IMMUNODIAGNOSIS OF PARASITIC INFECTIONS

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INTRODUCTION

A definitive diagnosis of parasitic infections is usually by demonstration of the parasite, generally in blood or faeces. A faecal examination is the routine diagnostic procedure for soil-transmitted helminths, e.g. *Ascaris lumbricoides* (large roundworm of man), *Necator americanus* and *Ancylostoma duodenale* (human hookworms), *Strongyloides stercoralis* and *Trichuris trichiura* (human whipworm). Examination of peripheral blood, either by thick- or thin-blood films, is carried out normally for cases of malaria and filariasis. There are instances, however, where parasitic infections are not easily detected. This is evident in light infections of malaria or filariasis where the parasites can be easily missed by the blood film examination. Extraintestinal involvement in amoebiasis, or cases of toxoplasmosis where *Toxoplasma gondii* invades internal organs cannot be easily confirmed. Aspiration or tissue biopsy for these latent parasites may yield negative results. In such cases, diagnosis is only suspected from clinical history and symptoms manifested by the patient. In these situations, immunodiagnostic procedures become useful.

It is generally accepted that the parasite or some product of it acts as antigen and may evoke an immune response from the host. Broadly speaking, the host may react by producing humoral antibodies, or a state of delayed-type hypersensitivity. The antibodies in the blood can be detected by a variety of serological tests such as gel-diffusion, haemagglutination, complement-fixation, and fluorescent antibody. Immediate or delayed type hypersensitivity is detected by the skin (intradermal) test.

Many difficulties are encountered in these tests. One of these is the use of crude antigen preparation of the parasite. This may often give rise to cross-reactions with other parasitic infections. To offset this, attempts have been made at antigen purification so that the test employed will be specific for only the particular parasite. Many of the parasites have complex life cycles. Not all stages of the cycle, however, can be usefully employed as antigens. For this reason, adequate antigenic material is often a problem. *In vitro* culture techniques are slowly being developed in this direction. Despite these attendant problems, great strides have been made towards immunodiagnosis of parasitic infections.

The following account gives a brief review of some of the diseases of local importance where immunological procedures may be employed as diagnostic tools.

Amoebiasis

An accurate diagnosis depends on the correct identification of the pathogen, *Entamoeba histolytica*. This can exist in the motile, trophozoite form and the resting, transmissible cyst stage. Very active haematophagous trophozoites can be seen in fresh stools from patients with acute amoebic colitis. In less severe cases, single examination of stools is often inadequate to demonstrate the amoebae. In extraintestinal amoebiasis, liver abscesses are commonly seen, less so in the lungs, brain and other areas of the viscera. Aspiration of the abscess often yields no amoebae. In such situations where *E. histolytica* is suspected but cannot be easily demonstrated, one must resort to serodiagnostic procedures. A wide range of tests can be usefully performed, but their sensitivity, cost and reproducibility varies. These include the least sensitive immobilization reaction to the most sensitive indirect haemagglutination (HA) test. Many of the problems associated with these tests have resulted from the use of *E. histolytica* trophozoite antigen contaminated with the bacteria often necessary for their growth in *in vitro* cultures. Diamond (1968) devised an axenic culture of *E. histolytica* which can be utilized as antigen free of microbial contaminants. Recently one of us employed cysts of *E. invadens* (a strain of amoeba pathogenic only to snakes) as antigen in the indirect HA test (Yap *et al*, 1970). From parallel tests using *E. histolytica* trophozoite antigen, it was found that a higher percentage of positives were obtained with the cyst antigen than with the *E. histolytica* trophozoite antigen, especially in acute amoebic dysentery.

Routine fluorescent antibody (FA) tests are performed locally with sera from patients with amoebiasis. This is most useful in confirming

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cases of hepatic amoebiasis, but the test is positive in only 20% of patients with amoebiasis without liver involvement (Yu and Lim, 1970).

Malaria

For malarial infection, a routine diagnosis is made by examination of blood films stained with Giemsa or Leishman. Very light infections (asymptomatic cases with low grade of parasitaemia) however, can be missed since there will be very few parasitized red blood cells in the peripheral blood. Use of a serological procedure is indicated in these situations. Serological tests could also be useful in mass screening for detecting specific antibodies to the malarial parasite in a given population. This provides a clue to the extent of exposure of the population to the parasite. Many problems are confronted when using serological tests in malarial infection. Some of these include the availability of antigen, *in vivo* binding of antibody and the doubtful specificity due to cross-reactions between different species of *Plasmodia*. Preparation of antigen from the parasites requires the use of large volumes of parasitized blood from a donor. At best, this can only yield small quantities of the crude antigen.

