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Serodiagnosis of parasitic infections

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Introduction

DETECTION OF the characteristic stages of parasites in the blood, tissues, stools, other excretions and secretions of the host is the proof of parasitic infections. Negative results, however, do not exclude the presence of parasites especially if they have been altered by drugs, or when they are present at subpatent levels as often is the case in very light, early or in late infections. At times, microscopic examination is impossible or impractical due to the location of the parasites in the deep tissues. Diagnostic techniques may be laborious and time-consuming. Tissue biopsy or aspirations for parasites may be traumatic and also may give negative results. Under such conditions, diagnosis is often made from the clinical history, symptoms and signs alone. The clinical picture of most parasitic infections is highly variable.

Serodiagnostic techniques have been found useful in supporting the clinical observation or to rule out these diseases. Although serological techniques have been used for diagnosis of parasitic infections from the early years of this century, only in the last few years have these tests received general acceptance. At present the interest in the serological diagnosis is fast increasing and such techniques are available for at least 24 parasitic infections (Kagan, 1976a).

Many reviews on the recent advances in the immunodiagnosis of parasitic infections are available (Miller and Brown, 1969; Kagan and Norman, 1970; Denham *et al.,* 1971; Singh and Yap, 1971; Fife, 1971; 1972; Kagan, 1974; 1976a; 1976b and Sadun, 1976). A number of serodiagnostic techniques both quantitative and qualitative, are employed for serodiagnosis of parasitic infections. However, none of these is universally accepted as the best single method. Each method has its uses, advantages and limitations. A combination of several tests and antigens are recommended for more accurate results (Kagan, 1974). This paper discusses briefly the serodiagnostic techniques that are commonly used for parasitic diseases, their applications and limitations.

Application

Serological techniques may be useful in diagnosing individual cases, particularly those infections where parasites are not readily detected by other methods as in toxoplasmosis, extra-intestinal amoebiasis, trichinellosis, hydatid disease, visceral larva migrans etc. These are also invaluable in the epidemiology of parasitic infections. These techniques have also been found useful in screening blood donors and to exclude infections like malaria and other parasitic infections in patients with manifestation such as pyrexia of unknown origin, hepatosplenomgaly, anaemia and nephrotic syndrome.

Limitations

Inspite of the great progress made in recent years, serodiagnosis of parasitic infections, still has many limitations. Most of the methods are laborious, time-consuming and difficult to perform. Only specially trained serologists can perform the tests, and expert knowledge is required for interpretations of the results. Certain tests require expensive equipment and reagents.

So far, no tests are available for the detection of early infections before production of detectable levels of antibody and as a result false negatives are common when dealing with early stage of infections. None of the available tests become negative immediately after an infection has been terminated, although in some infections like malaria and amoebiasis, titres generally decrease shortly after treatment. Therefore, a positive reaction may not indicate whether an infection is present or past, acute or chronic, primary or secondary. It may however, be possible to differentiate, an acute infection by rising titres over a few days or weeks. Often there is little or no correlation between severity of symptoms and level of antibodies. The results can be interpreted only with a thorough knowledge of the clinical picture and epidemiological data. Cross-reactions are often found among related species or at times even unrelated parasites.

Antigens

The most important prerequisite in serodiagnostic tests is the availability of sufficient quantities of specific antigen. Fife (1971) emphasized the fact that no serological test is better than the antigen used. The source, nature, purity, and specificity of the antigens are important factors in determining the specificity, sensitivity and reproducibility of the tests. Most parasitic antigens presently used are mixtures of specific and non-specific componants which have been specially prepared for use in one or more tests. These complex mixtures may contain antigens that are shared by other related or even unrelated parasites which results in cross-reactions. The cross-reactions have been shown (Kent, 1963) to be due to similarities in antigenic constituents that may be present in different organisms or due to the molecular rearrangement of antigens due to treatment during their preparation. Other factors that may further influence the suitability and efficiency of the antigen are the particular stages of the life-cycle of the parasite that are used for antigens and the procedures which are employed for preparing, quantitating and testing. Best results are obtained when pure antigens prepared from homologous species are used.

Although various developmental stages of a parasite can be used as antigen, the stages in the life cycle which are available in large quantities are often used. Due to the biochemical and structural alterations that occur in the parasite during its life cycle, these stages vary in their antigenicity as has been shown in trematodes (Sadun and Gore, 1967) and nematodes (Williams and Soulsby, 1970). Most of the serodiagnostic investigations have been done using sematic antigens prepared from whole or part of the various stages of the parasite. Wienstein (1959) has shown that antigens present in the secretions and excretions of living helminths evoke a considerably higher degree of protective immunity in laboratory animals than do somatic antigens. However, isolation and purification of sufficient quantities of excretary and secretory antigens are difficult and therefore, only very few studies have been made using these antigens.

