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Serodiagnosis of Parasitic Diseases

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INTRODUCTION

This review on the serology of parasitic diseases summarizes recent developments in and the present status of the field. Topics included are antibody detection, antigen detection, use of monoclonal antibodies (MAbs) in serodiagnosis, molecular biological technology, and skin tests. A survey of the literature from January 1985 to March 1990 was prepared, but some older references to the use of MAbs and molecular biological technology are included.

Except for the reference serum samples for toxoplasmosis, international standards for serologic antigens and antisera for parasitic diseases have not been developed. In published reports about the specificity of new assays, the studies frequently do not include adequate batteries of sera. Therefore, one cannot compare assays adequately from reports in the literature. Furthermore, the number of commercial laboratories offering serodiagnosis of parasitic diseases and the number of kits on the market are increasing. For these reasons, a specific assay cannot be recommended for each disease. However, this review will emphasize to the reader the need for requesting data about the sensitivities and specificities of the available assays for diagnosing parasitic infections to determine the best tests available.

The basic serodiagnostic tests, such as indirect hemagglutination, whole protozoan or antigen-coated particle agglutination, indirect immunofluorescence (IIF), radioimmunoassay, plate enzyme-linked immunosorbent assay (ELISA) with relatively crude antigens, and dot blot assays, continue to be used. Antigen detection in sera and body fluids is being more widely investigated. Recent advances in immunochemistry and molecular biology are resulting in assays of markedly improved sensitivity and specificity. This new technology is costly and not readily available in many lesser-developed countries in which most parasitic diseases occur (2). It is, therefore, of great importance that some approaches are being made to modify techniques to allow their use in field, epidemiologic, and chemotherapeutic studies.

ANTIBODY DETECTION

Applicability

For parasitic diseases for which parasitological proof of infection is difficult to obtain, serology with a sensitive and specific assay is a very helpful aid to diagnosis for the clinician. At the other end of the spectrum, serology is not indicated in those intestinal helminthic diseases in which parasite eggs are readily detected in fecal specimens. Table 1 lists parasitic diseases according to the usefulness of serologic diagnosis. Nonetheless, while not useful as a diagnostic tool for some parasitic diseases, serology may play an important role in epidemiologic and chemotherapeutic studies of these diseases. This is exemplified by the large number of reports directed to defining meaningful seroepidemiologic assays for malaria. These reports are discussed below in the section on recombinant antigens.

TABLE 1. Parasitic diseases listed by usefulness of antibody detection as an aid to diagnosis

Disease	Type of infection
Serology indicated	
Amebiasis	Extraintestinal
Chagas' disease	
Clonorchiasis	
Cysticercosis	
Hydatidosis	
Filariasis	Lymphatic; suspect cases when microfilariae cannot be identified in blood
Leishmaniasis	Cutaneous and visceral
Malaria	Transfusion malaria; suspect blood donors
Schistosomiasis	Ectopic cases; chronic cases when eggs cannot be demonstrated in feces or urine
Trichinellosis	
Toxocariasis	Visceral and ocular
Toxoplasmosis	IgM assays to identify congenital cases and recently acquired infection in pregnant women, immunocompromised patients, and the general population
Serology may be useful	
Amebiasis	Intestinal, particularly when well-trained technicians are not available to examine stools
Amebic meningoencephalitis	Caused by free-living amebae
Babesiosis	
Filariasis	Suspect cases when microfilariae cannot be detected
Giardiasis	
Gnathostomiasis	
Paragonimiasis	Eggs not detectable in sputum or feces
Serology not indicated	
Ascariasis	
Anisakiasis	
Capillariasis	
Cryptosporidiosis	
Hookworm	
Malaria (with exceptions listed above)	
Trichuriasis	

Availability of Commercial Products

Displays of commercial products at immunology and microbiology conferences indicate that a number of serologic kits are available for some parasitic diseases such as amebiasis and toxoplasmosis. In the United States, approval from the Food and Drug Administration (510K) is required to sell diagnostic kits. Unfortunately, a list of the approved products is not available. Serologic kits for parasitic diseases are also sold in South America, Europe, and Asia, but again, current lists of the products are not available. Simple, rapid tests have been designed to detect Chagas' infection in blood donors (42, 48).

Test Evaluation

Clinicians frequently have difficulty interpreting serologic data when the fundamental characteristics of the assay (significant diagnostic level of reactivity, sensitivity, and specificity) are not included in the report. The quality of the assay depends not only on the quality of the antigen but also on the selection of sera used to evaluate a new diagnostic procedure. Since no serologic assay is 100% sensitive and specific, a new diagnostic assay should not be evaluated against a previously used procedure. Instead, well-characterized batteries of serum specimens from parasitologically proven cases of homologous and other parasitic infections and from nonparasitic diseases which could present as

TABLE 2. Interpretation of serologic reactivity in assays for toxoplasmosis

Titer			Interpretation
IIF-IgG	IIF-IgM	Capture EIA[a]-IgM	
≤8	≤8	Negative	No evidence of exposure
>16	≤8	Negative	Infection probably acquired more than 1 yr ago
≥1,024	≥16	≥256	Infection probably acquired within the past yr
≥16	≥16	Negative	Past infection; negative EIA-IgM indicates false-positive IIF-IgM

[a] EIA, enzyme immunosorbent assay.

differential diagnostic problems should be tested. This procedure allows clear definition of sensitivity, specificity, and the diagnostically significant level of reactivity of each assay. For toxoplasmosis and toxocariasis, it is impossible to obtain batteries of serum specimens from parasitologically proven cases; therefore, clinical evaluation of the individual cases plays a role in selection of serum specimens to be tested (34).

