenicity are currently being investigated.

The gene encoding an exposed schistosomulum surface polypeptide which appears to represent the polypeptide backbone of the protective M_r 38 K molecule has been cloned. Antibodies against the gene product, expressed in *E. coli* as a fusion protein with β-galactosidase, bind to the schistosomulum surface demonstrating the availability of the polypeptide epitope to the external environment and its potential use in immunization. The cloning of genes for other surface associated proteins was reported in addition to the cloning of the genes for the M_r 28 K und 38 K antigens, which have been demonstrated by the use of monoclonal antibodies to be protective in both rats and mice. Two groups reported the cloning of developmentally regulated M_r 25 K proteins present in the surface membrane of adult worms. The sequence of the clones suggests that these are distinct molecules but may be exposed on lung stage parasites and thus potentially represent targets of protective immunity. Likewise, the gene encoding an antigenic M_r 85 K polypeptide, thought to be exposed on the schistosomulum surface, has been cloned which may be of value in the eventual immunization with synthetic molecules. The cloning of the gene corresponding to an adult schistosome gut associated molecule of M_r 31 K which has been shown to be of value in immunodiagnosis has also recently been initiated. It was reported at the meeting that more than 40 different *Schistosoma* gene libraries, representing the three major species of schistosome that infect man and most of their life cycle stages, currently exist in different laboratories throughout the world. It

is likely that these will not only provide the genes for all the protective antigens already defined, but also for a variety of novel poly-peptide antigens, the relevance of which to immunization or immunodiagnosis, will be subsequently assessed using the recombinant antigens themselves.

The use of molecular biology enables fundamental aspects of schistosome development to be investigated in this regard several groups have cloned highly expressed developmentally regulated genes which apparently encode the precursor protein for the schistosome eggshell and which are being used for the study of oogenesis. The long-term aim of this work is to identify means of interrupting this process and thus provide an alternative strategy for prophylaxis.

5. Vaccination of experimental animals with defined antigenic preparations

The successful immunization of experimental animals with antigenic preparations, a crucial step in the progress towards the production of a schistosome vaccine, has been achieved with a variety of purified and heterogenerous antigens in both rats and mice. The mouse has historically been the more difficult rodent host to immunize but this is now possible in several ways. Immunization with homogenates of various stages of the life cycle with alum (designed to specifically induce an IgE response), intradermally with BCG (which results in antibody independent T-cell responses) and subcutaneously with saponin (which appears to result in both antibody and delayed-type hypersensitivity responses) have all resulted in levels of protection which are comparable with that which results from immunization with irradiated cercariae. High levels of immunity have also been achieved by the repeated immunization of mice with frozen and thawed schistosomula in the absence of an adjuvant. In the case of immunization with BCG the protective antigens were identified as soluble proteins. Further analysis demonstrated the central importance of the M_r 97 K molecule which was subsequently purified, cloned and identified as paramyosin. Immunization with saponin appeared to require membrane bound antigens and was attributable, at least in part, to the recognition of polypeptide epitopes on the schistosomulum surface.

In the mouse, protection has been reported with the purified M_r 97 K paramyosin molecule, as well as the M_r 22 K and 28 K antigens derived from the schistosomulum surface. Only very small amounts of the latter two antigens could be purified. Nevertheless, immunization in the presence of BCG and Maalox, in the case of the M_r 22 K antigen, or as antigen-antibody complexes bound to protein-A sepharose beads in the case of the M_r 28 K antigen, resulted in 27% and 38% resistance, respectively, demonstrating the protective capacity of the molecules. In addition, immunization using the M_r 28 K antigen with aluminium hydroxide resulted in 40% protection. Antigens recognized by the protective monoclonal antibody, specific for the carbohydrate epitopes of the M_r 38 K and >200 K molecules, were isolated from the egg and, following

