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Immunology

Hybridoma-derived antibody with immunodiagnostic potential for schistosomiasis japonica

(*Schistosoma japonicum*/immunodiagnosis)

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ABSTRACT **A murine hybridoma-derived antibody (IPH.134) has been produced which has apparent high binding specificity for an extract of *Schistosoma japonicum* adult worms. No binding was detected to extracts of *S. japonicum* eggs or to extracts from other adult trematodes (*Fasciola hepatica*, *Paragonimus westermanii*, *Clonorchis sinensis*, and *Schistosoma mansoni*) and several other helminths and protozoa. When sera from two series of *S. japonicum*-infected Philippine patients (19 and 20 patients, respectively) were tested for inhibition of binding of ^{125}I-labeled IPH.134 to *S. japonicum* adult worm antigen, a low (10%) false-negative rate was obtained. In the 19-patient series, the 4 patients with highest fecal egg counts had high inhibitory activity in their sera. In the 20-patient series, the 3 patients with prominent disease had high inhibitory activity in their sera. Evidence was obtained that IgG anti-*S. japonicum* antibodies (rather than circulating antigens, immune complexes, or anti-idiotypic antibodies) were most likely to be responsible for serum inhibitory activity in the test. No false-positive reactions have been obtained with pooled or individual sera from patients infected with numerous parasites other than *S. japonicum*, although no information is yet available on the inhibitory activities of sera from *S. mansoni*- or *S. hematobium*- infected individuals. On the basis of the data obtained to date, it is a reasonable prediction that the molecule or determinant to which this hybridoma antibody is directed will be a useful immunodiagnostic antigen for schistosomiasis japonica in the Philippines. A test based on detection of serum antibodies to this antigen should have high specificity and may provide additional information on the level of infection or disease status in patients.**

Serological methods have provided a useful supplement to fecal examination for eggs in the diagnosis of schistosomiasis. In the Philippines, the anti-egg circumoval precipitin (COP) test (1) has found wide use in the immunodiagnosis of schistosomiasis japonica (2–4). The high sensitivity and specificity of this test have been demonstrated (5), but quantitative determination of COP anti-egg antibody level is difficult, standardization of egg batches to be used is difficult, and the test provides little information on presumed infection levels in individual patients. Moreover, a recent study using a radioimmunoassay (RIA) with extracted *Schistosoma japonicum* egg antigens and 24 sera from the Philippines showed that titers of anti-egg antibodies do not correlate with fecal egg output (6). In general, highest titers were found in infected teenagers and lowest titers were in older individuals. An immunodiagnostic test (IDT) for schistosomiasis japonica that is simple and quantitative and that provides some estimate of infection level (or vulnerability to disease) would be extremely valuable for epidemiological purposes and for monitoring the success of control programs based on selective or mass chemotherapy or environmental modification. There is a high probability that individuals living in an area endemic for *S. japonicum* would be exposed to, and produce antibodies against, other schistosomes of animal or bird origin. Thus, the IDT would most likely need to be based on detection of serum antibodies to or antigens of late life cycle stages such as eggs or adult worms.

Attempts have been made to develop new IDTs for parasitic infection by using mouse hybridomas that secrete antibodies reactive to antigens of various parasites (7–10). By this approach, crossreacting hybridoma-derived antibodies can be used for depletion of shared antigens in parasite extracts (unpublished data) or highly specific antibodies can be used in competitive binding assays with sera (7, 8) or, eventually, for purification of immunodiagnostic antigens. The respective merits of these methods have been discussed (10).

This paper reports that a hybridoma (designated IPH.134 or, more correctly, the doubly cloned IPH.134-18-6), selected from the fusion of modified myeloma cells with spleen cells from mice immunized against *S. japonicum* adult worms, secretes an antibody that has high immunodiagnostic potential. The binding of the labeled hybridoma-derived antibody to a crude adult worm extract (AWE) in a RIA is inhibited by at least 90% of sera from individuals known to be infected with *S. japonicum*, and no false-positive reactions have yet been detected.