Antigens used are mainly of two types, the soluble and the particulate antigens. The soluble antigens have been used in complement fixation tests, precipitation test, and to sensitise various particles such as erythrocytes, bentonite, latex, cholesterol-lacithin complexes, charcoal-particles, etc., which may then agglutinate or flocculate in the presence of specific antibodies. Soluble antigens are also used in skin tests. Whole or parts of parasites or particulate antigens are used in Indirect fluorescent antibody (IFA) and direct antibody (FA) tests.

Among the many serological techniques that are available for detection of antibodies to parasitic infections three test types are of greater importance and these are, the complement fixation (CF) tests, the indirect haemagglutination (IHA) tests and fluorescent antibody (IFA) tests. For many parasitic infections it is a question of choice, which test is employed and the reliability of the results may depend more on the quality of the antigen rather than on the particular test used.

Complement fixation

Among the serodiagnostic techniques used for parasitic diseases, complement fixation is the oldest and one of the more widely used techniques. When performed under strictly standardized and optimum conditions, it is one of the most reliable, reproducible and sensitive tests. This test, now uses the 50 percent haemolysis end point instead of 100 percent haemolysis and employ spectrophotometer instead of somewhat emperical procedures for standardization of various reagents. The modified techniques are far superior to the earlier ones. However, different laboratories use different techniques devised within the accepted concepts of immunehaemolysis and complement fixation. At least some of the variations of the CF tests, can be performed as a microtitration procedure.

The use of CF tests is, however, limited by the very delicate techniques and complicated test systems which need precise standardization. It is a very laborious technique which can be performed only by competent and experienced serologists. The tests

cannot be used under field conditions. Unless the sera are rapidly transported to the laboratory and stored under optimum conditions, a larger percentage of the specimens develop anticomplementary properties which make them unsuitable for testing (Sadun, 1976). Buck *et al.,* (1970) have shown that a large number of sera from donors with nutritional deficiencies and infectious diseases may have low serum albumin and high gamaglobulin levels which may be anticomplementary and unsuitable for CF tests. The results obtained from different laboratories are not often comparable and may be due to the differences in the quality of the antigens and the variations in techniques used.

The CF tests have been successfully employed for serodiagnosis of amoebiasis (Kessel *et al.,* 1965), toxoplasmosis, malaria, trypanosomiasis, filariasis, schistosomiasis, paragonimiasis and echinococcosis (Kagan, 1974).

Indirect haemagglutination

Today IHA test is one of the preferred routine tests in the serodiagnosis and seroepidemiological investigations of many parasitic diseases. Its procedures are simpler and easier than those of most of the serological tests and no specialised equipment is required for performing or reading the tests. The introduction of microtitre plates has facilitated easy reading and has reduced considerably the volume of reagents required. Large numbers of sera can easily be tested and these tests can be performed even under certain field conditions. The test is very sensitive and reasonably reproducible when performed under standardised conditions. A serious problem encountered in the IHA technique however, is its lack of specificity which may be due to it high degree of sensitivity (Fife, 1971; Sadun, 1976). Various workers treat the erythrocytes with different chemicals such as tannic acid, formalin, other aldehydes or chromium chloride before the antigen is absorbed on the cell surface. Variation in reproducibility of the tests therefore, may be due to the differences in the erythrocytes used and the variations in the reagents and techniques used to sensitise and stabilise these cells. It may be possible to overcome these problems by strict standardization of all reagents and the use of pure antigen.

This test has been used statisfactorily in serodiagnosis of malaria (Stein and Desowitz, 1964); amoebiasis (Kessel *et al.,* 1965); toxoplasmosis (Jacobs and Lunde, 1957); filariasis (Kagan, 1963); visceral larva migrans (Kagan *et al.,* 1959); trichinellosis (Kagan and Bargai, 1956); schistosomiasis (Kagan and Oliver-Gonzalez, 1958) and echinococcosis (Garabedian *et al.,* 1959).

Indirect fluorescent antibody

The IFA technique is considered as the most sensitive of the standard serodiagnostic procedures (Fife, 1971, 1972). It can be performed on serum or on blood collected by finger prick and dried on filter paper (Anderson *et al.,* 1961). This makes the collection of the samples much easier and enhances its value as a tool in the seroepidemiological studies. It is a relatively rapid and simple test where particulate antigens are used. The preparation of comparable batches of antigen is relatively simple (Sulzer, 1965; Sulzer *et al.,* 1969; WHO, 1974; Thomas and Ponnamapalam, 1975).