Specific Methods

Capture enzyme immunosorbent assay (toxoplasmosis). At the Centers for Disease Control (CDC), as technology becomes available the serologic assays used in the Parasitic Reference Serodiagnostic Laboratory are updated. For example, the double-sandwich enzyme-linked immunosorbent assay (ELISA) for specific immunoglobulin M (IgM) class antibodies was modified by using peroxidase-labelled soluble toxoplasma antigen to develop a capture enzyme immunosorbent assay (28). Interpretation of the data depends on the levels of reactivity of the serum specimen in IIF for IgG and in capture enzyme immunosorbent assay for IgM antibodies (Table 2).

FAST-ELISA (schistosomiasis, malaria, fascioliasis, and leishmaniasis). The newer techniques of FAST-ELISA (36) and immunoblot (Western blot) (97) have also been introduced in the CDC Parasitic Reference Serodiagnostic Laboratory, primarily for diagnosing schistosomiasis. FAST-ELISA is a quantitative assay in which a standard reference serum pool from patients with proven homologous infection is used to construct a standard curve in each microtitration plate. The reference serum is arbitrarily designated as having 100 activity units per μl. The unknown serum samples are tested at a single dilution, in triplicate, and the levels of reactivity are derived from the standard curve. Results are reported as activity units per microliter on a continuous-response scale. When diagnostically significant levels of activity are defined on the report, physicians readily accept this change from the classical reporting of reactivities as titers. For the schistosomiasis FAST-ELISA assay at the CDC, the purified *Schistosoma mansoni* adult worm microsomal antigen (MAMA) is used (98). Cross-reactivity with serum specimens from patients infected with *S. haematobium*, *S. japonicum*, or *S. mekongi* is lower than that with sera from patients with homologous *S. mansoni* infections. Identification of the *Schistosoma* sp. causing infection is made with specific immunoblots, using microsomal antigens of *S. mansoni*, *S. haematobium*, and *S. japonicum*, respectively.

FAST-ELISA is a very sensitive assay. Therefore, if crude antigens are used, cross-reactivity as well as specific reactivities are enhanced. FAST-ELISA is finding application in a number of serologic systems in addition to schistosomiasis. It has been used with synthetic peptide and recombinant antigens from *Plasmodium falciparum* (10) and undefined antigens for fascioliasis (40) and leishmaniasis (106).

Immunoblot (paragonimiasis, hydatidosis, and cysticercosis). In contrast to FAST-ELISA, specific immunoblot assays may be developed by using crude antigen extracts of some parasites because the antigenic components are separated during the gel electrophoresis stage of the assay. If probing the blot with serum specimens from homologous infections demonstrates a specific band(s) that is not detected with serum specimens from putative cross-reacting infections or diseases, no further purification of the antigen is necessary. At the CDC, immunoblot assays have been developed with crude extracts of *Paragonimus westermani* (86) and *Echinococcus granulosus* (59), with specificities of 98 and 100% and sensitivities of 96 and 98%, respectively. A second immunoblot study of *E. granulosus* antigens showed results similar to those obtained at the CDC (54).

Undoubtedly, the immunoblot for neurocysticercosis is the assay that most significantly improved diagnosis of a parasitic disease at the CDC (96). A semipurified extract of cysticerci of *Taenia solium* is used as the antigen. It has seven major glycoprotein bands with apparent molecular masses ranging from 50 to 13 kDa. A serum reaction with one or more of these bands is indicative of cysticercosis. The sensitivity is 98% and the specificity is 100%. Infection with a single parietal cyst is usually detectable. Subcutaneous infections without demonstrable neurologic involvement are also usually detectable with this assay.

FAST-ELISA and immunoblot methods (field studies). More recent modifications to reduce incubation times in the immunoblot assay from 1 h to 5 min have resulted in a RAPID BLOT assay without reduction of sensitivity and specificity (6). FAST-ELISA has been adapted for use in field studies. Antigen-coated beads that are stable for at least 6 months are reacted with finger prick blood collected in heparinized capillary tubes which are immediately dropped into tubes containing isotonic buffer and 0.2% sodium azide (75). No refrigeration is required before testing. The FAST-ELISA beads are washed with local nondeionized water, and readings are made with the unaided eye. Two other groups of investigators have found that capillary-collected blood transferred to diluent with preservative is superior to filter paper-collected blood in both field and large epidemiological studies. They used either phosphate-buffered saline and sodium azide (47) or disodium EDTA (80). Serologic investigation of AIDS has clearly shown that antigen-blotted strips, ready for probing with patients' serum samples, can be produced in large quantities and used in field studies. Thus, both FAST-ELISA and the immunoblot assay can be used in developing countries for primary diagnosis and epidemiologic surveys.

FAST-ELISA and immunoblot methods (monitoring therapy/fascioliasis). FAST-ELISA and immunoblot methods incorporating secretory and/or excretory antigens of *Fasciola hepatica* were used to study the specific antibody response of humans to infection with *F. hepatica* (40). The FAST-ELISA appeared to be sensitive and specific. Following chemotherapy with praziquantel, those patients who were cured showed an initial small rise in antibody level and then a gradual decrease. In contrast, the patients who were not cured maintained high antibody levels throughout the 2- to

3-year period of follow-up. In immunoblots, a specific band with an apparent molecular mass of 17 kDa was identified.

Relationship between Immunoglobulin Class or Subclass and Specificity of Response

Recently, the relationship between the class of immunoglobulins or subclass of IgG and the specificity of response to parasitic infections has been recognized. As a result, improved serodiagnosis has been reported in toxoplasmosis, several filarial diseases, strongyloidiasis, and hydatidosis, as discussed below.