MATERIALS AND METHODS

Mice and Hybridomas. BALB/c mice to be used as donors of spleen cells for fusion were injected with *S. japonicum* eggs (6) or lyophilized, mouse-derived *S. japonicum* worms or extracts in Freund's complete adjuvant (Difco) and given booster injections without adjuvant at least twice. Spleen cells were taken for fusion at 4 days after the last antigen injection. The mice were derived from a specific pathogen-free facility but were maintained conventionally in the Melbourne laboratory (11). Cells from mice infected with *S. japonicum* were also used for fusion in the Manila laboratory and cells in Costar trays were brought back to Melbourne for cloning and screening. The IgG antibody-secreting cloned hybridomas referred to in this paper were obtained from mice injected with worms and their extracts (IPH.134) or injected with eggs (SEF.85) and were selected by using extracted worm or egg antigens, respectively (see below). All methods used for production of hybridomas (12–14) by using NS-1 myeloma cells have been described in detail (7). Hybridoma-derived antibodies were prepared from bulk culture supernatants or from ascites fluids from pristane-injected mice and were purified on *Staphylococcus aureus* protein A-Sepharose

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Abbreviations: AWE, adult worm extract; COP, circumoval precipitin; IDT, immunodiagnostic test; P_i/NaCl, phosphate-buffered saline at pH 7.3; PRC, People's Republic of China; PVC, polyvinyl chloride; RIA, radioimmunoassay.

(Pharmacia) (7, 15). They were labeled with ^{125}I by the chloramine-T method to a specific activity of approximately 370 kBq/μg (i.e., 10 μCi/μg) by John Pye of this Institute.

RIAs. Three types of solid-phase RIA were used; the methods and sources of reagents have been described (7, 8, 16). For direct-binding RIAs, a 50-μl antigen titration was performed in borate buffer (pH 9.5) in polyvinyl chloride (PVC) plates and left for 3–4 hr at room temperature in a humidified box, unbound material was removed, and 50 μl of 0.5% bovine serum albumin in mouse tonicity phosphate-buffered saline (pH 7.3) (P_i/NaCl) was added to each well. After 1 hr, plates were rinsed in 0.05% Tween 20 in P_i/NaCl, and approximately 20,000 cpm (50 μl) of ^{125}I-labeled hybridoma antibody in Tween 20/albumin/P_i/NaCl was added to each well and left overnight at room temperature. Radioactivity in individual wells was determined in an Autogamma counter after the plates were rinsed and cut with a hot wire.

For competitive RIAs, an amount of stock antigen was chosen for coating to the plates which resulted in 50–80% of the plateau level of binding of labeled hybridoma antibody. The procedure followed was the same as that described above except that, in the final step, 25 μl of solution containing 20,000 cpm of ^{125}I-labeled hybridoma antibody was added to a 25 μl titration of antiserum (usually commencing at 1:20 dilution) in 0.05% Tween 20/0.5% albumin in P_i/NaCl. For selection of hybridomas, culture supernatants were treated for 3 hr with antigen-coated plates followed by overnight incubation with an affinity-purified ^{125}I-labeled sheep anti-mouse IgG antiserum which had Ig light chain as well as γ and some μ heavy chain reactivities (16). To examine the isotype of hybridoma antibody, labeled commercially available isotype-specific rabbit anti-Ig antisera were used (7) after purification on protein A-Sepharose.

