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Schistosomiasis research and the European Community

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Schistosomiasis research within the framework of the Commission of the European Communities 'Science and Technology for Development' (CEC/STD) Programme is targeted at three specific problems: diagnosis of infection and disease; the dynamics of transmission, immunity, and morbidity; and the need for improved tools and strategies for control. Several important advances have been made over the past decade. Improved methods of diagnosis by detection of circulating antigens are in an advanced stage of development and have already undergone field trials in several epidemiological settings. Treatment and reinfection studies combined with immunological observations have allowed the elucidation of possible mechanisms leading to acquired resistance, and have shown that repeated chemotherapy with praziquantel can substantially reduce morbidity. Other projects have studied the epidemiological and ecological determinants of transmission, infection and disease in various endemic situations and also in newly established, epidemic foci where remarkable observations on chemotherapeutic responses were made. Important advances have been made towards the development of a vaccine. The glutathione-S-transferases of the major species of schistosomes have been cloned, sequenced and expressed, and their biological function studied. In a variety of vaccine formulations and animal systems GST has been able to confer protection against infection and to reduce worm fecundity. GST and a series of other crude and defined antigens have been evaluated with varying results in Schistosoma japonicum *and* S. bovis *in cattle. Much work has yet been done, however. Recommendations as to possible future directions for research are provided.*

Key words: schistosomiasis, research, control, vaccination, policies

Studies on schistosomiasis within the Health Area of the Science and Technology for Development (STD) Programme of the Commission of the European Communities (CEC) target several objectives: improving diagnosis of infection and disease; elucidating the complex dynamics of transmission, immunity, morbidity, and control; and developing new tools for prevention and control, in particular vaccines. The increased pressure on the funding of science, and on tropical disease research in particular, has necessitated a review of how diminishing resources might be used to best effect. As a consequence, the EC have adopted a network approach for health research, with several main functional aims:

1 To facilitate communication among researchers in endemic and non-endemic areas in order to strengthen the capacity to conduct multidisciplinary bench and field research.

2 To transfer, through this linkage, knowledge and skills in both directions.

3 To foster the links between researchers and national disease control programmes.

4 To disseminate information through the network on new advances in field and laboratory research, and where appropriate to standardize research methodologies.

5 To promote further field and laboratory studies by appropriate expansion of the network to additional researchers, institutions, and countries.

The progress which has been made by the CEC/STD Network on Schistosomiasis can be judged from the reports of its IIIrd meeting in Noordwijk, The Netherlands, in September 1993, which are contained within this volume.

FUNDING FOR SCHISTOSOMIASIS RESEARCH

The contribution of the CEC to schistosomiasis research started a decade ago and came at a very opportune time. With other funding agencies modifying their inputs to tropical disease research, promising lines of research were threatened with suspension or even discontinuation. Increasing demands have been, and still are, being made on the limited resources of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR). The recent changes in the structure of WHO/TDR will also have an impact on schistosomiasis research, as its objectives for applied field research in particular have become less disease-specific, and more oriented towards directly applicable results. The Edna McConnel Clark Foundation's input to schistosomiasis research has been gradually reduced and is now

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restricted to the Strategic Plan for Vaccine Development, administered by WHO/TDR. The EC member states, through a variety of national agencies, continue to fund research on schistosomiasis but their commitment has been tested during this period of economic recession. As with WHO and EMCF, national programmes may involve bilateral agreements between laboratories in developed and developing countries, but as field work can be relatively expensive and little visible in the home country, it is an easy target for reduction when funds are restricted. Paradoxically these changes were coincident with advances, described below, which have brought a new impetus to schistosomiasis research and renewed hope that some intractable problems might be resolved. The CEC/STD programme, through its continued and increasing support, has made a major impact on research on schistosomiasis, but much remains to be done. A move towards coordination of the efforts of all involved supporting agencies, inside and outside the EC, would be one way of building on the progress made to date.

THIS REPORT

This report attempts to summarize the progress which has been made as a result of the CEC-input into schistosomiasis research in recent years, and to set this within the context of the progress reported by other researchers. It concentrates on three main, overlapping areas considered as priorities for research: diagnosis of infection and morbidity; (immuno-) epidemiology and control; and vaccine development. The progress made in these fields will be summarized, with some overlap, in the following sections.