intradermal and intramuscular immunization and boosting with BCG and Maalox, resulted in 37% immunity. Other studies, not presented at the meeting, have also reported immunization of mice with antigens purified with monoclonal antibodies which bind to the schistosomulum surface resulting in protection in the range of 20–30%. The same M_r 28 K antigen which produced 40% protection in mice resulted in protection of up to 70% in rats using either complete Freund's adjuvant or aluminium hydroxide and a mixture of M_r 22 K and 26 K antigens, released from young schistosomula in culture, produce up to 89% protection in the rat in the absence of adjuvant. One experiment has been published in which cynomolgus monkeys were immunized with a M_r 155 K antigen, isolated with the aid of a protective monoclonal antibody, resulting in a 30% reduction in worm burdens. These immunization studies, while not producing the levels of resistance that would be required of a human vaccine, do demonstrate the protective potential of individually defined antigens.

In the rat, immunization with epitopes recognized by the protective anti-M_r >200 K and 28 K antibody has been achieved using an anti-idiotype antibody raised against the monoclonal antibody which produced up to 76% protection. This demonstrates that a polypeptide, in this case in the form of the variable region of an antibody, can mimic a carbohydrate epitope which may point the way forward for the production of these epitopes in a synthetic form. Indeed, the experimental synthesis of antigens is a vital step since it is necessary to overcome the problem of shortage of antigenic material derived from the parasite itself. In this regard, the immunogenicity of peptides and a number of antigens, produced by cloned genes as discussed above, have been demonstrated by the induction of antibody in experimental animals. The next step is the demonstration of induction of protection with the synthetic antigens either produced in vitro or synthesized in vivo within a recombinant infectious organism such as Vaccinia virus or an attenuated *Salmonella* species.

6. Perspectives and recommendations

The results achieved in the last few years can be viewed as solid ground for optimism with respect to the feasibility of an antischistosomal vaccine. It is particularly encouraging that a consistent and coherent view of immunity in schistosomiasis and its stimulation is emerging from a number of different laboratories working with different experimental hosts. It is also beginning to be possible to compare results and conclusions obtained in studies of man and experimental animals and again a great deal of consistency is evident. This is both intellectually satisfying and important in that it reinforces the relevance of animal models. Much further progress is needed. The most obvious limitations of the work to date is the incomplete degree of protection which has been achieved in experimental animals. The areas of future research, most likely to contribute to the production of an antischistosomal vaccine for use in man, were reviewed at the meeting and are summarized below.

1. Studies of animal and human immunity. – a) Studies of immune effector mechanisms in experimental animals. Studies on the basic mechanisms of schistosome immunity have represented the backbone of the progress achieved so far in the field. Such studies should continue to be pursued with special attention to the immune effectors involved in protection, to the parasite stages which are targets of immune attrition and to the important area of isotype regulation and blocking responses. Antigen associations should be explored not only with respect to the epitope involved but also with respect to possibly different effector mechanisms elicited and different (possibly sequential) target stages. A great deal of work remains to be done in the area of T-cell recognition of antigens and the role of lymphokines and other mediators in the regulation of immunity need to be further elucidated. In addition, the use of outbred experimental animals must be considered and, if possible, a re-evaluation of primate models should take place. Finally, since there are indications of a positive interaction between chemotherapy and the immune response, this approach should also be explored.

b) Analysis of human resistance. More attention should be given to mechanisms operating in human resistance. Further studies are needed to assess the relative importance of immunological responses versus behavioural or genetic factors in determining resistance to reinfection in man. Numerous factors such as age, intensity of previous exposures, cross-exposure to other species, nutritional factors, chemotherapy and possible MHC influences, should continue to be investigated for their possible effects. In order to carry out these studies, long-term field projects are necessary and it is possible that such projects could constitute an addition to existing control programmes in endemic areas. Finally, the problem of how to evaluate the efficacy of a future vaccine should begin to be tackled. In this respect separate immunological markers for immunity and infection must be identified and the means to rapidly identify different strains and populations of the same species of parasite improved.

c) Interaction of animal and human studies. Recent steps to assess the importance in human immunity of particular antigens and effector mechanisms, which have been well defined in animal models, must continue. Studies which combine human and animal derived antibodies and cells for antigen analysis are of importance and the study of human immune factors such as antibodies and cells in experimental systems may be a fruitful approach.

