PRACTICAL METHODS IN CLINICAL IMMUNOLOGY

IMMUNOLOGICAL INVESTIGATION OF TROPICAL PARASITIC DISEASES

VACLAV HOUBA

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Immunological Investigation of Tropical Parasitic Diseases

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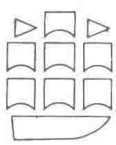

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Foreword

This second volume of the series Practical Methods in Clinical Immunology, by Dr Houba and his illustrious co-authors, deals with a group of diseases of major topical importance.

The Foreword to the first volume of this Series proclaimed that, 'The explosive development of clinical immunology and the increasing breadth of its medical horizons during the last decade have outstripped the availability of practical texts to provide up-to-date information for hospital laboratory workers and clinicians. This has created a need for rapidly published specialized books detailing technical laboratory procedures and explaining their clinical relevance.' The Series aims at satisfying this need by books about individual body systems, or groups of diseases, or immunological events in disease. The range of topics so far decided is given in the list on p (ii).

The present volume deals comprehensively with one such topic. The authors are well qualified for the task having themselves pioneered the immunological investigation of parasitic diseases. In this book will be found brief descriptions of the principal tropical diseases, practical details of immunological tests currently employed for investigating and analysing them, and clear guidance to help the physician and epidemiologist select appropriate tests and interpret their results.

Melbourne, 1980 R.C.N.

Preface

Millions of people living in tropical and subtropical countries are affected by tropical diseases which do not occur in non-tropical areas. Parasitic diseases, such as malaria, schistosomiasis, filariasis, leishmaniasis, African and South American trypanosomiasis, are the main burden among them. Many of these infections are chronic diseases which do not necessarily kill but debilitate a significant proportion of the victims. Extensive national and international eradication programmes were introduced and carried out in different parts of the world in the past in order to interrupt the complicated cycle of these infections; these included new antivector compounds (e.g. insecticides, molluscicides, etc.) or new drugs for therapy of individual infections. However effective in some areas, the measures used in the past did not solve the problem of control of these infections in global scope and, therefore, a search for other control measures has been instituted. Immunological studies represent one of the new trends in this approach as it has been believed that, at least in some of the tropical parasitic infections, immunology could substantially contribute to their control by several means, i.e. (1) to improve the diagnostic criteria (new, more sensitive, specific and simple immunodiagnostic tests), (2) to prevent pathological consequences of the infections (better understanding of pathogenesis of the lesions) and, hopefully, (3) to prepare effective vaccines.

All these infections induce an immune response in the host, but this response is not effective when compared with bacterial and/or viral infections. For this reason special attention has been given in recent decades to studies of host–parasite relationships and especially to mechanisms by which parasites evade the immune response of the host and why the response of the host is not effective enough.

The purpose of this book has been to review immunological investigations of tropical parasitic diseases occurring in man with the main emphasis on immunodiagnostic and other immunological measures relevant to clinical investigations and pathological consequences. The use of vaccines in man is still more dream than reality and, therefore, studies related to this subject have not been included.

Other parasitic diseases, occurring in temperate climates, have been left out, with the exception of amebiasis which has a special significance for the tropics.

Together with several collaborators, authors of individual chapters, we have tried to provide the readers not familiar with the subjects with a short description of parasites and their life-cycles, and with a description of clinical manifestations

and/or pathological consequences relevant to immunological studies in individual infections. The main part of the text has been focused on immunological investigations, on comprehensive reviews of immunodiagnostic tests and on detailed descriptions of tests recommended for routine use.

W.H.O., Geneva
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Introduction

V. Houba

Host-parasite relationships have long been of interest to scientists, but until about 1960 most of the studies were more or less descriptive only, and did not focus on basic questions of how the parasites are able to survive in immune hosts and why the hosts are unable to mount effective defence against invading parasites. We now have better understanding of this relationship (Ciba Foundation Symposium, 1974), but many more studies are required before we shall be able to manipulate immunological reactivity of the host for effective protection against parasites, especially in the tropics.

Natural (innate) immunity

This plays an enormous role in the resistance of the hosts to parasitic infections; although several thousands of species of protozoa parasitic for various hosts have been identified, only a few of them are pathogenic for man (Cohen, 1974). Several possible mechanisms of this type of resistance have been described and recent observations have confirmed an essential role of genetic factors in the susceptibility of hosts.