DIAGNOSIS OF INFECTION

Although standard parasitological techniques are adequate for many purposes, improved and alternative diagnostic methods are required to screen populations in areas where health services have limited recources, and to assess the effectiveness of control programmes, particularly where infections are being reduced to low levels of intensity. Furthermore, more accurate methods for worm burden assessment are necessary to study the population dynamics of schistosomes and eventually to evaluate vaccine trials. Research topics on diagnosis within the EC/STD schistosomiasis programme include immunodiagnosis through the detection of specific antibodies and of circulating antigens, and assessment of morbidity by ultrasound.

Antibody detection assays are often sensitive, but lack specificity and quantitative power, and fail to distinguish past from present infection. Recent reports indicate, however, that an ELISA using partially purified *Schistosoma mansoni* egg antigen, designated CEF6, gave antibody responses which correlated well with egg counts in young children and could be useful in detecting new infections.[1] CEF6 ELISA was also found to be relatively effective in detecting the conversion from the infected to the uninfected state after treatment. Additional testing of this assay is required but it is restricted by the need to purify the native antigens from schistosome eggs. Crude soluble egg antigens for immunodiagnosis tend to give less satisfactory results than more defined antigen preparations.[2] The production of recombinant molecules may resolve this problem. No such difficulties occur with keyhole limpet haemocyanin (KLH), a commercially available antigen derived from the keyhole limpet, which is antigenically cross reactive with schistosomes.[3] KLH has been exploited in ELISA for differentiating patients with acute schistosomiasis mansoni in Egypt and Brazil.[4,5] Other antigens such as Sm21/22 and SmE16 may also become of value in immunodiagnosis by antibody detection.[6]

Despite some successes of antibody detection assays, the detection of circulating antigens currently has the edge on other methods. Monoclonal antibodies against several circulating antigens have been produced, of which the most promising and extensively studied are circulating anodic antigen (CAA) and circulating cathodic antigen (CCA).[7] These can be applied in sensitive, relatively simple assays such as ELISA. A CCA-assay has been developed for urine specimens, also in *S. mansoni* infection. Such a non-invasive assay would have the advantage that population screening would become much more feasible, and that patient compliance could be more easily obtained, especially during repeated surveys in control programmes. The circulating antigen assays have also opened up new opportunities for epidemiological investigations. Comparison of circulating antigen levels with egg output may allow the relationship between worm burden and egg output to be studied more closely.[7,8] For example, the assays could be of value in determining whether the human immune response exerts the sort of anti-fecundity effects on adult worms which have been observed in animal models of infection. Their value for vaccine evaluation would thus be quite important.

Serum detection of CAA by ELISA has now been extended to *S. japonicum*.[9] Particularly in China, where parasitological screening is considered a major problem in the control programme, a sensitive, field applicable antigen detection assay is keenly anticipated. Preliminary trials have been reported with parallel detection of serum CAA and urine CCA in Egyptian *S. mansoni* patients with sensitivity reaching 98%.[10] False-positive results by CAA ELISA in non-endemic controls have so far not been observed.[7] Positive results with this assay in individuals with negative egg counts, were therefore interpreted as evidence that the assay detected infections where adult worms were present but eggs were not being produced at detectable levels. One of the next important steps is the development of a simplified format which can readily be applied in field conditions. In the future, a combination of

different antigen detection assays, perhaps even combined with antibody assays, may be used in a single 'dip-stick' test kit. Also a magnetic bead antigen capture ELISA, for which it was not necessary to pre-treat samples with TCA, has been used successfully.[11,12] Further developments of circulating antigen detection assays, which may include the candidate vaccine molecule Sm28GST, are described in this volume.[7,13]

MORBIDITY

Since many control programmes are now geared towards reducing the disease rather than preventing infection, monitoring the levels of morbidity has become as important as diagnosing infection. Surprisingly little is known about the dynamics and importance of morbidity associated with schistosome infection, and the impact of treatment on disease. There is evidence of an association between egg counts and morbidity, but strong correlations have not been reported from all studies. This may be a consequence of single egg counts being unreliable indicators of worm burdens, and of the time lag between (heavy) infection and the development of severe, chronic pathology.[14] Furthermore, simple approaches to the assessment of morbidity, such as hepatomegaly in *S. mansoni* infection may not be good indicators of functional disease. In the case of *S. haematobium* infection, urinalysis reagent strips for the detection of haematuria and proteinuria have been used extensively, but it is also not clear to what extent these results reflect significant morbidity.[15]