In spite of these advantages, the IFA procedure has a few inherent limitations and great care must be taken in performing, reading and interpreting the reactions. The quality of the conjugate and the degree of conjugation of the antiserum influence the result and therefore care must be taken to standardise these factors. The microscopist must have considerable competence and experience in the fluorescent microscopy. The readings are subjective and are interpreted in relation to known positive and negative controls. Due to a higher sensitivity, this technique tends to give more false positive reactions than other less sensitive techniques. This can, however, be minimized by selecting a higher dilution as diagnostic titre. The test occasionally gives false negative reactions especially in early infections in children.

The IFA seems to be the preferred technique for the diagnosis of malaria (Wilson *et al.,* 1971, 1975, Collins and Skinner, 1972); toxoplasmosis (Miller and Brown, 1969; Remington *et al.,* 1968); leishmaniasis (Duxbury and Sadun, 1964) and trypanosomiasis (Fife, 1972). This technique has also been used in infections like amoebiasis (Ambroise-Thomas and Truong, 1969); visceral leishmaniasis (Araujo and Mayrink, 1968); schistosomiasis (Kagan *et al.,* 1965); facioliasis (de Azevedo and Rombert, 1965); trichinellosis (Sadun *et al.,* 1962) and filariasis (Mantovani and Sulzer, 1967).

In recent years, the availability of immunoglobulin class specific conjugate for use in the diagnosis of parasitic infections has greatly increased the usefulness of the immunofluorescent test. In addition to its use for serodiagnosis, the IFA procedure has the unique potential for basic studies on the immune response to parasitic infections. It has been shown (Reminton and Miller, 1966 and Remington *et al.,* 1968) that the IgM fluorescent antibody test is useful in diagnosis of acute acquired and congenital toxoplasmosis. Similar investigations

may be useful for other parasitic diseases and would possibly improve the pathognomonic value of immunodiagnostic technique. However, the specificity and the purity of the immunoglobulin class-specific conjugate must be insured for reliable results.

The IgM class-specific tests for toxoplasmosis, however, is not always disease specific and false positive results were obtained for patients with rheumatoid factor (Camargo *et al.*, 1972) and with antinuclear antibodies (Araujo *et al.*, 1971).

Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA) is a newly developed procedure which is a modification of IFA technique. In this technique, fluorescein isothiocyanate is replaced by alkaline phosphatase enzyme or horse-radis-hperoxidase in the anti-immunoglobulin conjugate. After the interaction between antigen, serum and enzyme conjugate has taken place, a suitable substrate is added which causes the enzyme in the antigen-antibody complex to become coloured. The colour change can be measured by a colorimeter or by eye. The intensity of the colour is directly related to the amount of antibody in the test serum. The assay has been used in Chagass disease (Ferreira *et al.*, 1975; Voller, 1977) toxoplasmosis (Ourth *et al.*, 1974); amoebiasis, filariasis and hydatid disease (Bout *et al.*, 1975) and trichinellosis (Ljungstrom *et al.*, 1974). The application of enzyme-linked immunosorbent assay has been discussed by Voller *et al.*, (1976).

Soluble antigen fluorescent antibody

The soluble antigen fluorescent antibody (SAFA) procedure developed by Toussaint and Anderson (1965) and Toussaint (1966) is a recent advance in the serodiagnosis of parasitic infections. This test is also a modification of the indirect fluorescent antibody technique and the soluble antigen is fixed on an artificial matrix (cellulose acetate filter paper disc) and the tests are read on a fluoremeter. The SAFA technique has certain inherent advantages over the IFA procedure in which intact organisms are used as antigen. The procedures are much simpler to perform and the fluorometer reading of the results eliminates subjectivity. In addition there is no "fade out" during reading of the tests. This test seems satisfactory for the serodiagnosis of a number of parasitic diseases like American trypanosomiasis (Toussaint *et al.*, 1965), amoebiasis (Gore and Sadun, 1968a); filariasis (Duxbury and Sadun, 1967; Schistosomiasis (Toussaint, 1966); echinococcosis (Gore *et al.*, 1970); and trichinellosis (Gore and Sadun, 1968b). The specificity and sensitivity of the SAFA tests for these infections are reported to be equal or superior to those of other standard serologic tests.