Toxoplasmosis. The capture IgM ELISA (28) is a technically simple assay for identifying acute and congenital cases of toxoplasmosis, as discussed above. A double-sandwich ELISA for detecting specific IgM antibody (DS-IgM-ELISA) was developed for toxoplasmosis (82) and has a wide application. A single dilution of serum is tested in triplicate, and the level of reactivity is determined from a calibration curve constructed from positive and negative sera, the same principle as FAST-ELISA (36). These steps resulted in increased reproducibility. The DS-IgM-ELISA allowed differentiation of early- and late-stage infections with *Toxoplasma gondii*. Recently, a DS-IgA-ELISA, performed in a similar way, also differentiated acute from chronic disease in patients with toxoplasmosis (89). Specific IgA class antibodies were demonstrated in eight of nine congenitally infected fetuses and in acute but not chronic infection in adults. IgM class antibodies could not be detected in some of these specimens. Only 1 of 20 AIDS patients with biopsy-proven toxoplasmic encephalitis was positive. The authors recommended that tests for IgA antibody be included in assays for toxoplasmosis in pregnant women and congenitally acquired infections in the newborn when the disease is suspected.

Bancroftian filariasis. In bancroftian filariasis, clinical manifestations of the disease correlated with antibody subclass recognition of antigens of different molecular weights (44). Microfilaremic patients showed stronger IgG4 reactivity in immunoblot to antigens with molecular masses of >68 kDa than did patients with chronic lymphatic pathology. The latter group had little IgG4 reactivity to any antigens. In a plate ELISA the use of a peroxidase-labelled anti-IgG4 conjugant eliminated the nonspecific cross-reactivity with phosphocholine, which is a widely occurring molecule in filarids (52). Initial studies with two recombinant DNA *Brugia malayi* antigens indicated differential recognition of these antigens by IgG and IgE classes (104). Studies of the IgG isotypes remain to be performed.

Onchocerciasis. The IgM class and IgG isotype responses to *Onchocerca volvulus* antigens of patients with generalized manifestations of onchocerciasis and of others with Sowda were investigated in immunoblots (8). Responses of all patients were essentially similar except that the Sowda patients had a very marked IgG3 response to two major antigens with apparent molecular masses of 72 and 9 kDa, respectively. The reaction with the 9-kDa antigen was detected more consistently than that with the 72-kDa antigen. The IgG4 response resembled that of total IgG and showed cross-reactivity in the sera of patients with generalized onchocerciasis, bancroftian and Malayan filariasis, and, to a lesser extent, with *Mansonella ozzardi* and other helminthic infections. Subsequently, this group of investigators used low-molecular-mass (16.2- and 12.8-kDa) antigens from the surface of *O. volvulus* adult worms (9) in direct-plate ELISA and an inhibition plate ELISA. In the latter, a MAb recognizing 15.6- and 25.9-kDa *O. volvulus* antigens was used to study the same sera in the report discussed above. ELISA using the low-molecular-weight antigen fraction and anti-IgG4 conjugate as well as the MAb inhibition ELISA improved specificity of the detection of *O. volvulus* infection. However, cross-reactivity with bancroftian filariasis and, to a lesser content, *M. ozzardi* infection remained.

Strongyloidiasis. Detection of IgG4 reactivity in ELISA also resulted in improved sensitivity of the assay for *Strongyloides stercoralis* infection (32), in both uncomplicated infections and, to a lesser extent, immunocompromised patients with strongyloidiasis.

Hydatidosis. When crude preparation of hydatid cyst fluid was used as antigen in plate ELISA, detection of IgE class antibodies in patients with hydatidosis resulted in high test sensitivity (85). When evaluated with serum from 78 patients with other parasitic infections, the specificity was 81%. However, 11 of the 15 patients with false-positive reactions had markedly elevated total IgE levels.

ANTIGEN DETECTION

Because antibody usually persists for a year or more after the acute phase of parasitic diseases, demonstration of antibody rarely can differentiate between acute and chronic infection. For the same reason, measurement of antibody levels before and after chemotherapy gives little or no indication of treatment efficacy. Measurement of circulating parasitic antigen or antigen in urine or feces would more appropriately indicate the presence of active infection and, furthermore, might indicate parasite load. In other instances, demonstration of specific parasite antigen in lesion fluid, such as amebic liver abscess "pus" or fluid from a hydatid cyst, would provide definitive diagnosis of the infecting organism.

The literature up to 1984 on the detection of antigens in serum samples and other body products for diagnosing parasitic diseases in humans has been reviewed recently (58). Eighty-seven additional reports identified from January 1985 to May 1990 are grouped in Table 3 by disease and type of specimen examined. The types of assays used in these studies are shown in Table 4. Since 1984, there has been a pronounced shift away from radioimmune assays that use isotopes to ELISAs. Not all 87 reports are referenced in this review, but to summarize developments in antigen detection, reports on diseases for which a number of studies were made are discussed below.

Amebiasis

Use of a sandwich ELISA to detect *Entamoeba histolytica* antigen in amebic liver abscess pus showed promise, with sensitivity and specificity of 93 and 94%, respectively (1). By using blot ELISA, the need to concentrate serum by precipitation with polyethylene glycol prior to detection of circulating antigen-antibody complexes was eliminated (33). The test was positive for 75% of 47 patients with amebic liver abscess. In a radioimmunoassay which detected immunologically bound, but not free, amebic antigen, all 10 patients with amebic liver abscess had detectable circulating immune complexes (65). However, only 79% of 24 patients who were stool positive for *E. hystolytica* had circulating amebic immune complexes.