An important advance in the diagnosis and evaluation of disease is the application of ultrasound technology.[16] Modern, field-applicable ultrasound techniques allow an objective assessment of community-based morbidity, its relationship with prevalence and intensity of infection, and its regression after treatment. It should now be possible to mount a series of cross-sectional and longitudinal studies on age-related infection and morbidity patterns, and to compare these between different endemic areas. Geographical differences in morbidity, sometimes on a very focal level, have indeed come under increasing scrutiny in recent years, from researchers in a variety of disciplines.[14,17] It is not yet fully known whether the differences result from variations in transmission dynamics, genetic, immunological or parasite-related factors, or the interaction with other infections such as malaria.[14,17]

As the use and interpretation of ultrasound requires trained personnel, and as even portable apparatus may be prohibitively expensive in most endemic settings, the search for simpler ways of determining morbidity and monitoring the effects of control interventions continues. A novel approach to the assessment of *S. haematobium* morbidity, based on the eosinophil response to the eggs in the bladder wall, examines the potential of eosinophil cationic protein (ECP) in urine as an indicator of disease, and is reported upon in this issue.[18]

TREATMENT

Whilst oxamniquine for *S. mansoni* and metrifonate for *S. haematobium* have been available for some time, the most significant advance in the treatment of schistosomiasis has been the development of praziquantel, now in widespread use for over ten years.[19] It has proved to be safe and effective, but its cost, though decreasing, remains an obstacle in the design and implementation of treatment-based control programmes. Reinfection after chemotherapy, a problem common to all drugs, remains a second important drawback of this strategy. Nevertheless, this modern approach to the control of schistosomiasis is supported by several demonstrations that pathology can be prevented by intermittent drug treatments.[14,19–21] On the other hand, there has been at least one report on *S. japonicum* in the People's Republic of China, in which repeated treatment was not effective in preventing morbidity.[22]

Results of chemotherapy have also led to a revision of ideas with regard to the fibrolytic process resulting in collagen degradation in lesions which were so far considered irreversible.[23] Schistosomiasis in experimental animals and in human patients has thus provided a valuable model for the study of fibrogenesis and fibrolysis.

Within the STD and other CEC-supported programmes the effectiveness of praziquantel for control purposes is being monitored in different epidemiological situations.[8,24,25] Factors which are being monitored include compliance, treatment failures, the effect of chemotherapy on morbidity and on transmission. Eventually, it is hoped not only to make recommendations for optimal treatment schedules in particular endemic and epidemic situations, but also how to best apply this strategy in a sustainable way through regular health services. For the interpretation of such complex data sets, mathematical modelling of the epidemiology, transmission and morbidity are required and several approaches to this application are being investigated.[26,27]

Preliminary reports on the use of praziquantel in a new, epidemic focus in Senegal indicate lower efficacy and more side-effects than in other endemic areas.[8] These findings may be due to extremely intense transmission; also, the recently infected population may still lack the immune reactions which act synergetically with praziquantel, and which remove antigens released from drug-damaged parasites. However, reduced susceptibility of the local parasite strain to praziquantel cannot be excluded and is a sufficiently worrying prospect to warrant further investigations.

(IMMUNO-)EPIDEMIOLOGY

Work on *S. haematobium* in The Gambia, and *S. mansoni* in Brazil and Kenya, has provided evidence of an association between the specific IgE response to the parasite and resistance to reinfection.[28–30] The relationship between IgE and schistosome infection in animals has been known for many

years, and suggested that the IgE response, usually associated with harmful allergic reactivities, might have evolved as a specific immune defence against helminth infection.[31,32] The human field studies provided evidence that specific IgG4 antibodies may interfere with the potentially protective effects of the IgE antibodies, delaying the development of resistance to infection.[28,33,34] Also IgM and IgG2 were shown to act as such blocking antibodies.[35]

A 22KDa antigen present in the tegument of adult worms has been identified as one of the principal targets for IgE antibodies in the serum of individuals infected with *S. mansoni*.[30] Reinfection was less frequent and less intense in those with the anti-22KDa IgE antibodies than in those without. Though this association cannot be taken as definitive evidence of a causal relationship, there is now strong support for the role of specific IgE antibodies in protection against schistosome infection, and for further investigating the 22KDa as a potential vaccine candidate.[30]