Antigens for the RIAs were as follows. The *S. japonicum* AWE was prepared by homogenizing and sonicating lyophilized worms (derived from mice or rabbits) in borate buffer at pH 9.5. Any mouse Ig-binding molecules in the 12,000 × *g* supernatant (15 min) of this crude aqueous extract were depleted by passage through a normal mouse Ig-Sepharose column; the run-through was collected and frozen at −20°C until used. This antigen-processing step was taken because of difficulties experienced in using crude worm extracts in the selection of hybridomas in various parasite systems in the past, difficulties that could be accounted for by binding of Ig to Fc receptor-like molecules in worm extracts. (Subsequently it was found that ^{125}I-labeled IPH.134 bound equally well to processed and unprocessed *S. japonicum* AWE.) Preparation of the *S. japonicum* aqueous egg extract has been described (6) as have *Echinococcus granulosus* cyst fluid and pepsinized protoscolex antigen mixtures plus *Taenia saginata* and *Fasciola hepatica* whole worm extracts (8). Lyophilized *Schistosoma mansoni* adult worms were obtained from Ken Mott (Special Programme for Research and Training in Tropical Diseases, WHO, Geneva) and *Angiostrongylus cantonensis* worms were from R. Ko (University of Hong Kong) and M. Kamiya, (Hokkaido, Japan). They were homogenized and sonicated by using the method described above for *S. japonicum* worms. *Clonorchis sinensis* and *Paragonimus westermanii* skin test antigens (also AWEs) were obtained from Huang Sung-ru and Zhong Huei-Lan (Beijing Friendship Hospital, Beijing, PRC). A *Leishmania tropica* extract was prepared by E. Handman (Hall Institute) by freezing and thawing of culture-derived LRC-L137 *L. tropica* promastigotes. *Plasmodium falciparum* antigens were prepared by concentration of culture supernatants of PNG isolates of *P. falciparum* in long-term Trager and Jensen cultures or by sonication of purified schizonts and were provided by G. V. Brown (Hall Institute). Protein content of antigen mixtures was determined by using the Hartree modification of the Lowry method (17) and A_{280} measurements were performed on purified hybridoma antibodies.

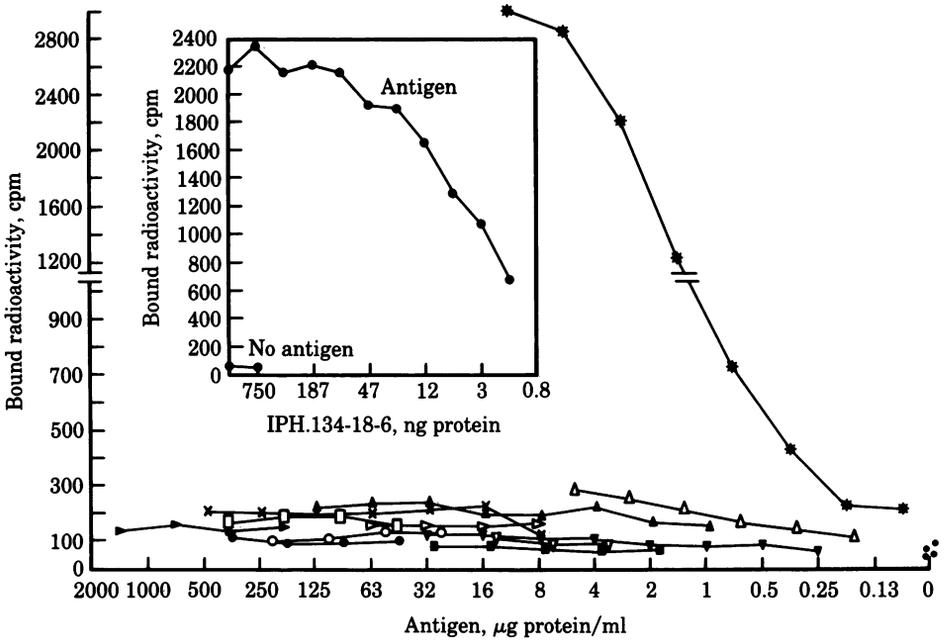

FIG. 1. Binding of ^{125}I-labeled IPH.134-18-6 (2 ng) in a solid-phase RIA using various amounts of parasite antigens to coat the plate wells: *, *S. japonicum* processed AWE; ●, *E. granulosus* cyst fluid antigen; ○, *E. granulosus* pepsinized protoscoleces; ×, *T. saginata* whole worm extract; □, *F. hepatica* whole worm extract; △, *A. cantonensis* AWE; ▲, *S. mansoni* AWE; ▽, *C. sinensis* skin test antigen (AWE); ▼, *P. westermanii* skin test antigen (AWE); ■, *L. tropica* promastigote extract; ▶, *P. falciparum* concentrated culture supernatant; ▷, *P. falciparum* schizont sonicate. The amount of ^{125}I-labeled IPH.134-18-6 added was 20,000 cpm; volumes of antigen and labeled hybridoma were 50 μl per well. (*Inset*) Plate wells were coated with *S. japonicum* processed AWE (5 μg/ml) or diluent only and allowed to react with varying amounts of protein A-purified IPH.134-18-6 from bulk culture supernatants prior to addition of 20,000 cpm of ^{125}I-labeled sheep anti-mouse IgG antibody. The data in both portions indicate that, with reference to the homologous system, the assay has high sensitivity for both the hybridoma antibody and the *S. japonicum* AWE antigen to which it is directed.