The most practical and widely accepted test to measure circulating antibodies to malarial parasites is the indirect FA test (Tobie and Coatney, 1961). 95% detection rates have been found in an evaluation of the thick smear antigen in the indirect FA test for malarial antibodies (Sulzer *et al*, 1969) while Gleason *et al* (1970) found it useful for determining the species of *Plasmodium* causing clinical malaria. Perhaps the main problem in this test appears to be the high level of non-specific fluorescence especially if the donor's serum contains specific anti-malarial antibodies. It is therefore necessary to wash the parasitized erythrocytes before preparing a thick smear. Recently, Collins *et al* (1968) in their study of the ecology of malaria in Malaysia observed that in areas of hyperendermic malaria, good correlation was found between the antibody response and active parasitaemia using indirect FA test with *P. falciparum*, *P. vivax*, *P. malariae* and *P. fieldi* antigens.

A modification of the fluorescent antibody test, the soluble antigen fluorescent antibody test (SAFA test) has recently been developed for the serodiagnosis of human malaria (Sadun and Gore, 1968). This requires the use of *P. falciparum* parasitized erythrocyte lysates from experimentally infected chimpanzees as antigen. This antigen may be either a metabolic product of the parasites or an altered host element which is released after the infected erythrocytes are lysed. This test appears to show a high degree of sensitivity, specificity and reproducibility. The use of a lysate antigen in SAFA test makes it possible to conduct a large number of tests. Approximately 50,000 screening tests can be performed with the amount of antigen normally collected from one infected chimpanzee. This test has possible application in mass screening of population, for epidemiological studies of malaria and also for mass screening of potential blood donors.

Toxoplasmosis

In both the acquired and the congenital forms of this disease, the isolation of the causative agent, *Toxoplasma gondii* is the most certain proof of infection. This, however, is not possible in most cases. The organism may reside in sites like the eye or the brain where a biopsy is impossible. Also, the organism may have disappeared after the acute phase of the infection. In most cases, a serological diagnosis is necessary.

Of the many serological tests used, the Sabin-Feldman dye test (or the methylene blue dye test) is commonly used (Sabin and Feldman, 1948). In this technique, *Toxoplasma* trophozoites are harvested from the peritoneal exudate of infected mice 3 days after prior intraperitoneal infection. Standard numbers of these organisms are mixed in several dilutions of test sera. A fresh antibody-free serum of human origin is added to serial dilutions of the test sera to provide the complement-properdin component (called the "accessory factor"). The organisms are incubated for 45 to 60 mins. at 37°C. Alcoholic methylene blue at pH 7·0 is added after incubation. In the presence of specific antibody in the test serum the cytoplasm of the *Toxoplasma* trophozoite becomes modified and does not stain intense blue. The test is used quantitatively by finding the highest dilution of test serum which will modify 50% of the standard number of *Toxoplasma*. The dye test has been shown by many investigations to be a very sensitive and specific indicator of Toxoplasma antibodies in both man and animals. This test, however, has its disadvantages. It requires the use of viable, virulent organisms and also the need for an accessory factor which is not present in all human serum. Moreover, the end point interpretation is very subjective.

The indirect HA test for toxoplasmosis is also a sensitive and specific technique (Jacobs and Lunde, 1957). The antigen used is the peritoneal exudate lysate of mice infected with *T. gondii.* Good qualitative agreement is found between the indirect haemagglutination and the dye test. The IHA test is also useful in sero-epidemiological studies.

The FA test has also been applied to the diagnosis of toxoplasmosis. In particular, the indirect FA test has been shown to be useful in demonstrating IgM antibodies to *T. gondii* in cases of acute congenital toxoplasmosis (Remington *et al*, 1968). Serologic diagnosis of infection in the newborn is complicated by the presence of maternal antibodies (of the IgG class) transferred placentally. The formalin-fixed antigen on slides is stable for 3-4 months and the reagents are also readily available. The main disadvantage, however, lies in the subjective reading of the end point in titration.

Skin tests have been attempted in toxoplasmosis but have found only restricted use. Infection of toxoplasmosis cause a delayed type hypersensitivity response. This is shown by injecting an extract of *Toxoplasma* intradermally (Frenkel, 1948). Reactions are of the delayed tuberculin type i.e. erythema of several cm. diameter or with oedema and induration. A good correlation has been reported between a positive skin test and a positive dye test at the titres of 1:8 or more. The skin test, however, does not provide any clue to how recent the infection was. In very recent infections, it can be negative.