Ongoing or planned investigations in recently exposed, naive populations may shed light on the dynamics of resistance as they will allow the study of epidemiological patterns and human immune responses without interference of age effects and past histories of exposure.[7,24]

That such studies are possible is a sad reflection of the continued influence that agricultural and industrial development programmes have on the distribution of schistosomiasis, leading to the emergence of new foci. The CEC/STD network can contribute to solving some of the problems posed by the dichotomy between economic development and health. EC scientists have been working closely with Senegalese public health officials in their attempts to investigate an epidemic of *S. mansoni*.[8] Apart from the important information on transmission dynamics, immunology and morbidity that can be gained from studies of such a naive population, this research should also help to develop a suitable control programme, with which research interests should not conflict.[36]

Of great importance in the study of the interaction between environment and health remains the investigations on host-parasite relationships. Recent advances in molecular biology have provided exciting new tools for addressing old problems, such as the focal distribution of certain schistosome species and strains and intermediate hosts. Interesting work on this subject has been initiated in Senegal, Mali and Zambia.[37] Obviously, new studies on these issues can lead to improved understanding of transmission patterns, and possibly to strategies for avoiding the potential adverse impact of environmental changes. Thus, a new impetus has been given to the fields of applied malacology and parasitology, areas which have been starved of resources in the past decade and in which available expertise has reached critically low levels.

The information gained from epidemiological and control interventions may be particularly valuable for improving existing mathematical models and vice versa; a network of researchers can provide data from diverse epidemiological situations for the development of more robust models, which can then serve to evaluate and improve control strategies. Such collaboration between epidemiologists and modellers have contributed to solving the problem of the day to day variations in egg counts in stool samples, and the statistical estimation of 'true' prevalences of infection in communities.[38–40] The recent advances in epidemiology and immunology are being applied to develop and fit comprehensive models, which may become very useful to test scientific hypothesis, identify knowledge gaps and predict the outcome of alternative control strategies.[26,27]

VACCINE DEVELOPMENT

Among disparate scientific disciplines, the EC programme has fostered an awareness that an integrated coherent research strategy is essential to resolve many of the problems in schistosomiasis. Researchers in immunology, molecular biology and vaccine development are now much more aware that investigations on transmission, pathology and diagnosis also remain important. On the other hand, current control strategies, however succesful in reducing morbidity, are in the longer term likely to be insufficient to control infection and transmission. There is no room for complacency; 76 countries are now endemic for schistosomiasis, two more than in 1984, and new foci related to water resources development continue to arise.[19] Clearly, adequate primary health care, water supply, sanitation and health education remain the pillars of long-term control. However, the necessary socio-economic development to fully implement such strategies will not come soon in many endemic countries. Therefore, the development of new preventive tools, in particular vaccination, remains of the highest priority.

Significant progress in schistosomiasis vaccine development has been reported from the group in Lille. Their work has centered on an enzyme of *Schistosoma mansoni*, identified as a glutathione-S-transferase (GST), for which the encoding gene has now been isolated, cloned and sequenced.[41] Recombinant DNA technology has enabled the insertion of the parasite gene into *Escherichia coli*, and the production of quantities of GST. The exact metabolic role of the enzyme is yet to be established; in infected animals, monoclonal antibodies against the GST reduce worm fecundity and egg viability. Studies in laboratory animals have shown that an effective immune response can be stimulated against both native or recombinant GST. The immunization procedure induces high levels of specific IgE, IgA and IgG. Fewer parasites from a challenge infection reach maturity in animals vaccinated with GST; those that survive are stunted and their fecundity is reduced.[13] Subsequent studies have been geared towards the identification of the immunogenic fragments of