Sera. Blood was obtained from patients presenting at the Institute of Public Health, Manila, and the serum was removed; after COP tests were performed, the sera were stored at −20 or −70°C except during transport to Melbourne. Fecal egg counts were performed by using the Kato–Katz technique (18). Control sera were obtained from personnel within the Institute of Public Health and were negative in the COP test. Lyophilized sera from patients proven to have cysticercosis or paragonimiasis were obtained from Beijing (PRC). Two serum samples from patients infected with *F. hepatica* were obtained from P. S. Craig (University of Melbourne Veterinary Clinical Centre). Other human sera were obtained from D. I. Grove (University of Western Australia, Perth; elephantiasis and other sera from an area of the Philippines where bancroftian filariasis is endemic), G. V. Brown (Hall Institute; malaria sera from the hyperendemic Madang region of Papua New Guinea in which *Wuchereria bancrofti* infections are also common), M. D. Rickard (University of Melbourne Veterinary Clinical Centre, Werribee; hydatids sera from four patients with strong positive reactions in *E. granulosus* serological assays), and R. S. Hogarth-Scott (ICI Australia Pty. Ltd; sera from three children with high eosinophilia and suspected toxocariasis). Some of the schistosomiasis sera were fractionated by affinity chromatography on protein A-Sepharose or by gel filtration on Sephacryl SF-300 (Pharmacia).

RESULTS

Binding of ^{125}I-Labeled IPH.134 to Parasite Antigens. The hybridoma-derived antibody IPH.134-18-6 (an IgG2a protein prepared from bulk cultures), when labeled with ^{125}I, was found to bind to a processed AWE of *S. japonicum* but not at all to a *S. mansoni* AWE or to other crude antigen mixtures from helminths and protozoa that can infect man (e.g., *F. hepatica*, *P. westermanii*, *C. sinensis*, *A. cantonensis*, *E. granulosus*, *T. saginata*, *P. falciparum*, and *L. tropica*). Some of these antigens were used in the solid-phase RIA at a protein concentration 1000 times that of *S. japonicum* AWE (Fig. 1).

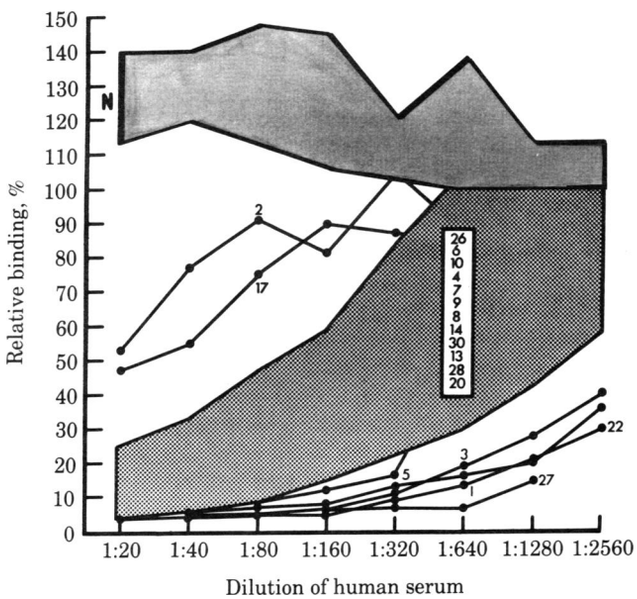

FIG. 2. Inhibition of binding of ^{125}I-labeled IPH.134-18-6 to *S. japonicum* processed AWE by sera from 19 *S. japonicum*-infected individuals from the Philippines, expressed relative to binding of the labeled hybridoma-derived antibody in the absence of human serum (100%). This method of expression of the data has been used to show the slightly >100% binding of the labeled antibody in the presence of relatively high concentrations of sera from uninfected (N) individuals—four pools of three or four individuals each (and collected from personnel of the Institute of Public Health, Manila, and the Hall Institute, Melbourne) were used as control sera (blackened area). Assays were performed on COP test-positive sera from patients with various egg outputs (see Table 1), the serum numbers are indicated, and the ranking of 12 clustered sera (no. 20, relatively high, to no. 26, relatively low) is shown within the stippled area. In the assay, 20,000 cpm of ^{125}I-labeled IPH.134-18-6 in 25 μl and processed AWE at 5 μg/ml were used with doubling serum dilutions (25 μl) from 1:20 to 1:1280 or 1:2560.