Examining stool specimens for amebiasis is unreliable unless done by technicians skilled in recognizing intestinal protozoa. An alternative is a specific assay for detecting

TABLE 3. Diseases and specimens discussed in 87 reports on antigen detection in parasitic diseases (published January 1985 through March 1990)

Disease	Specimen	No. of reports
Protozoan		
Amebiasis	Serum	5
	Liver abscess fluid	2
	Feces	5
Chagas' disease	Serum	5
	Urine	1
Giardiasis	Feces	7
Leishmaniasis	Serum	2
Malaria	Serum	5
	Urine	2
Pneumocystosis	Serum	3
Toxoplasmosis	Serum	7
	Urine	1
Helminthic		
Cysticercosis	Cerebrospinal fluid	3
Echinococcosis	Serum	7
	Cyst fluid and serum	1
Fascioliasis	Serum	2
Filariasis, bancroftian	Serum	11
	Urine and serum	2
	Urine	1
	Hydrocoele fluid	1
Filariasis, Malayan	Serum	1
Onchocerciasis	Serum	3
	Breast milk	1
	Urine	2
Schistosomiasis	Serum	4
Toxocariasis	Serum	3

TABLE 4. Assays discussed in reports on antigen detection in parasitic diseases (published January 1985 through March 1990)

Assay	No. of reports
ELISA	53
Polyethylene glycol ELISA	7
Immunoradiometric assay	8
Polyethylene glycol immunoradiometric assay	2
Dot blot	4
Countercurrent immunoelectrophoresis	5
Gel diffusion	2
Immunoblot	2

antigen in fecal specimens. One assay that used a MAb for capture and a rabbit anti-*E. histolytica* polyclonal antibody for a probe in an ELISA was 82% sensitive when used to test 22 stools from patients known to be positive for *E. histolytica* (99). The specificity was 98% in terms of patients harboring other parasites. Another ELISA for antigen detection used a polyclonal anti-*E. histolytica* serum for capture and an *E. histolytica*-specific MAb for a probe (62). Of the 116 individuals in whose feces antigen was detected by ELISA, *E. histolytica* was detected in only 52 by feces examination or rectal smear. The authors concluded that the antigen detection ELISA was more sensitive than examination of specimens for demonstration of the organism.

Giardiasis

The above approach is also being investigated for giardiasis since the intermittent passage of cysts and the resultant need to examine multiple stool specimens are problems, particularly in outbreak studies. *Giardia lamblia* antigen was detected in fecal samples more frequently during patency of the infection than were cysts detected by microscopic examination (69). The investigators also conclude that the antigen detection assay was easier to perform than microscopic examination. Antisera directed against *G. lamblia* cysts were used in an ELISA to detect antigen in stool eluates (91). Antigen could be demonstrated in both formalinized and unfixed stool specimens. Changes in antigen levels were useful in monitoring the effect of chemotherapy for giardiasis. A MAb was subsequently developed against *G. lamblia* cysts for an antigen capture test (90). The sensitivity of the resulting ELISA was 97% with formalinized stools, but it dropped to 82% with unfixed specimens. The assays appear to be specific for *Giardia* spp. Studies of the capture antigen indicated that it was heat stable, periodate resistant, and probably a carbohydrate occurring in multiple molecular weights.

Malaria

The level of sensitivity for detecting circulating antigen in malaria correlated with a 0.001% level of parasitemia regardless of whether conventional plate ELISA (27) or dot blot (57) was used. Trials are under way for the use of dot blot in malaria field studies. Double-sandwich dot blotting of unconcentrated urine was positive in 91% of 45 patients infected with *Plasmodium falciparum* and in 86% of 35 patients infected with *P. vivax* (49). However, the levels of parasitemia were not given.

Toxoplasmosis

The increased number of reports that demonstrate circulating antigen in persons with toxoplasmosis may reflect the increased recognition of the need to confirm active infection in patients who are immunosuppressed, pregnant women, and congenital cases. Simultaneous detection of IgM antibodies to *T. gondii* and of circulating antigen indicates early active infection (11, 55, 100). More recently, the need for rapid diagnosis of acute toxoplasmosis in AIDS patients has prompted the development of an assay for antigenemia in stage III and IV AIDS patients in Austria (37). Antigen was detected in the serum but not in the cerebrospinal fluid of 26% of patients with stage IV and only 6% of patients with stage III of the disease. Immunoblot studies indicated that circulating antigen consisted of at least two parasite components with apparent molecular masses of 27 and 57 kDa, respectively.

Visceral Leishmaniasis

A competitive ELISA was developed for detection of circulating antigen in kala azar (84). Patients' sera competed with peroxidase-labeled leishmania antigen for reactivity

with hyperimmune serum preabsorbed to ELISA plates. This initial study detected circulating antigen in 100% of 20 parasitologically proven cases of kala azar. The assay appeared to be species specific as antigen was not detected in the sera of six patients with cutaneous leishmaniasis. In another study, antigen-specific circulating immune complexes were precipitated with polyethylene glycol and tested in an ELISA (88). Immune complexes were detected in 100% of 27 parasitologically proven cases of kala azar, 64% of clinically diagnosed cases, 59% of treated cases, and 5% of healthy controls. The author concluded that the assay was potentially useful for monitoring the effect of chemotherapy.

Hydatidosis

Echinococcal antibodies were found in serum samples from only 25 to 40% of Turkana patients with hydatidosis (15). However, specific immune complexes in serum specimens from five of seven antibody-negative patients indicated that antigens were complexing with and masking antibody in the serum. This study also indicated that specific IgM immune complexes were associated with active infection.

Cysticercosis (Cerebral)

Although the diagnosis of cerebral cysticercosis has been markedly improved by the introduction of computerized tomography and magnetic resonance imaging, demonstration of specific *Taenia solium* antigen in cerebrospinal fluid would be a useful adjunct. Two antigens with apparent molecular masses of 190 and 230 kDa were demonstrated in the cerebrospinal fluid of 14 of 18 patients with suspected neurocysticercosis (24). Nine of these cases were confirmed by histopathology.

Bancroftian Filariasis

A comparatively large number of studies report detection of antigen in serum specimens, urine, or hydrocoele fluid in bancroftian filariasis, because it is difficult to obtain a parasitologic diagnosis of the chronic lymphatic form of the disease. Indian investigators have been particularly active in this field, using a *B. malayi*-specific rabbit antibody in an immunoradiometric assay (73). Urine and finger prick blood specimens collected on filter paper were found to be suitable for antigen detection in patients with microfilaremia; specimens from patients with chronic infections were negative. However, in another study, circulating antigen was found in 60% of serum samples from amicrofilaremic patients with manifestations of obstructive lymphatic filariasis (43).