the molecule. In experiments with synthetic peptides in mice, immunization with the carboxyl terminus of the molecule decreased challenge worm recoveries, egg production and hatchability.[42,43] Interest in GST as a vaccine candidate has further been fostered by evidence that the molecule is recognized by residents of an endemic area of Kenya, and that their immune response might be associated with susceptibility or resistance to infection.[44] In particular, the development of the IgA antibody response to recombinant Sm28GST and to one of the synthetic peptides was found to parallel the development of resistance to reinfection. IgA antibodies from patients were also found to neutralize the enzymatic activity of GST. Studies on the structural and functional relationships of GST will be aided by the availability of the recombinant molecule in a form suitable for use in x-ray crystallography.[45] The genes for GST from *S. haematobium*, *S. bovis* and *S. japonicum* have also been cloned, sequenced and expressed.[46] The vector pGEX which capitalizes on the high levels of expression of *S. japonicum* GST, allows the expression of the proteins from incorporated DNA sequences as fusions. Peptides of interest can now be cleaved from the GST by a simple one-step procedure.[47]

Outwith the EC programme several other schistosome molecules which also are considered to have vaccine potential are being investigated. Triose-phosphate isomerase (TPI), another parasite enzyme, and Paramyosin have been studied extensively.[48–50] Monoclonal antibodies against TPI interfere with the catalytic activity of the enzyme and induce up to 40% protection in mice. TPI has now been cloned, sequenced and expressed in a functional form.[51]

The 37kDa antigen of *S. mansoni*, identified as glyceraldehyde–3–p-dehydrogenase has been identified as another potential vaccine candidate. Earlier work with this antigen demonstrated that recognition of the molecule by IgG in the serum of Brazilians was associated with resistance to reinfection.[52,53] An integral membrane protein of *S. mansoni* Sm23 has been identified, cloned, sequenced and expressed and may have potential as a vaccine candidate.[54] Preliminary studies using the recombinant antigen and synthetic peptides indicate that parts of this molecule are highly immunogenic and are identified by serum from all strains of mice which have been tested. The antigen is present in various stages of the parasite but of particular importance may be its presence on the surface of lung stage worms, important targets of effector responses in mice. Other parasite antigens, such as rIrV–5, a cloned surface protein, and the polypeptide Sm14 have also been reported to have immunogenic potential.[55,56] Sm14, Sm23, Sm28 (GST), TPI, Paramyosin, and rIrV–5 have now been identified by a World Health Organization (WHO) committee on Strategies for the Development of a Schistosomiasis Vaccine as having the potential for use in humans, and are currently undergoing independent testing in mice.

Recently, it was shown that IgA antibodies against *S. mansoni* in rats prevent maturation of parasites and have an effect on female worm fecundity.[57] IgA may act directly on one or more of the components, perhaps enzymes, which are critical for the synthesis of eggs, or through other effector mechanisms not yet known. This would open up the possibility of oral immunization against schistosomes, which would not protect from (re-)infection, but reduce egg deposition and thus pathology, while allowing continued immunological boosting by incoming larval parasites. Whether vaccine-induced reduction in worm fecundity would also alter the level of transmission is more speculative. GST and a series of other crude and defined antigens have been evaluated with varying results in *S. japonicum* and *S. bovis* in cattle.[58] Besides providing a valuable model for vaccination in human schistosomiasis, particularly for schistosomiasis japonica and for studies of anti-fecundity effects, these investigations may lead to an animal vaccine which would strengthen the economic basis of vaccine development and help to control transmission from animal reservoirs.

CELLULAR IMMUNE RESPONSES

Over the last thirty years cellular immune responses in human schistosomiasis have received much attention but there have been few studies of their relation with resistance to reinfection. In a recent study cell-mediated immunity was examined in 50 Brazilian residents of an area endemic for *S. mansoni*, considered to have similar exposure to infection.[59] Although they all responded to non-specific immune stimulators PPD, Tetanus toxoid and PWM, 56% did not make a lymphoproliferative response to *S. mansoni* antigen. An inverse correlation was recorded between lymphocyte proliferation and infection intensity. When stimulated with *S. mansoni* antigens the interferon-gamma responses of peripheral blood cells was impaired whereas responses to PHA were unimpaired. This work offers support for the view that resistance to infection in people is associated with Th2- rather than Th1-type responses.