Table 1. Results with 19 Philippine sera

Ranking of sera*	Patient data: Age; sex	Eggs, no./g feces	Other intestinal helminths†	RIA antibody levels against egg antigen‡
27	45; F	3040	H, T	I
1§	19; M	2010	H, T	H
22	18; M	110	H, T	VH
3§	25; M	2090	H, T	I
5	16; M	480	H, T	I
20	16; F	70	H, T	I
28	32; M	50	H, T	I
13§	13; F	150	A, H, T	H
30	38; M	90	A, H, T	L
14	34; F	60	A, H	L
8§	20; M	70	A, H	I
9	14; F	50	T	H
7	55; F	160	H, T	L
4	21; M	50	A, H	L
10	50; F	70	H	I
6	65; M	150	T	I
26	31; M	90	None	I
17	16; M	120	A, H, T	H
2	47; M	60	H, T	L

Competitive RIA with ^{125}I-labeled IPH.134 and a *S. japonicum* processed AWE, and an indirect RIA with ^{125}I-labeled anti-human Ig and a *S. japonicum* egg antigen extract.

* Based on competitive RIA. Shown as serum identification number.

† Eggs in feces: A, ascaris; H, hookworm; T, trichuris.

‡ See ref. 6. I, intermediate; VH, very high; H, high; L, low. Four of the five individuals with high egg outputs had high hybridoma inhibitory levels in their sera; the fifth (serum 22) had been shown previously to be unusual in having a very high level of antibody against egg antigens. Teenagers have high or intermediate levels of anti-egg antibodies; older individuals have intermediate or low levels (6).

§ Patients known to have prominent splenomegaly.

Inhibition of Binding of ^{125}I-Labeled IPH.134 to *S. japonicum* AWE by Sera from Infected Patients. COP-positive sera were available from 19 known infected individuals in the Philippines: they had been tested previously for anti-egg antibodies in an RIA (6). They were screened for inhibitory activity by using ^{125}I-labeled IPH.134-18-6 and the *S. japonicum* AWE. No false-negative reactions were observed, although sera differed markedly in their inhibitory activity. Four pools of sera (three from the Philippines and one from Melbourne), made up from individuals known not to have schistosomiasis japonica, were negative in the assay (Fig. 2). When sera from the infected patients were ranked according to level of inhibitory serum activity (Table 1), the four patients with relatively high fecal egg counts had high inhibitory activity in serum (i.e., sera 27, 1, 3, and 5). The other serum with high inhibitory activity (serum 22)

^{125}I-Labeled IPH.134 was found not to bind to a *S. japonicum* egg antigen extract in the solid-phase RIA. An IgG1 anti-egg hybridoma antibody, SEF.85-5-3, did bind to this same antigen preparation (or to *S. japonicum* AWE), indicating that at least one antigen was present in the egg extract. Moreover, this same egg antigen preparation had been used successfully for screening human sera in a previous study (6).

was found previously to differ from all others in having a very high anti-egg antibody titer (6).

Serum 26 (low inhibitory titer) and serum 27 (highest inhibitory titer) were fractionated (100 μl) on protein A-Sepharose; the bound Ig was eluted with pH 3 buffer. The amount of serum protein in the protein A-binding fraction was 5 times greater in serum 27 than in serum 26 but the inhibitory titer [as in the assay with whole serum (Fig. 2)] was >50 times higher. No inhibitory activity was found in the run-through fraction of the protein A-Sepharose column. Serum 3 (high inhibitory titer) was fractionated on SF-300, and again a marked hypergammaglobulinemia was evident. When fractions were assayed in the competitive RIA, inhibitory activity coincided precisely with the large IgG peak, and none was detected in the IgM or albumin regions of the protein profile. The lack of inhibitory activity in fractions other than those containing IgG proteins in the SF-300 fractionation and the protein A-Sepharose run-through fraction does not support the notion that inhibitory activity in these sera is mediated by immune complexes or circulating antigen in the schistosomiasis sera (19, 20) (see refs. cited in 21).