Acquired immunity

It is generally agreed that parasites and their products are immunogenic for the hosts and this has played an important part in the evolution of host–parasite relationships. However, the fact remains that in the vast majority of parasitic infections the immune response does not protect the host effectively enough, and in some of them not at all (Mitchell, 1977, 1979). For this reason, the significance of specific acquired immunity in parasitic diseases has been disputed for a long time.

According to clinical manifestations of acquired specific resistance, parasitic infections can be divided into three main categories outlined by Cohen (1976):

1. *Absence of an effective immune response*

In this case subjects do not develop any effective immunity although signs of the hosts' immune response are present, e.g. specific antibodies. Typical examples are African trypanosomiasis, visceral leishmaniasis, and amebiasis.

2. *Non-sterilizing immunity*

The majority of parasitic infections induce an immune response which is only

partially effective. In protozoa this phenomen has been referred to as 'premunition' with persistence of a controlled level of parasites (e.g. malaria). In helminthiasis, it corresponds to 'concomitant immunity', which protects the host against reinfection but is inactive against established worms from primary or previous exposures (e.g. schistosomiasis).

3. *Sterilizing immunity*
This is very rare in human situations, perhaps seen in cutaneous leishmaniasis, but it has been demonstrated in animal model infections.

Diagnosis from immune status
It should be noted that whatever status of immunity develops, the immune response is usually species-specific and often strain- or stage-specific. The response is an inadequate index of present infection; for parasitological diagnosis, demonstration of the parasites or their products (e.g. eggs) is still the only satisfactory way of confirming infection. In some situations (e.g. acute malaria) this may be quite easy.

However in other situations, especially in relation to chronic infections and to therapy control, the detection of parasites may be extremely difficult in spite of the refinement of parasitological techniques, such as the use of concentration methods (blood protozoa on columns, eggs on filters), cultivation techniques *in vitro*, injection of materials into neonatal or irradiated rodents, etc. For these, immunodiagnostic tests have proved useful to support clinical diagnosis (Warren & Mahmoud, 1978), apart from their value in mass screening studies, epidemiological surveys, screening for blood transfusions, etc.

Since their introduction into diagnostic services of parasitic diseases, immunological techniques have been based on the detection of specific antibodies or antigens, either free or bound in immune complexes, and on reactivity of immune cells of the host.

Detection of antibodies
Recognition of antibodies reacting with parasite components, is the classical way by which serological techniques were applied since the beginning of this century. Both primary and secondary reactions to parasitic antigens have been studied. Of the spectrum of techniques described in the literature the main immunodiagnostic tests were precipitation, agglutination, indirect hemagglutination and complement fixation. Later on, immunoelectrophoresis and its modifications were introduced as well as artificial particles, latex and bentonite to replace red blood cells. More recent developments using labelled reagents include immunofluorescence, enzyme-immunoassays, enzyme-linked immunosorbent assays and radioimmunoassays. The techniques detecting antibodies reacting with parasite antigens have been gradually improved to increase their sensitivity and some have reached a high level in this respect. Unfortunately, their specificity is still not satisfactory in the majority of the tests. The reasons are several, but the main problem is the lack of defined antigens.

The nature and source of the antigens selected for immunological tests has to be carefully considered with respect to the very complex mixture of substances

possessed or produced by parasites during their life-cycles and their tendency to survive in the host. Many of the antigens used in the past, but still available for serological tests, are 'ill-defined heterogeneous mixtures' (WHO, 1975). It is therefore not surprising that in many cases 'cross-reactions with unrelated parasitic conditions are the rule rather than the exception in the serology of parasitic diseases' (WHO, 1975). Therefore, the need to develop biochemical and physicochemical techniques for preparation of better antigens and to use purified antigens has been repeatedly stressed. A typical modern development is the isolation of MSA_1 fraction from crude soluble egg antigen of *S. mansoni* and its use in radioimmunoassay which has greatly increased the specificity of antibody testing. Similar efforts are being made for other antigens and there is hope that in a few years purified fractions or even pure antigens will be available to improve the specificity of many immunodiagnostic tests.