2. Improvement of vaccine efficiency. – a) Antigen idenficiation and analysis. It is desirable that an increased number of schistosome antigens be analyzed for their ability to stimulate protective immunity. Protective antigens should be fully characterized, both in terms of amino acid sequence and carbohydrate structure, where applicable. The epitopes involved in protection should be defined as closely as possible and epitopes inducing protective and suppressive effects dissected. To do this, further production of monoclonal antibodies and T-cell clones should be encouraged as well as the synthesis of overlapping sets of

peptides. Increasing use should be made of predictive computer technology for the identification of likely epitopes. Exchange of reagents and information is likely to help in recognizing overlaps in the antigens identified and is to be encouraged. The question of whether it is desirable or safe to use egg antigens in vaccination must be seriously addressed. In addition, the related topic of vaccination which achieves a reduction in morbidity as well as parasite burden should be considered. In this respect, the male-dependent female schistosome maturation and subsequent egg production represents an, as yet, unexploited target of immune intervention and basic molecular biological and biochemical studies aimed at a fundamental understanding of these events are of potential importance.

b) Antigen synthesis. The use of products from cloned genes as immunogens in protective experimental vaccination is an essential step in further progress foreseeable in the near future. Carbohydrate epitopes which cannot be obtained in sufficient quantity from the parasite life cycle may conceivably be obtained by gene expression in eukaryotic cells or they might be substituted by anti-idiotype antibodies. This approach has already been successful in producing protection and should be investigated further.

The chemical synthesis of polypeptide structures mimicking the configuration of carbohydrate epitopes ("mimeotopes") is an interesting novel approach which should be exploited. Serious consideration must be given to the use of combinations of antigens as a means to increase vaccine efficiency, particularly with regard to antigens that may lead to immune killing later in the life cycle. The engineering of synthetic molecules containing multiple epitopes from diverse sources possibly directly linked to carriers with adjuvant effects must also be considered.

c) Antigen presentation. Apart from the nature of the antigen(s) employed in vaccination, the method of antigen presentation is of great significance. In this respect, the physical state of the antigen should be considered as well as its association with adjuvants and with other molecules, as for example in the so called immunostimulating complexes (ISCOMs). The site of antigen application is also a decisive factor in orienting the immune response towards the humoral or the cellular effector mechanisms and this should be systematically explored. Hybrid molecules obtained by expression of genes in foreign organisms represent interesting new ways of antigen presentation and the approach of gene incorporation into *Vaccinia* virus is an important example of the new chapter to be explored for schistosome immunogens. Both this and other systems for presentation of antigens within a heterologous infection should be thoroughly exploited. As the immune response against the same antigen has been shown to present wide variations in different animal species, a range of experimental models must be tested. In particular, vaccine trials in primate hosts must be undertaken in the medium term. In addition, the age of the host should be taken into consideration in view of the potential administration of the vaccine to

newborns or infants and, at this stage, studies on the duration of protection achieved by vaccination should be initiated.

3. Studies of S. haematobium and S. japonicum. – There is a very urgent need to encourage parallel work with *S. haematobium* and *S. japonicum* to that being undertaken with *S. mansoni.* In this regard gene cloning approaches which circumvent the need for a continuous life cycle for antigen analysis should be exploited.

7. Summary

The general consensus of opinion expressed at the meeting was that the recent rapid advances in the field of schistosomiasis and the effort which has been put into the precise identification of means and sites of parasite killing have resolved many contentious issues and led to a coherent view of worm attrition. Moreover, the diversity of methods, and indeed antigens, now successfully used to immunize experimental animals, has led to real optimism that acceptable levels of protection may be achieved by the combination of these approaches and that the extension of these achievements to man is a realistic goal. In this respect, the biological characteristics of the infection, in particular the lack of reproduction within the definitive host coupled with the apparent lack of antigen variation of diversity, make the production of an effective vaccine all the more likely. It is likely that the expanded use of the new technologies, which have already been to a large extent assimilated by the field, will contribute to reaching these goals in the near future.