Flocculation

Flocculation tests have been used for the routine diagnosis of a few parasitic infections. Flocculation tests are similar to the IHA technique except that inert particles such as latex (Fischman, 1965), bentonite (Kagan *et al.*, 1963) or lecithincholesterol crystals (Anderson, 1960) are used as antigen carriers instead of intact red blood cells. These are simple tests which can be formed within a few minutes and need no special equipments. The antigen-sensitized particles can be stored for a few weeks without deterioration. However, false negative reactions are common, a one way cross-reaction between schistosome antigen and trichinella antibodies is known to exist (Anderson *et al.*, 1963).

Flocculation tests are extensively used in the routine serodiagnosis of schistosomiasis, trichinellosis and echinococcosis (Fife, 1971).

Preciptin and immunodiffusion

Precipitating antibodies are often shown in a gel diffusion system in which antibodies and antigens are allowed to diffuse towards each other from wells cut in slides or plates coated with agar. The reactants diffuse in all directions and only the small amounts meeting in the area between the wells can form precipitation lines. This method is thus very slow and insensitive. The test is relatively inexpensive and technically simple to perform and requires very little special equipments. This procedure has been successfully used for the serodiagnosis of anoebiasis (Maddison *et al.*, 1965) and other parasitic infections.

Counter-current immunoelectrophoresis (CIE) is a recent development in which both antigens and antibodies move towards each other when an electric current passes through the slide or plate. This increases the sensitivity and speed of the reaction. A double system in which the specimen for the test is placed in a central well with the antiserum on the anodal side and the antigen on the catodal side. The equipment needed for a CIE test system are simple and cheap. This test has been found to be more sensitive than simple gel diffusion to detect antibodies in amoebiasis (Kaupp, 1974). Its use in other parasitic infections has been discussed by Draper (1976).

Methylene-blue dye test

This is one of the oldest and most useful serodiagnostic methods for toxoplasmosis. It is based on the principle that when live *Toxoplasma gondii*

trophozoites are exposed to immune serum in the presence of an "accessory factor" they fail to stain with alkaline methylene blue (Sabin and Feldman, 1948).

Trophozoites and Toxoplasma are harvested from the peritoneal exudates from mice infected intraperitoneally 3 days earlier. Standard numbers of the washed trophozoite are mixed in serial dilutions of the test sera in the presence of an optimum concentration of accessory factor. The accessory factor is a heat-labile substance found in antibody free sera of certain donors. The organisms are incubated for 1 hour in a water-bath at 37°C. Alcoholic methylene blue at pH 7.0 is added after incubation.

In the presence of specific antibody in the test serum and the accessory factor, the cytoplasm of the *Toxoplasma* trophozoite becomes modified and partially lysed and does not stain blue. In this test the alkaline methylene blue does not take part in the reaction but is used as an indicator to distinguish between unmodified parasites modified organisms modified by antibody. It is a quantitative test and the highest dilution of the test serum which modify 50% of the *Toxoplasma* is considered the end point. The test can be done on slides and end points determined with the help of a microscope. It is a very sensitive and specific test for toxoplasmosis and cross-reaction with other protozoa are minimal.

However, the test has certain draw-backs. It requires the use of live organisms which make the test dangerous and this test is difficult to perform and laborious to read. Special care has to be taken by those who handle the parasites for the test. Very careful attention must be paid to many technical details including standardization of the "accessory factor" and the number of parasites per dilution.

Intradermal tests

Intradermal tests have been used for diagnosis of a variety of parasites especially for helminthic infections. These tests, however, are subject to serious inherent draw-backs which limit their value. Although sensitive, these tests are among the least specific of the immunodiagnostic techniques and show cross-reactions and false positive reactions. Therefore, these have limited value of the diagnosis of individual cases although these tests are still used for epidemiological surveys of infections such as filariasis (Sawada *et al.,* 1962), echinococcosis (Kagan *et al.,* 1966), trichinellosis (Kagan and Zaiman, 1964) paragonimiasis, colonorchiasis (Sadun *et al.,* 1959a and 1959b), schistosomiasis (Anderson and Naimork, 1960) and leishmaniasis (Imperate and Bradrick, 1969). Immediate hypersensitivity reactions are employed for the diagnosis of helminthic infections and delayed hypersensitivity reactions for protozoan diseases.

In conclusion, it must be stressed that serodiagnosis of parasitic diseases is far from being perfected and standardized. Although for diagnosis of certain diseases like toxoplasmosis, chagas disease, amoebiasis, trichinellosis and schistosomiasis are accepted, the serodiagnostic techniques for other infections are still in developmental stages. These require further evaluation and standardization before they are universally accepted for routine serodiagnosis of parasitic disease.

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