Experiments in animals have provided many of the basic ideas on which studies of human immunity to schistosome infection have been based. However, there is an increasing awareness that results from animal models may not precisely mirror the human situation. This is best illustrated by the lively debate on the relative roles of Interleukins during schistosome infection. Murine studies indicate that interferon-gamma, a product of the Th1 subset of T lymphocytes may be important for the development of immunity.[60,61] This immunity appears to be linked to schistosome-reactive T cells which are recruited to participate in focal $CD4^+$ rich pulmonary cellular responses. Following immunization with irradiation attenuated schistosomula, schistosome-reactive T cells are recruited to the lungs, where they persist.[62,63] The rapid secondary

response to challenge infection in vaccinated mice is not associated with the enhanced recruitment of cells and appears to be linked to this persistence of reactive T cells from the immunization. The precise mechanism which results in parasite loss in the lungs has yet to be established; it has been postulated that the inflammatory foci force parasites to leave blood vessels and enter the alveolar space where they are unable to survive.[64] Interestingly, variations in the level of irradiation used to attenuate the immunizing infections indicate that the irradiation doses which give the best protection are those which lead to the production of interferon-gamma.[65] In contrast, the pathology of infection in mice is associated with $CD4^+$ responses classified as Th2. The eggs of *S. mansoni* are a potent stimulus of Th2 and cells of this type can be readily identified in the lymph nodes draining sites of egg deposition.[66] The influence of *S. mansoni* infection on immune responses is powerful enough to reduce Th1 responses and to elevate Th2 responses to an unrelated antigen, sperm whale myoglobin, and to delay the clearance of vaccinia virus by up to three weeks in infected animals, an effect thought to be due to the reduction of $CD8^+$ cytotoxic T lymphocytes.[67,68] The egg antigens which induce granuloma formation have been investigated with SEA-fraction coupled beads.[69] In acutely infected mice seven fractions of egg antigens ranging from less than 21kDa, to fractions greater than 200kDa, all induced granuloma formation. In chronically infected mice granuloma formation was restricted to four antigen fractions, all of which had molecular masses greater than 66kDa. Differential cytokine production in response to different antigen fractions was reported with the 32kDa fraction inducing mainly interferon-gamma and IL–2 production, and the 35 and 38kDa antigens inducing gamma interferon and IL–4. Although human T cells have yet to be classified in quite the same way as those from mice, the results from studies in The Gambia, Kenya and Brazil all support the view that human immunity to *S. haematobium* and *S. mansoni* infection may be dependent upon interleukins, produced by lymphocytes classified as Th2. Perhaps Th1 responses may provide protection by alternative mechanisms, not normally activated in a naturally infected host.[70] One implication of the work on cellular responses is that the target antigens of protective immunity in immunized animals are present in the lung stage parasites. Attempts are now being made to identify these antigens.

Differences in the immune responses of different mouse strains to vaccination with radiation attenuated parasites have also been exploited in an attempt to identify relevant antigens. Most notably a comparison of moderate/low responder CBA/J mice with high responder C57BL/6J mice revealed similarities in recognition of three antigens GST, an integral membrane protein Sm23 and cathepsin B. Moderate/low responder mice also recognized Sm32 and paramyosin but failed to recognize the 70kDa heat shock protein which was strongly recognized by the C57BL/6J mice.[71]

Important advances have recently been made by the use of severe combined immuno-deficient (SCID) mice in studies of *S. mansoni*.[72] If human cells are transplanted into SCID-mice, then SCID-hu mice are created with a functional 'human' immune system. By reconstituting SCID mice with spleen cells from infected major histocompatibility complex (MHC)-compatible BALB/c mice, granuloma formation can be restored.[73] Since schistosome eggs are a stimulus for the activation of murine, Th–2 class, $CD4^+$ helper cells which produce a characteristic cytokine profile, the role of these cytokines in granuloma formation could be studied. It was demonstrated that the injection of SCID mice with purified recombinant TNFα alone led to a dose-dependent formation of granulomas around schistosome eggs. Crude supernatants of Th–2 cells preabsorbed with monoclonal antibody specific for TNFα lost this ability. Treatment of SCID mice with TNFα produced a dose-dependent increase of egg recovery. A dose-dependent, direct effect of TNFα on worm fecundity was shown by incubation of female worms in the presence of TNFα *in vitro*, whilst worm growth, survival and motility was unaffected. Since egg laying by adult female worms is stimulated by TNFα and the granulomatous response may assist the movement of eggs through the tissues, this led to the hypothesis that the host response induces parasite replication and transmission, and that TNFα may play a role in concomitant immunity and perhaps also in parasite migration to the definitive site in the mammalian host.[74] TNFα may also play a role in schistosomiasis related oncogenesis.[75]

CONCLUSIONS

Based on the progress of the above reported research, the participants at the IIIrd CEC/STD schistosomiasis meeting in Noordwijk/Leiden drew up a list of conclusions and recommendations. These can be summarized under the following headings.