A MAb-based ELISA for detection of circulating *Wuchereria bancrofti* antigen was more sensitive than detection of microfilariae in patients with active infection (103). In addition, unlike microfilarial counts, levels of circulating antigen did not fluctuate between night and day, and the assay was useful in monitoring the effects of chemotherapy. When finger prick blood specimens were used in this assay, whole blood samples were satisfactory, but use of specimens dried on filter paper resulted in reduced test sensitivity (80). To establish microfilarial prevalence rates and levels in an endemic area, the authors suggested that the assay for antigenemia using whole blood collected during the day be used as a screen. All positive reactors should then be bled at night for microfilarial counts. In a hyperendemic area in Mexico, an antigen detection ELISA for *O. volvulus* showed correlation between microfilaria levels observed in skin snips and antigen levels detected in serum and urine (81). It has not yet been determined whether the circulating antigens are associated with both microfilariae and adult worms.

Toxocariasis

By using a MAb, an ELISA for *Toxocara canis* circulating antigen differentiated between *T. canis* and *T. cati* infections in mice (77). In a small study of human infections, these investigators detected antigen in some sera from patients with visceral larva migrans or ocular toxocariasis. The assay is being fully evaluated.

Schistosomiasis

The most widely recognized and studied circulating antigens in human and murine infection with *S. mansoni* are the circulating cathodic antigen and the circulating anodic antigen (CAA) (18). These polysaccharides are gut associated in adult *S. mansoni* worms. Circulating cathodic antigen shared some characteristics with the phenol sulfuric test active peak antigen but was shown to differ in comparative studies with MAbs (68). With similar techniques, CAA and the gut-associated proteoglycan were shown to be identical. The level of CAA correlated well with egg counts in feces (21) and showed a rapid drop within 10 days of chemotherapy with praziquantel (19). In addition, CAA was detected in the urine of 81% of patients with *S. mansoni* intestinal infections and 97% of patients with *S. haematobium* urinary infections (20). *S. haematobium* egg counts ranged from 5 to 3,000 eggs per 10 ml of urine. However, urine had to be concentrated fivefold and dialyzed before antigen testing. The assay would, therefore, be of greater practical value in epidemiologic and chemotherapeutic studies of *S. mansoni* infections than *S. haematobium* infections. However, the use of a homologous antigen might increase the sensitivity of the assay for schistosomiasis haematobium and eliminate the cumbersome urine concentration procedures. Detection of CAA in ELISA by using MAbs was reported (17). A second, stable, gut-associated polysaccharide has been detected in the urine of patients infected with *S. mansoni* or *S. haematobium* (76). This polysaccharide, however, differs from those described above (68) in that, in addition to being gut associated in adult worms, it also occurs in large amounts in *Schistosoma* eggs. Antigen in urine was demonstrated by a hemagglutination inhibition assay. Erythrocytes were sensitized with an egg extract antigen. Concentrated urine samples and diluted MAb were allowed to react in a microtiter plate for 30 min before the sensitized cells were added. As counts of *S. mansoni* and *S. haematobium* eggs were not recorded in this study, the intensity of infection was not indicated. Antigen was detected in the urine of 57% of patients passing *S. mansoni* eggs in their stools and in 47% of patients passing *S. haematobium* eggs in their urine. Antigen was not demonstrable in 200 urine samples from individuals who had never been in an area endemic for schistosomiasis.

USE OF MAbs IN SERODIAGNOSIS

MAbs have been produced against antigens from most parasites known to be pathogenic to humans. The production of most MAbs has involved fusion of antigen-stimulated mouse cells with a mouse myeloma cell line. Recently, Epstein-Barr virus-activated B cells from *S. mansoni*-infected or vaccinated humans were fused with a mouse-human heteromyeloma cell line. The resulting five human

MAbs are presently being characterized (35). Most investigations with MAbs have been directed to studying host-parasite relationships, particularly, malaria and schistosomiasis. Nonetheless, MAbs have been used in three ways to improve serodiagnosis.

Identification and Purification of Antigenic Components

The immunoblot method is used to identify MAbs that react with antigenically important components of crude or partially purified parasitic extracts. The MAbs are then used to construct immunoaffinity columns for purifying the specific antigenic components from parasitic extracts (79) or from the supernatant fluids of cultured parasites. Biochemical and immunochemical characterizations of the purified components are then possible.

By using this approach, two antigens were purified from *Leishmania donovani*. Their molecular masses were approximately 70 and 72 kDa (46). When used in a dot blot assay with sera from patients with visceral leishmaniasis, the test sensitivity was 90 and 100%, respectively. The specificity was 98 and 93%, respectively. Cross-reactions were not observed with the sera of patients with cutaneous or mucocutaneous leishmaniasis or Chagas' disease. The assays could readily be standardized by measuring the level of reactivity of a visceral leishmaniasis reference serum pool with each new batch of antigens.

Competitive Inhibition Assays for Antibody

Schistosomiasis. In a competitive inhibition assay, the extent to which a patient's antibody inhibits the reactivity of any MAb with crude parasite antigen is determined in ELISA, radioimmunoassay, or indirect hemagglutination. This type of ELISA, although more cumbersome than the usual serum dilution ELISA, may result in assays with improved specificity. A MAb specific for adult worm *S. japonicum* antigen was used in a competitive ELISA, using a crude adult worm antigen. Positivity correlated well with the classical circumoval precipitin test, and the assay provided quantitative information important for monitoring the effects of chemotherapy (64).

Chagas' disease. The above approach has also been successful in the development of an assay for visceral leishmaniasis (45). An inhibition assay with a MAb against an M_r-25,000 epimastigote antigen of *Trypanosoma cruzi* was positive with sera from 41 patients with chronic Chagas' disease in Brazil, Paraguay, and Ecuador (94). No cross-reactivity was observed with sera from 5 leishmania patients or 24 patients with other protozoal disease.