In addition, ^{125}I-labeled IPH.134 did not bind to sera 27 and 14 from infected individuals when coated to plates in an RIA; anti-idiotypic antibodies in human sera, and directed against IPH.134, are thus unlikely to be responsible for inhibition in the assay although these negative direct binding data do not rule out a contribution of anti-idiotypic antibodies in serum-mediated inhibition. Also, if antigen to which IPH.134 is directed is present in the serum, then it at least does not bind to PVC plates in detectable quantities.

In another series involving 20 COP-positive sera (Table 2), inhibitory titers again varied widely. Unlike the previous series (Table 1; Fig. 2), no suggestive association between high egg output and high inhibitory serum activity was detected. However, the three individuals with both splenomegaly and hepatomegaly (i.e., prominent disease) had the highest levels of inhibitory activity in their sera. Four of the sera were negative in the assay. Thus, the false-negative rate is approximately 10%, based on a total of 19 + 20 + 3 additional sera (i.e., 42) screened. None of the four false-negative individuals had high fecal egg outputs and no disease was recorded in the two from whom clinical data are available (Table 2).

Search for False-Positive Reactions. In addition to the control Philippine and Australian serum pools used (Fig. 2), pools or individual sera from 33 patients with elephantiasis (3 sera), malaria (10 sera as a pool), paragonimiasis (4 sera as a pool), fascioliasis (2 sera), hydatids (4 sera), suspected toxocariasis (3 sera), or cysticercosis (5 sera) from Papua New Guinea, Peoples Republic of China, Australia, and the Philippines were screened for inhibitory activity. No inhibitory activity (even at 1:10 or 1:20 dilution of serum) was detected in the assay using *S. japonicum* AWE and ^{125}I-labeled IPH.134 except that, at the lowest dilution, the three sera from children with suspected toxocariasis had low inhibitory activity (<20%).

When three other hybridoma-derived antibodies with binding activity for *S. japonicum* AWE were used in the competitive RIA with ^{125}I-labeled IPH.134, no inhibition of binding was found. These antibodies presumably are directed against antigenic determinants other than the determinant to which IPH.134 is directed.

Table 2. Results with 20 Philippine sera

		Patient data			
Ranking of sera	Approximate titer*	Age; sex	Eggs, no./g feces	Disease†	Other intestinal helminths‡
894	>1:2560	58; M	180	+	H, T
938	>1:2560	44; M	50	+	H
940	>1:2560	15; M	460	+	A, H, T
1060§	1:2560	16; M	550	NR	A
914	1:1280	16; F	1470	0	A, H, T
911	1:1280	20; F	20	(+)	H, T
959	1:1280	12; F	350	(+)	A, H, T
800	1:1280	16; M	250	NR	H, T
954	1:640	20; M	40	NR	H, T
904	1:320	19; M	180	NR	H, T
860	1:160	31; M	50	NR	A
993	1:160	40; F	20	(+)	T
1046	1:80	47; M	20	NR	T
994	1:80	46; M	160	(+)	T
872	1:40	39; M	1400	(+)	A, T
863	1:40	55; M	50	(+)	T
b-8	<1:20	22; M	90	0	None
851	<1:20	18; M	120	NR	A, H, T
b-7	<1:20	22; M	250	0	T
853	<1:20	44; M	50	NR	A, H, T

Competitive RIA with ^{125}I-labeled IPH.134 and a *S. japonicum* processed AWE.

* Dilution of serum resulting in 50% inhibition of binding of ^{125}I-labeled IPH.134 in a titration using dilutions of 1:20 to 1:2560.

† Disease classification: +, hepatomegaly and splenomegaly; (+), hepatomegaly or splenomegaly; 0, neither; NR, no record.

‡ Eggs in feces: A, ascaris; H, hookworm; T, trichuris.

