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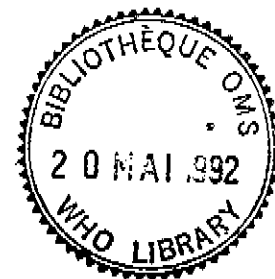
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REPORT OF A MEETING ON APPLICATION OF IMMUNODIAGNOSIS
IN SCHISTOSOMIASIS

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Ce rapport exprime les vues collectives d'un groupe international d'experts réuni par le PROGRAMME SPECIAL PNUD/BANQUE MONDIALE/OMS DE RECHERCHE ET DE FORMATION CONCERNANT LES MALADIES TROPICALES (TDR). Il ne représente pas nécessairement les vues du TDR/OMS et, en vue d'une diffusion accélérée, il n'a pas été l'objet d'une mise en forme particulièrement soignée. En outre, les noms géographiques utilisés dans le présent rapport n'impliquent, de la part du TDR ou de l'OMS, aucune prise de position quant au statut juridique de tel ou tel pays, territoire, ville ou zone, ou de ses autorités, ni quant au tracé de ses frontières.

1. INTRODUCTION

Parasitological diagnostic techniques continue to form the basis for identifying individuals with schistosomiasis and they are essential for the planning, execution and evaluation of national control programmes. On the other hand, apart from being labour-intensive, these methods are relatively insensitive and influenced by the daily fluctuations in the rate of egg excretion which tend to render results equivocal, particularly in areas characterized by low intensities of infection. There is, therefore, a need for improved diagnostic procedures.

Collaborative studies have been carried out with the objective of comparing immunodiagnostic test systems suitable for diagnosis and monitoring of schistosomiasis. The results of three, WHO-coordinated multi-centre trials suggest that such assay systems can be standardized and ultimately used in control programmes to assess infection. Whilst detection of excreted eggs and determination of antibodies specific for schistosome antigens are viewed as complementary approaches, techniques for antigen-detection may eventually supersede parasitological examination.

This consultation was convened to review the current status of immunodiagnosis in schistosomiasis and to identify its main future applications. An additional goal was to provide guidance and encouragement for the development and implementation of appropriate immunodiagnostic assays. While all avenues of approach were welcomed, it was felt that priority areas of emphasis would need to be selected.

2. ROLES FOR IMMUNODIAGNOSIS

The multiple uses to which immunodiagnostic systems can be applied require various approaches. For example, the specifications for methods intended for support of control activities differ from those suitable for vaccine development and separate test designs may be needed for central vs. peripheral use. Taking into consideration the importance of distinguishing between past and current infections, the following areas of application were identified:

2.1 control programmes, specifically for:

- a) identification of communities and subjects for treatment; and
- b) monitoring efficacy of chemotherapy, transmission control and prophylaxis

2.2 assessment of morbidity

2.3 monitoring the state of specific immunity (degree of protection)

2.4 vaccine evaluation, specifically for:

- a) quantitation of worm-burdens; and
- b) quantitation of egg-loads

The consensus was that, in regard to need and feasibility, the highest priority should currently be assigned to developing tests for use in control programmes.

3. ANTIBODY-DETECTION

Although detection of antibodies against specific schistosome antigens does not currently differentiate between active and past infection, this approach was considered useful as an adjunct to parasitological methods in areas of low-intensity infection. There are indications that the class and isotype of antibodies directed against certain antigens can serve as markers of given conditions or situations, i.e. as correlates of acute vs. chronic disease in untreated populations and of resistant vs. susceptible states.

Continued production of specific antibodies after cure makes antibody-detection impractical for monitoring the outcome of chemotherapy. However, certain antibodies, for example, IgG anti-CEF6 and IgG₄ anti-SEA, decrease progressively after cure and may therefore be diagnostically useful. New assays, reported to specifically demonstrate acute infection by detecting anti-schistosome antibodies cross-reacting with keyhole limpet haemocyanin (KLH), urgently need evaluation. In addition, recent results suggesting an association between a reduction of egg-induced pathology and the presence of IgA to parasite glutathione S-transferase (GST) might form the basis for future immunological evaluation of morbidity.

The persistence of IgG₄ against SEA or adult worm antigens may mark a state of relative susceptibility to reinfection, whereas an increase in IgE to adult worm antigens may indicate a degree of resistance. It would be worthwhile to examine these aspects further with the aim of facilitating the development of assays geared to determining the host immune status in relation to schistosomiasis. Further research should be directed towards protective antibodies useful for assessment of vaccine efficacy and/or resistance and susceptibility to infection.