In Bolivia, where both Chagas' disease and mucocutaneous leishmaniasis occur, a MAb inhibition assay with a "component 5"-enriched antigen of *Trypanosoma cruzi* eliminated cross-reactivity of antibody from the leishmania cases (53). As the antigen preparation did not require complex technology, the authors advocated the assay for mass studies in areas where the two causative organisms coexist.

Measurements of Antigen in Serum, Body Fluids, or Excretions

A third way of using MAbs is as capture antibodies in ELISA to detect circulating antigen (25, 26) or antigen in body fluids or feces (99). The captured antigen can be detected and quantitated by probing with an enzyme-conjugated, antigen-specific antiserum. If the capture MAb recognizes a repeating epitope on the antigen molecule, the same MAb may be used as the indicator probe. If the epitope does not repeat, a second MAb recognizing a different epitope on the same antigen molecule or a polyvalent antiserum is used as a probe to ensure accessibility to the second epitope site. An alternative is to reduce the size of the probe molecule by preparing $F(ab')_2$ from the antibody used as a probe. If different species of animals are used to produce the capture and indicator immunoglobulins, reduction of the capture antibody to $F(ab')_2$ will eliminate any cross-reactivity between the capture and probe antibodies (51). Recently developed assays with MAbs as capture antibody for antigenemia include those for bancroftian filariasis (80, 103), schistosomiasis (17–19, 21, 76), toxocariasis (77), amebiasis (99), and giardiasis (69, 90, 91). These have been discussed in the preceding section.

MOLECULAR BIOLOGICAL TECHNOLOGY

Many molecular studies of parasites pathogenic for humans are directed to mapping the genome and to gene regulation of parasite development stages (83). Some studies have resulted in production of DNA probes from parasites. When hybridized to DNA from organisms causing human infection, the probes are highly specific diagnostic and epidemiologic tools (105). This subject has recently been reviewed extensively (4) and will not be discussed here because it is not a serologic technique.

Other molecular biological studies have concentrated on protective antigens (71) and vaccine production (3, 92). The subject of control of parasitic diseases by vaccines has also been reviewed recently (61).

Antigen Production (Hybridization)

***Trypanosoma cruzi* and *Leishmania mexicana*.** A new approach to antigen production is the hybridization of viable living parasite cells with mouse myeloma cells. The epimastigotes of *T. cruzi* were fused with mouse myeloma P3-x63-Ag8 cells. The hybridomas expressed *T. cruzi* antigen during 14 weeks of serial cultivation (16). Promastigotes of *L. mexicana* were successfully fused with mouse myeloma SP2/0 cells. *Leishmania* antigen could be detected in initial cultures over a period of 45 days (95). The hybrid cells, however, died after this period. Attempts to passage them both in vitro and in vivo were unsuccessful.

***Echinococcus multilocularis*.** Two attempts to develop helminthic antigens from hybridomas have been reported. *E. multilocularis* germinal cells were fused with the mouse myeloma cell line P3-x63-Ag8.653 (23). Colonies of hybridized cells remained viable for up to 10 weeks, and parasite antigens could be demonstrated by IIF on the surface of 87% of cells and appeared to be specific for *E. multilocularis*. Murine antigens were observed in 65% of the cells. Neither continuous cultures of the hybridized cells nor in vivo cultures in mouse peritoneal cavities could be established.

***Fasciola hepatica*.** Continuous cultures of hybridized cells of egg-free female *F. hepatica* adult worms and SP2/0 mouse myeloma have been maintained for over 1 year (39). Treatment of the hybridized cells with anti-*F. hepatica* serum and a fluorescein-labeled conjugate followed by flow cytometry showed that 57% of cells from a 7-month-old culture had combined with the specific antibody. Mice immunized with antigen expressing hybridoma cells developed antibodies

against *F. hepatica*. The sera of these mice when tested in immunoblot assays against *F. hepatica* excretory-secretory antigens reacted with a 57-kDa antigen. This study clearly shows that parasite antigens can be produced by a hybridization technique similar to that extensively used for production of MAbs.

Antigen Production (Recombinant DNA Technology)

The use of recombinant DNA technology to produce serodiagnostic antigens has resulted in reagents of diagnostic promise for a number of parasitic diseases.

Echinococcus multilocularis. A recombinant polypeptide, II/3, was identified as a potentially sensitive, specific diagnostic antigen for *E. multilocularis* (101). By using the immunoblot assay, specific bands of 33 and 31 kDa were identified. In further studies (67), to obtain a large quantity of purified antigen, a shortened fragment of antigen II/3 encoding cDNA in a plasmid vector was expressed in *Escherichia coli*. The purified product (antigen II/3-10) was used in an ELISA. Tests with sera from well-documented cases of alveolar hydatidosis (n = 88), cystic (*E. granulosus*) hydatidosis (n = 108), 110 patients with other helminthic infections, and 355 Swiss blood donors showed 90% sensitivity and 99% specificity. The authors thus considered antigen II/3-10 to be a potentially useful serodiagnostic antigen.

A second team of investigators (38) produced two recombinant *E. multilocularis* antigens, EM2 and EM4. EM2 had an apparent molecular mass of 70 kDa, and EM4 was identified as 62-, 49-, and 144-kDa bands in immunoblot. The native antigens of both clones are present in *E. granulosus* protoscolices and *Taenia solium* cysticerci but were not detected in *E. granulosus* cyst fluid. This may explain the absence of responsiveness of sera from patients with *E. granulosus* or *Taenia solium* infections to these cloned antigens.

Schistosoma mansoni. In preliminary studies with infected mouse and human serum specimens, an in vitro translation product, p40, from *S. mansoni* egg poly(A)$^+$ mRNA (14) and a fusion product of part of a 31/32-kDa *S. mansoni* adult worm purified antigen (50) showed good serologic activity.

Onchocerca volvulus. A species-specific recombinant antigen derived from adult female worms of *O. volvulus* has been isolated (56). The antigen has an apparent molecular mass of 16 kDa and is a potentially useful serodiagnostic reagent.