§ To facilitate comparison between the two series, serum 5 of Table 1 and Fig. 2 had a serum inhibitory curve identical to that of serum 1060 in this series.

DISCUSSION

That hybridoma-derived monoclonal antibodies would be of value in the development of specific IDTs for parasitic infection was demonstrated previously in a model system involving a natural larval cestode parasite of mice (7). The test involved the inhibition of binding of a labeled hybridoma (McH.105) to a crude parasite antigen mixture by sera from infected mice in a solid-phase RIA. No false-positive reactions were observed, and the only false-negative reactions obtained were with sera from infected hypothymic *nude* mice. (Attempts to replace the crude parasite antigen mixture in the RIA with a large pool of antiidiotypic antibodies, raised against the immunodiagnostic hybridoma, were only partially successful.) The parasite used in the model system proliferates in the mouse, and the strong chronic antigenic stimulation leads to marked hypergammaglobulinemia (22).

In the expectation that sensitivity problems would arise in the use of a single-specificity IDT with low-level parasitic infestations (10), an alternative strategy for the use of monoclonal antibodies in immunodiagnosis has been developed. Cross-reacting hybridoma-derived antibodies have been used to deplete crude parasite antigen mixtures of determinants shared with other parasites; an improved IDT for *Echinococcus granulosus* infection in sheep has been developed in this way (unpublished data). Despite the theoretical difficulties with single-specificity IDTs, a prototype hybridoma-based IDT has been developed for experimental infection with a veterinary cestode, *Taenia hydatigena* (8). Moreover, in the present studies, a hybridoma has been produced which secretes an antibody with high immunodiagnostic potential for schistosomiasis japonica infection in man. In a competitive RIA with approximately 40 clinically defined human sera from the Philippines, a low false-negative reaction rate was obtained. Most importantly, sera from patients in the Philippines or neighboring countries with various other parasitic infections or not known to be infected with any parasite were unequivocally negative in the assay.

Of particular interest is the result that, in one series, the sera from 4 schistosomiasis patients (out of 19) with relatively high fecal egg counts, had very high inhibitory activity in the assay. If high fecal egg counts reflect high worm burdens in human schistosomiasis japonica, then the hybridoma antibody IPH.134 in an IDT may be useful not only for the detection of *S. japonicum* infection but also for assessing the level of infection. However, in another series of 20 sera, the association between high inhibitory serum activity and high fecal egg output was not seen. Nevertheless, in this series, the three patients with prominent disease had high inhibitory levels in their serum. Concerning the association between inhibitory serum titer and fecal egg output in the two series, eight of nine patients with egg counts >300/g had titers of 1:1280 or greater. A correlation in some individuals between worm burden and serum inhibitory activity would be readily explained if circulating parasite-derived molecules were being detected in the assay. However, all available evidence indicates that anti-*S. japonicum* antibodies are the inhibitors in sera. Affinity purification of the antigen to which IPH.134 is directed and binding assays with sera will establish whether this is indeed the case.

The monoclonal antibody IPH.134 bound to an *S. japonicum* AWE but not to a *S. japonicum* egg extract or a *S. mansoni* AWE. The failure to bind to the *S. mansoni* antigen was unexpected and further tests are obviously required to define the life cycle stage and species specificity of the IPH.134 antibody. It failed to bind to antigenic extracts from a range of trematodes, other helminths, and protozoa that can infect man. Despite apparent high specificity, we have proven in other systems that clinically defined sera from monospecific parasite-infected individuals are of far greater value in assessing the parasite specificity and immunodiagnostic potential of hybridoma-derived antibodies than are various parasite antigen mixtures. Presumably, the amplification provided by the host immune response reveals the presence of minority antigens in parasites not detected in *in vitro* binding assays and the relevant parasite life cycle stage for antigen preparation simply may not be available. In this regard, therefore, it is considered particularly significant that sera from patients with filariasis, malaria, paragonimiasis, fascioliasis, hydatids, cysticercosis, or suspected toxocariasis were negative in the competitive RIA. Without sera from *S. mansoni*- or *S. hematobium*-infected individuals, it is not yet possible to state whether these other schistosomes do or do not contain the antigen to which IPH.134 is directed. Neither *S. mansoni* nor *S. hematobium* is present in the Philippines.

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