The diagnostic potential of schistosome antigens produced by the recombinant DNA technique should be tested using antibody-detection assays and existing anti-schistosome monoclonal antibodies (Mabs) should be evaluated in competitive ELISA applications. The possible use of antigens from different parasite developmental stages needs to be investigated. A recently-developed ELISA-formatted immunoassay directed at parasite enzymes is of potential importance in antibody-detection.

4. ANTIGEN-DETECTION

A number of circulating antigens, both from the adult worm and from the egg, have been adequately characterized for use in antigen-detection assays. Such tests would prove useful in determining rates of infection, efficacy of chemotherapy and reinfection since they provide quantitative information and show less variability than currently used parasitological techniques. Available laboratory-based assays appear to have sufficient sensitivity for these purposes but research on field-applicable formats needs encouragement and support.

Anti-schistosome Mabs are desirable for basic and applied research on immunodiagnosics and their production and dissemination should be further encouraged, including comparison in multi-centre trials. To date, on the other hand, there is no clear-cut evidence that antigen-based assays can provide useful correlation to levels of morbidity.

Antigen-detection assays are essential for future vaccination trials since adult worm antigens and egg antigens permit measurement of the levels of infection and fecundity, respectively. Preliminary studies indicate that current assays may be adequate for this purpose.

Recent studies show that schistosome ecdysteroid hormones excreted into the blood of the host cannot be diagnostically exploited.

5. ROLE OF IMMUNE COMPLEXES

Antibodies belonging to most immunoglobulin classes and subclasses and with specificities for antigens of the major schistosome species infective to man have been shown to form immune complexes with their corresponding antigens. The presence of such complexes may confound determination of circulating parasite antigens through partial or complete masking of relevant epitopes. This risk would be increased in antibody excess which, due to the minute amounts of antigens released, is the most likely form of complex. Additional studies of circulating immune complexes are warranted in order to improve our understanding of clearance of antigens and to provide a rationale for pre-treatment of samples other than the currently used trichloroacetic acid (TCA) precipitation that might permit detection of protein antigens.

6. ASSAY SYSTEMS

Depending on the intended application, two general types of diagnostic assay were identified:

- 6.1 An uncomplicated, robust, portable and rapid "dip-stick" type for field use which should not require sophisticated equipment or a cold-chain.
- 6.2 A batch-operated or automated, quantitative approach for large-scale studies and reference diagnostic centres in endemic areas.

Public-Health organizations should be encouraged to contemplate integrating immunodiagnosics into existing health delivery systems and to facilitate links between field activities and reference diagnostic centres.

The relative insensitivity of parasitological tests and the high daily fluctuations in egg outputs renders egg-counting unsuitable for reference purposes, particularly in the case of uninfected individuals in endemic areas. New strategic approaches geared to developing alternative selection criteria of an optimal standard should receive priority.

The development of rapid qualitative antigen-detection tests, e.g. dipsticks or assays based on magnetic beads, is expected to prove useful, particularly in large-scale monitoring of therapeutic interventions. In contrast, a quantitative approach is preferable for research and vaccination studies.

Assays should be designed according to goal-oriented specifications and the personnel who would eventually use the assays should be consulted during the development phase. Input should be obtained from the commercial "research & development" sector and the new TDR Product Development Unit.

7. TECHNICAL VALIDATION

Before the practical implementation of any assay, its characteristics must be appraised in terms of sensitivity and specificity. Insufficient attention to this and ignorance of the relation between prevalence and test results contribute to failure of accurately assessing the performance of an assay under any given conditions. Consequently, a mechanism for quality control and standardization must be incorporated into all assay systems. Validation should be performed at all levels of test development, taking into account both qualitative and quantitative results.

Although currently available assays commonly display high specificity, attention should be paid to the possibility of cross-reactions with antigens from other helminths whose endemicity coincides with that of schistosomes. In order to ensure test reliability in areas characterized by a low level of transmission, a high degree of sensitivity is required.

Direct comparisons of different assays, be they based on antibodies or antigens, have a high priority. A well-defined bank of reference diagnostic specimens and reagents is therefore essential. Collection teams in different endemic foci should be considered for obtaining specimens. The specimens, together with essential clinical data, would subsequently be despatched to a centre responsible for quality control, aliquoting, storage, inventory control and distribution of specimens as requested by researchers. In order to increase motivation for sample acquisition, supported scientific collaboration should be offered to scientists involved in specimen collection. Extended funding permitting the continuous addition of new specimens would assure long-term operation of the specimen bank which should optimally include:

- a) specimens from subjects with relevant and defined pathology;
- b) longitudinally procured specimens;
- c) specimens taken before and after exposure (migration samples);
- d) specimens taken before and after therapy (treatment samples);
- e) specimens from subjects infected with other helminths (heterologous infection samples); and
- f) specimens from endemic and non-endemic negative control subjects.