Filariasis

The intradermal test has been used in the epidemiological assessment of filariasis by several workers (Desowitz *et al*, 1966). The most popular antigen used is prepared from the dog heartworm, *Dirofilaria immitis*. The results, however, are difficult to interpret. The reactivity does not indicate acute or past infection of the patient. Moreover the antigen used is generally a crude preparation of the worm. The purified antigen is known as the Sawada antigen (Sawada *et al*, 1965). The specificity of the reaction is questionable but this test does find usefulness in epidemiological surveys of filariasis in selected populations.

A SAFA test has been adapted to detect circulating filarial antibodies (Duxbury and Sadun, 1967). This utilizes a soluble lipid-free somatic antigen preparation from adult *D. immitis* worms. Results on confirmed cases of filariasis indicate a high degree of sensitivity and specificity (Colwell *et al*, 1970).

Visceral Larva Migrans

This is an infection produced by larval nematodes that persist for prolonged periods in tissues of man, particularly in the liver and lungs. Several nematode species have been incriminated. Of these, the dog ascarid, *Toxocara canis* is considered the most frequent causative agent. This disease, as produced by *Toxocara*, occurs primarily in children having close contact with domestic pets. Infection is acquired by accidental ingestion of eggs passed in the animal faeces. Clinically, the disease is characterized by persistent hypereosinophilia, hepatomegaly, eosinophilic granulomatous lesions in various organs and some degree of pulmonary infiltration (Beaver, 1969). The larvae have a predilectation for the eye producing retinal granuloma or chronic endopthalmitis. This condition is usually suspected on clinical grounds. As biopsy is often difficult and chancy, one usually resorts to serologic tests.

An intradermal test for the diagnosis of toxocariasis using whole powdered adult *T. canis* in saline as antigen has been employed (Woodruff and Thanki, 1964). These investigators claim that this is a valuable aid in diagnosis of toxocariasis in man, giving rise to positive reactions in past or present infection. It does not appear to give cross-reactions in the presence of other helminthic infections. 0·1 ml. antigen is injected intradermally into the forearm of the patient. A positive reaction is indicated by a small wheal. The area of the wheal is measured immediately and again after 20 mins. A reaction is read as positive when the total area of induration is doubled or more during the 20 mins. In a negative reaction, the induration disappears or there is no enlargement of the original wheal size. The authors do not attach any significance to the presence of pseudopodic erythema.

At the moment there is no reliable serologic test available. The HA test is liable to provide cross-reactions with ascariasis and strongyloidiasis. This is especially marked in tests using the crude antigen preparation from adult stages. Choice of adult stage is decided by the scarcity of larval stages. Since *T. canis* occurs as a larval infection, antigens prepared from larvae or larval products should have greater sensitivity in serological tests (Kagan, 1968). This has been shown in IHA tests, in which antigens of *Toxocara* larvae or infective eggs proved more sensitive as detectors of antibody than extracts of *Toxocara* adults, *Ascaris* adults or *Ascaris* larvae (Aljeboori and Ivey, 1970) but a small degree of cross-reaction was still evident.

Hydatid Disease

This disease is acquired through the ingestion of eggs of the dog tapeworm, *Echinococcus granulosus*. Though the disease is not common locally, it is encountered in patients who have spent some time of their lives in those foreign countries where the disease is endemic and widespread.

The most frequently employed immunologic test is the intradermal test introduced by Casoni (1911) and referred to sometimes as Casoni's test.

In this test injection of 0·2 ml. of sterile hydatid fluid into the skin of the forearm produces a wheal up to 5 cm. in diameter with pseudopodia in about 20 mins. in positive cases. Skin reactivity is generally high compared with clinical evidence of infection. Sensitivity is good in proven cases. The test, however, has not been standardized as to the required amount of fluid injected and method of reading the reaction. Also, the test does not indicate if the hydatid is viable or calcified. The antigen cross reacts with persons having schistosomiasis (Cherubin, 1969). Fractionation, however, has produced more specific antigens (Kent, 1963). It is recommended that the antigen be diluted to a point where specificity is high and nonspecific response is at a minimum.

In addition to the Casoni skin test other serological methods employed are the complement fixation test, HA test, bentonite flocculation, latex agglutination and FA test. Procedures of choice at present include the HA test, bentonite flocculation and latex agglutination (Kagan, 1968).

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