Another development is the application of hybridoma technology to prepare pure parasite antigens. However, it must be realized that isolation of highly specific antigens from individual strains (clones) of parasites may not meet the criteria necessary for practical immunodiagnosis. Typical examples are surface variant antigens in African trypanosomes, which are important for research investigations but difficult to use for routine immunodiagnosis.

Detection of antigens

Antigen detection for diagnostic purposes is a more recent development. The presence of soluble antigens in the circulation and/or in excretions of the host has been demonstrated in several parasitic diseases. The main problem is that the concentration of these substances in body fluids (serum, milk, urine) is usually very low, and highly sensitive techniques are needed for their measurement. Such techniques are now available and it is expected that antigen detection may be preferred in the immunodiagnosis of parasitic diseases for several reasons:

(i) Detection of antigens produced by living parasites confirms the presence of infection.

(ii) Half-life of some parasitic antigens is less than the persistence of antibodies in circulation.

(iii) Some antigens (e.g. polysaccharides of schistosomes) are not good immunogens and circulate free, not bound to antibodies in immune complexes.

(iv) Some antigens are excreted into urine, milk, etc; obviating the need for blood samples.

(v) Studies are in progress to establish the relationship between the amounts of circulating or excreted antigens and the worm burden, etc.

These tests have the same problems of specificity and cross-reactivity as discussed above. However, monoclonal antibodies produced by hybridomas hold promise of an improved situation.

Antigen–antibody (immune) complexes

Immunodiagnostic procedures described above, i.e. the detection of antibodies or antigens, may be complicated where antibodies already present in the host bind with parasite antigens to form complexes, which may sometimes remain in circulation for prolonged periods. The presence of immune complexes,

circulating in the blood either in antigen- or antibody-excess has been reported in many tropical parasitic infections. It is perhaps the main reason for 'anti-complementary' activity of sera, which seriously hampers the diagnostic value of complement fixation tests in these diseases.

Many attempts have been made to use the detection of circulating immune complexes diagnostically (WHO, 1977). Unfortunately, the majority of the routine techniques for immune complex recognition lack antigen specificity and, therefore, cannot be used for the diagnosis of the diseases; new trends, identifying antigen or antibody in the immune complexes are more promising.

Hypersensitivity tests

Antibody-dependent tests for Types I and III hypersensitivity have been used for diagnostic purposes for a long time. Their sensitivity and specificity will be discussed later in connection with individual diseases; one of their main disadvantages is uncertain suitability of the antigens used.

Cellular immunity tests, i.e. delayed type or Type IV hypersensitivity and *in vitro* tests, have also been applied. Skin tests in man were evaluated in diagnosis and epidemiological surveys of several parasitic diseases. *In vitro* tests recently introduced are still used more for research than routine procedures.

Comment

Immunodiagnosis represents only one minor part of immunological investigations of tropical parasitic diseases. The main thrust of such investigations has been directed towards better understanding of host–parasite relationships with two principal objectives. Firstly, we wish to identify the effector mechanisms of the immune response to seek effective protection of the host against parasitic infections with the final goal of vaccine preparation and any other way of better disease control. An example is the antibody-dependent cell-mediated reactions which have proved effective against multicellular parasites (schistosomula) *in vitro*. Study of their relevance to *in vivo* situations hopefully may lead to new approaches for infection control. Secondly we wish to study participation of immunological mechanisms in clinical and pathological manifestations of these diseases, e.g. nephropathies due to the localization of immune complexes in glomerular basement membranes of the kidney as in malaria, African trypanosomiasis, schistosomiasis, or cell-mediated reactions around the schistosome eggs trapped in the liver leading to the granuloma formation.

Many studies are in progress to prepare vaccines against tropical parasitic diseases, and experimental models have shown promising results. However, there is still a long way to go before we have safe and effective vaccines for man, and this topic is not discussed further.

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Malaria

A. Voller and V. Houba

INTRODUCTION

Malaria is caused by parasites of the genus *Plasmodium*. Four species infect man: *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. These give rise to diseases which differ somewhat in their clinical course, pathology and to some extent in their treatment. In addition to the human malaria parasites there are many other plasmodia which infect apes, monkeys, birds and rodents.