Specimens from individuals with similar ethnic, socio-economic, and cultural backgrounds from non-endemic areas should be used as negative controls in multi-centre studies.

8. SPECIMENS

Every effort should be made to adapt assays to working with small test volumes, for example, the 10-20 μ l serum that can be provided by finger-prick puncture. Samples not tested in the field should be transported and frozen within a few hours. A temperature of -20°C would be sufficient for storing specimens for up to a month, whilst long-term storage would require -70°C or lower.

Whilst determination of specific antibodies and immune complexes must, by necessity, be carried out on serum samples, attention should be paid to the fact that some schistosome antigens, circulating in serum, are excreted in the urine.

9. RECOMMENDATIONS

9.1 Establishment and maintenance of an international specimen (serum and urine) bank with samples from individuals defined by a multitude of parasitological examinations to belong to one of the following categories:

- a) moderate to high egg-counts (>100 eggs per gram faeces or per 20 ml urine);
- b) low egg-counts (<50 eggs per gram faeces or 10 ml urine);
- c) negative controls

Specimens before and after treatment should be procured and, whenever possible, also longer series of longitudinal samples. Negative control specimens should preferably come from ethnically matched negative controls from endemic and non-endemic areas. Specimens from subjects infected with helminths other than schistosomes should be included for evaluation of specificity.

In addition to providing access to individual well-characterized sera, the establishment of reference serum pools with various predetermined levels of anti-schistosome antibodies and antigens is needed for standardizing the sensitivity of locally used assays.

- 9.2 Multi-centre laboratory trials geared to testing specificity and sensitivity of assays capable of demonstrating circulating schistosome antigens in serum, and subsequently urine, are a priority.
- 9.3 Further development of field-applicable assays for antigen and antibody-detection should be encouraged and compared with existing laboratory-based assays.
- 9.4 The kinetics of excretion of circulating antigens into the urine should be further investigated with the aim of developing reliable non-invasive diagnostic methods.

10. LIST OF PARTICIPANTS

CESARI Dr I., Laboratorio de Immunoparasitologica, Centro de Microbiologica y Biologica Celular (IVIC), Caracas, Venezuela

COLLEY, Professor D.G., Vanderbilt University School of Medicine, Veterans Affairs Medical Center, Nashville, Tennessee, USA

DEELDER, Professor A.M., Laboratory for Parasitology, Faculty of Medicine, State University of Leiden, Leiden, The Netherlands

GUNDERSEN, Dr S.G., Dept. of Infectious Diseases, Ullevaal Centre for International Medicine, Oslo, Norway

PIESSENS, Professor W.F., Harvard School of Public Health, Boston, Massachusetts, USA

DE SAVIGNY, Dr D., Health Science Division, International Development Research Centre, Ottawa, Ontario, Canada

SHAKER, Dr Z.A., Immunology Laboratory, Theodor Bilharz Research Institute, Giza, Egypt

SHIMUZU, Dr S.H., Faculdade de Ciencias Farmaceuticas, Universidade de Sao Paulo, Sao Paulo, Brazil

SNOWDEN, Dr K., Liverpool School of Tropical Medicine, Liverpool, UK

STRAND, Dr M., Department of Pharmacology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

TSANG, Dr V.C.W., Division of Parasitic Diseases, Centers for Disease Control, Atlanta, Georgia, USA

WEISS, Dr N., Department of Diagnostics, Swiss Tropical Institute, Basel, Switzerland

Observers

DE BRUYCKER, Dr M., Commission of the European Communities, Science and Technology for Development (STD), Brussels, Belgium

COOK, Dr J.A., Tropical Disease Research Program, The Edna McConnell Clark Foundation, New York, NY, USA

HALL, Dr L., Parasitology and Tropical Diseases Branch, National Institutes of Health (NIH), Bethesda, Maryland, USA

MECASKEY, Mr J.W., Tropical Disease Research Program, The Edna McConnell Clark Foundation, New York, NY, USA

TURNER, Dr G., Dana Center for Preventive Ophthalmology, Wilmer Institute, Baltimore, Maryland 21205, USA

WHO Secretariat

Dr N.R. Bergquist, SCH

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