Toxocara canis. Examination of in vitro translation products from mRNA of *T. canis* by immunoprecipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis indicated that a 27-kDa polypeptide reacted strongly with a serum specimen from a patient with toxocariasis (93).

Malaria. Extensive investigations of synthetic peptide and DNA recombinant antigens have been conducted by malariologists. Antigens have been derived from sporozoites, infected erythrocytes, and merozoites. The antigens have been developed over 5 years and have been evaluated with the sera of large groups of individuals in endemic areas mainly in Central Africa and to a lesser extent in Asia and South America. The ages of the patients ranged from 2-month-old infants to adults.

Results of several serologic studies on the use of the repetitive sequence of the *P. falciparum* circumsporozoite (CS) protein (NANP) as antigen have been reported. The frequency of positivity increased with age in patients from both Kenya (14) and Gabon (13). Three synthetic peptides derived from ring-infected erythrocyte surface antigens were tested in parallel with the CS synthetic peptide in an endemic area (10). The frequency of antibody reaction against one of the ring-infected erythrocyte surface antigen-derived peptides did not increase with age of the individuals. FAST-ELISA was used in this study.

A fusion peptide, 31.1, corresponding to a merozoite surface antigen has been described previously (31). When tested in parallel in ELISAs with a 41-kDa polypeptide associated with rhoptries of merozoites [organelles at the end of the merozoite as it enters the erythrocytes; they possibly secrete a substance(s) enabling penetration of the erythrocyte], the two assays allowed quantitative determination of antimalarial antibodies in children and adults.

A recombinant peptide, R32tet$_{32}$, with the structure MDP [(NANP)$_{15}$ NVDP]$_2$ fused to 32 amino acids of a tetracycline resistance gene was derived from the CS protein repeat region (7). The R32tet$_{32}$ peptide was used in an ELISA and compared with a sporozoite IIF in a study of acute and convalescent sera from Thai patients. IgG antibody responses were determined in both assays. Parallel response curves in the two assays were observed with the individual patients. The authors concluded that the R32tet$_{32}$ peptide was suitable for detection of *P. falciparum* infections in both acute and convalescent stages.

A World Health Organization-based comparison of IIF, using *P. falciparum* sporozoites, peptides (NANP)$_3$, (NANP)$_{40}$, F2-CAT (chloramphenicol acetyltransferase), and M6-DHFR (dihydrofolate reductase), and recombinant peptides R32tet$_{32}$ and R32LR, was made with sera from individuals living in malaria-endemic areas in Africa, Asia, and Central and South America (22). ELISAs using peptides that contained the highest number of NANP repeats showed good correlation with and were more sensitive than IIF. The (NANP)$_3$ peptide showed closer agreement with the IIF. The NANP-based peptide antigens provide a useful tool for epidemiologic and control studies.

Recombinant antigens F2-CAT and M6-DHFR derived from merozoites of *P. falciparum* were used to study patients with acute symptoms of malaria presenting at a hospital in Germany after travel in Africa. ELISA plates were sensitized with F2-CAT and M6-DHFR and reacted with patients' sera. To avoid reaction of the sera with bacterial antigenic components in these merozoite recombinant antigens, aliquots of the antigens were fused to β-galactosidase. Specific antibodies in the sera reacted with and bridged the F2-CAT/M6-DHFR-adsorbed antigens and the β-galactosidase fusion antigens. Enzyme activity of the β-galactosidase indicated positive reactions. The resulting β-galactosidase bridge ELISA appeared to be sensitive, with very little background activity. Recombinant *P. falciparum* peptides were compared in ELISA by using sera of individuals living in The Gambia. A mixture of the three peptides, (i) a conserved portion of the 190- to 200-kDa schizont merozoite component, (ii) the carboxy-terminal part of *P. falciparum* aldolase, and (iii) the 5.1 antigen, derived from the erythrocyte stage and having one or more epitopes cross-reactive with the CS protein, showed the highest sensitivity (87). The assay is potentially suitable for detection of antimalarial antibodies directed against asexual blood stages of the parasites in children as well as in adults.

The dynamics of antibody responses in children living in a mesoendemic area for *P. falciparum* in Gabon was studied (12). The three antigens used were (i) the synthetic peptide (NANP)$_{40}$ representing the CS antigen, (ii) the fusion protein 31.1 representing the 190- to 200-kDa glycoprotein of merozoites, and (iii) a mixture of infected fixed erythrocytes for the surface antigen (ring-infected erythrocyte surface anti-

gen) and unfixed infected erythrocytes for the intraerythrocytic asexual form antigen. The first two antigens were used in ELISA and the third was used in IIF. In infants up to 2 months of age, the percentages with antibodies against the intraerythrocytic asexual form, $(NANP)_{40}$, and 31.1 antigens were 50, 26, and 21%, respectively. This represents passively transferred maternal antibody. No anti-ring-infected erythrocyte surface antigen antibodies were detected. A decrease in antibody levels occurred between the ages of 2 and 65 months and thereafter increased progressively. By 15 years of age, the levels of antibodies were approaching those of adults. However, antibodies to the intraerythrocytic asexual form antigen developed earlier than did those to $(NANP)_{40}$.

Trypanosoma cruzi. A recombinant antigen, associated with trypomastigotes but not epimastigotes of *T. cruzi*, was recognized by sera from patients with acute Chagas' disease (29). A second fusion antigen was recognized by sera from patients with chronic Chagas' disease. Neither antigen cross-reacted with sera from patients with leishmaniasis. If further studies confirm these observations, a useful serologic test for distinguishing the two stages of Chagas' disease would be available.