1 The dynamics of the host-parasite relationship

It is still not yet fully understood which mechanisms determine the relation between parasite and host populations in various endemic and epidemic situations. On one hand, immunological, genetic and possibly neuro-endocrinological factors have been found to determine resistance or susceptibility to infection, but clearly intensity of transmission and of exposure to infection are also important factors. Obviously, rational choices in control and research strategies (snail control or vaccines?) cannot really be made until the contribution of the various factors and mechanisms has been more properly quantified. Further field-based studies on immuno-epidemiology, transmission and exposure in various situ-

ations, including epidemic foci, are therefore of the highest relevance.

Studies of exposure to infection are costly, labour-intensive and methodologically difficult; varying field methods can deeply affect epidemiological results; the interpretation of immunological responses also depends on applied assays and reagents. Standardization of the methods and protocols used by different research teams would simplify comparisons and increase confidence in conclusions.

Mathematical modelling of the (immuno-)epidemiology, transmission and control of infection would be highly useful in testing scientific hypothesis against field data, and to identify gaps in our current knowledge. They may also be of use in investigating the possible outcome of alternative intervention strategies in control programmes. In order to develop robust models, data from different field situations will be needed, for which the CEC/STD network provides ideal opportunities.

There is a paucity of studies on cellular immune responses to schistosome infection. Part of this stems from the technical and logistic difficulties inherent in this work, the high cost and restricted access to some cytokine reagents being a significant factor. Alternative approaches to meeting the costs and extending access to these reagents should be investigated.

2 Morbidity

Ultrasound now provides an opportunity to objectively document schistosomiasis morbidity, and to assess the relevance and impact of various control strategies in different endemic conditions. Moreover, the individual, ecological and ethnic factors which determine the clearly varying morbidity in schistosomiasis can now be better studied; to that end, standardized field studies in various areas would be highly useful.

Such studies should also include the interaction of schistosomiasis morbidity with other parasites and infections, and conversely the influence of schistosomiasis on responses to other infections.

In spite of the availability of ultrasound, non-specific morbidity such as general ill-feeling, loss of appetite and physiological capacity, and reduced growth and scholastic performance in children, should be properly defined and its public health implications documented.

The search should also continue for alternative markers of morbidity which might permit rapid screening of populations, perhaps through urinalysis. Such an approach would prove valuable in assessing the impact of control interventions.

3 Diagnosis and treatment of infection

The development of simplified, rapid, antigen detection assays such as dipsticks, magnetic bead ELISA and Dot ELISA can become valuable for individual diagnosis, population screening and monitoring control interventions. Antigen detection assays also have an important role to play as epidemiological tools for direct assessment of worm burdens and for monitoring reinfection following chemotherapy and vaccination. The diagnostical potential of other antigens such as egg antigens and Sm28GST, in diagnosis should be explored.

It is not realistic nor necessary to pursue the search for new antischistosome drugs under the STD programme. The use of currently available drugs for control purposes has been extensively investigated, though integration aspects and the impact on morbidity and transmission have remained underemphasized. The possibility of emergence of resistant parasite strains would be of sufficient importance to warrant close monitoring and investigation of suspect situations. The availability of reference laboratories and parasite banks would be highly useful in this respect.

4 Vaccine development

Schistosomiasis vaccine development are in an exciting phase and phase I clinical trials come into sight. The main immediate challenge is to select the most promising molecules or mix of molecules, and the optimal presentation. However, many other problems remain to be overcome.

At present all aspects of transferring the development of vaccines to industrial production have to be arranged by individual research groups. Mechanisms might be established for supporting this stage of vaccine development separate from the contracts for research on the earlier stages.

It is certainly also time for a detailed consideration of the public health and epidemiological implications of vaccine use, to which again mathematical modelling could substantially contribute.