Until recent times malaria was one of the important 'killer' diseases in the world. It was widely spread throughout the tropical belt and into the subtropical lands and even to the edges of the temperate zones. Extensive national and international malaria eradication programs, co-ordinated by the World Health Organization, were instituted in the years following World War II. These programs, largely based on the use of the new insecticides, such as DDT, were extremely successful and malaria was eradicated from many parts of the world. However, in due course malaria vectors developed resistance to the available insecticides and other means of control such as chemotherapy were sought.

At the same time the malaria parasites began to exhibit drug resistance, notably to chloroquine, one of the most widely used antimalarials. These 'biological problems', combined with financial and administrative difficulties have necessitated a reappraisal of the global malaria eradication strategy. It is now recognized that malaria will be with us for some considerable time and this means that renewed efforts are required for diagnosis, surveillance, prevention and treatment of the disease. It is recognized that immunology has a major part to play in the new approach to malaria control. Ultimately it is hoped that an effective vaccine will be developed (Cohen, 1979).

THE MALARIA PARASITE IN THE VERTEBRATE HOST

When an infected mosquito feeds on a susceptible individual, sporozoites are introduced into the bloodstream. These circulate for only a few minutes before invading the liver parenchyma cells where the exo-erythrocytic (EE) stage of development occurs over a period of a week or two. Eventually the EE schizonts burst to release thousands of merozoites into the bloodstream. These invade erythrocytes to initiate the erthrocytic asexual stage of the cycle. The parasites begin as tiny rings, develop to trophozoites and then nuclear division occurs to produce a mature schizont containing 8–24 merozoites. At this stage the host

erythrocyte bursts and the released merozoites invade new erythrocytes and so continue the multiplication in the asexual cycle. Eventually the parasitaemia increases to such an extent that the host dies or an immune response occurs (the crisis) which leads to a sudden drop in the number of parasites. Often there are later relapses or recrudescences when parasite numbers increase again to be followed by a crisis. Eventually parasitemia may stabilize at a low level or the parasites may disappear completely. The sexual forms of the parasites, the gametocytes, can appear during the initial attack or at any later time.

THE IMMUNE RESPONSE

Until recently it was thought that there was no immune response to the sporozoite injected by the mosquito. However, Nussenzweig et al (1972), Spitalny & Nussenzweig (1973) and Golenser et al (1977) were able to detect antibody to sporozoites in rodents bitten by infected mosquitoes, and Nardin & Nussenzweig (1978) found anti-sporozoite antibody in the sera of people in malaria-endemic areas.

The exo-erythrocytic stages again do not appear to provoke a detectable immune response. Such forms will, however, react in serological tests *in vitro* with antibody induced by blood forms of the malaria parasite.

The major immune response is undoubtedly induced by the erythrocytic asexual phase of the malaria parasite. Soon after the blood is invaded, antibodies can be detected by any of the conventional serological methods, and these rapidly rise to high levels and there are even substantial increases in the total IgG and/or IgM levels of the sera. That at least part of the immunoglobulins show antibody specificity has been established convincingly (Cohen et al, 1961).

The serological response to malaria is characterized by the production of antibodies and other factors of varying specificities. On one hand we have the highly specific antibody which agglutinates schizont-infected cells (SICA). This antibody is specific to each variant, e.g. the parasites isolated from each attack or relapse in a single individual, induce distinct SICA antibody. Then there is the antibody detected by inhibition of merozoite reinvasion in culture. This antibody appears to be strain-specific correlating well with clinical immunity to challenge. Of wider reactivity are the antibodies detected by conventional serological tests such as immunofluorescence, indirect hemagglutination and enzyme immunoassay. These antibodies are malaria genus-specific but not completely species-specific. Finally, there are the non-specific factors such as heterophile agglutinins, antinuclear antibodies, and rheumatoid factors which may be evoked by malaria but do not react specifically with material of malarial origin.

Cellular mechanisms of immunity have been studied in experimental animals. Thymectomized animals seem to be more susceptible to subsequent infection, and antithymocyte serum has reduced the resistance of rats to infection with rodent malaria. However, the majority of malarial antigens require co-operation between T and B lymphocytes for induction of specific antibody and, therefore, these data do not necessarily relate to cell-mediated mechanisms alone; failure of co-operation may reduce the antibody response (Cohen et al, 1974). Similar findings are reported in human subjects, e.g. marked involution of the thymus