SKIN TESTS

For some diseases, parasitologic diagnosis is rarely possible without biopsy or surgery, and skin tests have been recognized as simple inexpensive aids to diagnosis. An example of an immediate hypersensitivity reaction was a positive Casoni test for hydatidosis. The antigen was crude hydatid fluid. Delayed hypersensitivity reactions to leishmanin antigen (whole promastigotes of *L. donovani*) occurred in patients whose Montenegro tests were positive for visceral leishmaniasis. However, several problems have been associated with skin tests for parasitic diseases. Reference standards for skin test antigens and for performance, including reading, of the tests have not been developed. Most antigens are crude extracts of parasites and are neither highly sensitive nor specific. The danger of provoking anaphylaxis by injecting skin test antigen, particularly in patients with helminthiases, must be considered. Skin tests may also induce serologically detectable antibody which could complicate subsequent serologic evaluation of a patient. More recently, the ethics of injecting foreign protein antigen into an individual, particularly in epidemiologic studies, has been questioned.

In the United States, no parasite skin test antigens have been available commercially or from the CDC for the past 5 years. It has not been possible to determine which parasite skin test antigens are available commercially or from reference centers in Europe or Asia.

Toxoplasmosis

In the 4-year survey conducted for this review, several interesting reports of progress in the area of toxoplasmosis diagnosis were identified. An approach to a standardized skin test was clearly defined for detecting *T. gondii* infection in women before they become pregnant (78). An excretory-secretory antigen was prepared in accordance with biologic safety standards and delivered with a multipuncture ring. Optimal reading to avoid false-positive reactions was at day 7. The authors concluded that skin testing could play an important role in the prevention of congenital toxoplasmosis.

Leishmaniasis

A crude extract of cultured *L. donovani chagasi* promastigotes was substituted for whole promastigotes in a leishmania skin test antigen widely used in South America (74). Reactions were observed in 97% of persons with a 2-year history of visceral leishmaniasis. Cross-reactivity occurred at a low level (8%) in patients with Chagas' disease, but not in patients with other diseases. In contrast, extracts of promastigotes were insensitive as skin test antigens in two other studies in South America. Whole promastigote antigen was also found to be insensitive in Egypt (66) and Brazil (102). In India, skin test positivity to whole promastigote antigen was not demonstrable until 5 months after the acute phase of visceral leishmaniasis (70). At this time the positivity rate was 20%, and it increased to 86.6% after 8 months. A fusion protein derived by molecular expression cloning of an African strain of *L. donovani* was found to be highly sensitive and specific for visceral leishmaniasis (5). The authors suggested it as a putative skin test antigen.

Hydatidosis

Reports of Casoni tests for hydatidosis came from India (60), People's Republic of China (63), and Saudi Arabia (72). The sensitivity varied from 58 to 86%. False-positive reactions were observed in the Indian study; specificity data were not included in the other studies.

CONCLUSIONS

Kits for antibody detection in some parasitic diseases are available commercially in the United States, South America, Europe, and Asia, but lists of these kits are unavailable. Similarly, a number of commercial laboratories in various countries perform serodiagnosis for parasitic diseases, but it is not possible to obtain lists of these laboratories. Because international or national standard reference antigens and antisera have not been developed, and because the batteries of homologous and potentially cross-reactive serum specimens used to evaluate assays often have been inadequate, it is impossible to compare serologic assays and recommend a particular one for a particular disease. These deficits in parasitic serology apply equally to kits for antigen and antibody detection and to quality control programs with laboratories.

The need for assays that would be applicable to field, epidemiologic, and chemotherapeutic studies in tropical countries, where most of the human parasitic diseases occur, is important. Technically, both FAST-ELISA and immunoblot with prepared antigen strips can be used for such studies. However, the main drawback is the unavailability of adequate amounts of appropriate affordable antigens. The finding that finger prick blood samples can be collected in capillary tubes and stored in buffer or preservative without refrigeration is a major step forward. More than one capillary tube of blood could be collected from each individual with well-documented disease, which could thus form the basis of much needed blood (antibody) collections for evaluation of sensitivity and specificity of new antigens and assays. The reluctance to perform venipuncture and the cost of expensive syringes would be bypassed.

Skin testing is playing a markedly diminished role in the diagnosis of parasitic infections. A number of reasons include lack of reference standards, lack of sensitivity and specificity, and the danger of provoking anaphylaxis.

Serodiagnosis at the CDC now includes detection by capture ELISA of specific IgM antibodies in toxoplasmosis, application of a truly quantitative FAST-ELISA for schistosomiasis, and specific immunoblot (Western blot) assays for species identification in schistosomiasis and for diagnosis of cysticercosis, hydatidosis, and paragonimiasis.

It is becoming increasingly evident that insight into the disease state of patients with some parasitic diseases can be gained by determining the immunoglobulin class or IgG isotype or both of each individual antibody response to the infecting parasite.

Although few assays for antigen detection are, as yet, being used for routine diagnosis of parasitic disease, the number of research reports on antigen detection is increasing. MAbs are used in many of the studies. Isotopic assays for detection and measurement of antigen in circulation, body fluids, or excreta have largely been superseded by ELISA.

MAbs are widely used in serodiagnosis and have three main roles: (i) antigen purification and characterization, (ii) competitive antibody assays with enhanced sensitivity and specificity, and (iii) antigen detection and measurement. The application of DNA technology to parasitic serodiagnostic antigen production is expanding at a rapid rate. Particularly in the field of malaria diagnosis, significant advances have already been made in delineating antigens that are important in seroepidemiologic and chemotherapeutic studies. Although it is still in its infancy, the concept of producing parasitic antigen-secreting hybridomas is exciting.

With the implementation of new technologies for purifying antigens and for assay development, progress in serodiagnosis and seroepidemiology of parasitic diseases is assured. The limiting factor is having adequate batteries of serum specimens for evaluation of new assays. Collaboration between laboratories involved in advanced technologic research and laboratories or field programs in tropical areas could help to eliminate or reduce this deficit. Furthermore, it would be advantageous to the entire field if standard reference antigens and serum specimens, as they become available, were catalogued and distributed by the World Health Organization.

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