5 Host-parasite and ecological interactions

New tools such as random amplified polymorphic DNA (RAPD) analyses provide new opportunities to study snail intermediate host and parasite strain variations, which may have important implications for schistosome transmission and disease morbidity. New comparisons of fresh field isolates and established laboratory strains are needed. Reference parasite strains and banks would be an important asset in such research.

The impact of environmental aspects and changes on tropical diseases, and schistosomiasis in particular, and the application of new tools such as remote sensing techniques, merit further attention and new studies. This subject should be taken in its broadest sense, and include particularly the social, cultural and economic determinants of infection and disease.

Host-parasite interactions in animals are particularly important for *S. japonicum*, the parasite about which we know least and which poses a major disease problem complicated by its zoonotic aspects. Also for human schistosomes 'natural' an-

imal models such as S. *matthei* and S. *bovis* in cattle can be relevant to study the dynamics of transmission and immunity.

6 Intervention studies

The eventual application of newly developed control tools and strategies must be evaluated in the real-life context of existing health services. Conversely, the characteristics, resources and limitations of these services should steer the objectives of applied and even basic research. These fundamental aspects have been neglected to a surprising extent so far, and available expertise and methodologies for intervention studies are in fact quite limited. It is of crucial importance that the gap between public health workers, health system researchers and biomedical scientists be narrowed and eventually closed. The network approach of CEC/STD is an important step in the right direction, but further collaboration or even integration of networks, across diseases and disciplines, should be pursued, as well as interactions with development programmes funded by the CEC or member states.

7 Network activities

Several areas in which the progress of schistosomiasis research funded by the CEC might be enhanced through the coordination of the efforts of the separate contract holders were identified during the meeting:

1) Mathematical modelling of the epidemiology, transmission, immunology, morbidity and control of schistosomiasis could draw on the data from diverse epidemiological situations. A number of groups have already identified their willingness to share their data in such an endeavour. In addition, the collaboration of researchers with different approaches to mathematical modelling would capitalize on their wide expertise.

2) Antigen detection assays are proving to be of great importance and there is now, more than ever before, a need for their widespread application both in research programmes and in monitoring control interventions. Demand far outstrips current supply capacity. The situation will only improve when some mechanism is found to enable the large-scale, standardized production and distribution of the necessary reagents.

3) The establishment of a parasite 'strain bank' would have benefits for all of the research programmes supported within the network. The geographical distribution of the research programme of the network would make the collection of material a relatively simple task. However, some thought will need to be given by scientists as to what will be required of such a reference source and exactly how it should be set up.

4) An HLA reference resource would facilitate the study of the contribution of genetic factors to resistance and susceptibility to infection and disease, and would benefit many of the ongoing research programmes.

5) The coordination of ultrasound studies to ensure comparability and document morbidity variations and their determinants is highly desirable.

The coordination and standardization of such network activities might be addressed through periodic workshops which offer training in specific topics.

Scientific training is a key component of the network's activities but at present this is organized on a contract by contract basis. Alternative approaches should be reviewed and some consideration given to a coordinated formal training programme.

8 Other interactions

Interactions with other networks within the CEC/STD programme should be pursued. Control and development programmes supported by the CEC and by individual member states function independently of the CEC/STD programme. Interaction with these programmes holds potential benefits for all of those involved, and mechanisms to that end should be established.

In recent months there has been progress towards establishing a dialogue between the CEC and other international scientific bodies, such as WHO/TDR, NIH and EMCF. Further progress in these contacts and coordination between funding agencies was considered to be important to the scientific community involved in tropical disease research.

In the last ten years financial constraints on national and private funding bodies have led to an increasing number of directed, goal-oriented research programmes, geared towards specific questions and results of immediate utility. This rational approach to deal with dwindling resources also has negative aspects. In science success is mostly achieved through the gradual acquisition of data over a prolonged period with researchers building a coherent body of information from which logical progressions are investigated. If the CEC schistosomiasis programme is to continue to make an impact, it must also have the capacity to fund research of a more speculative type in which direct scientific benefits might be enormous but cannot be guaranteed. An appropriate example is vaccine development research. Scientists may appreciate the relevance of the intermediate steps leading towards vaccine production. It is essential, however, that a wider public and political appreciation be ensured together with an indication of the likelihood that these developments will produce results within a reasonable period. Clearly, this may be easier to achieve against the background of a research programme for which the strategic goals have been unambiguously defined and explained.

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