Clin. exp. Immunol. (1986) **65**, 223–231.

Detection and quantification of circulating antigen in schistosomiasis by a monoclonal antibody I. Specificity analysis of a monoclonal antibody with immunodiagnostic capacity

J. A. NOGUEIRA-QUEIROZ, C. LUTSCH, MONIQUE CAPRON, J. P. DESSAINT & A. CAPRON *Centre d'Immunologie et de Biologie Parasitaire, Unité Mixte INSERM U 167–CNRS 624, Institut Pasteur, 1 rue du Professeur Calmette, 59019 Lille Cédex, France*

(Accepted for publication 25 February 1986)

SUMMARY

Monoclonal antibodies were obtained after immunization of mice with *Schistosoma mansoni* excretory/secretory antigen, previously shown to contain the circulating cathodic (M) antigen. Among these, the 40:B1 monoclonal antibody proved to be specific for the schistosome genus and to detect only adult worm-derived antigens as shown both by immunoprecipitation and with a two-site immunoradiometric assay using the monoclonal as both the solid-phase and the labelled antibody. The two-site immunoradiometric assay allows a sensitive measurement (detection limit: 5 ng) of circulating schistosome antigen in blood and in urine from patients with schistosomiasis. The amount of circulating schistosome M antigen is correlated with schistosome egg excretion in stool.

Keywords circulating antigens schistosomes radioimmunoassay

INTRODUCTION

A major characteristic of parasitic infections is the presence of a variety of antigens in the circulation of man and other infected hosts. In schistosomiasis, various parasite-derived antigens have been detected in biological fluids in human and experimental infections (Okabe & Tanaka, 1961; Berggren & Weller, 1967; Gold, Rosen & Weller, 1969; Nash, 1974; Carlier *et al.*, 1975; Deelder *et al.*, 1976; Santoro *et al.*, 1978; Abdel-Hafez, Phillips & Zodda, 1984).

Schistosomes excrete or secrete many antigenic specificities in the bloodstream of their hosts. Among these, only a few are suitable for immunodiagnosis, because of their cross-reactivity with other parasites or of their rapid removal and degradation by the host, as it is found with some polysaccharide antigens of short biological half life, eliminated in the urine as non-antigenic materials (Nash, 1982; 1983). This is fortunately not the case for all circulating antigens. A comparison of polysaccharide antigen from schistosomes showed that *Schistosoma mansoni* releases in the host circulation two main molecular species, the circulating anodic antigen (Deelder *et al.*, 1976) and the 'M' antigen (Carlier *et al.*, 1975) with cathodic mobility. *S. japonicum* in contrast was found to produce seven circulating molecules, among which the circulating anodic antigen is the major molecule, but is immunologically identical to the *S. mansoni* anodic antigen (Quian & Deelder, 1983). The circulating cathodic (M) antigen of *S. mansoni* is detected in the urine,

Correspondence: Dr J. P. Dessaint, Centre d'Immunologie et de Biologie Parasitaire, Institut Pasteur, 1 rue du Professeur Calmette, 59019 Lille Cédex, France.

serum and milk of infected individuals (Carlier *et al.*, 1975; Santoro *et al.*, 1977; 1978; Carlier *et al.*, 1980b), and has been characterized as a polysaccharide originating from the cells lining the schistosome gut. It is absent in schistosome eggs (Andrade & Sadigursky, 1978; Carlier *et al.*, 1980a).

In the diagnosis of parasitic infections, circulating antigens may represent interesting tools (Mott & Dixon, 1982; Dessaint & Capron, 1982) in two aspects: both qualitatively, because of the difficulties sometimes encountered in the direct detection of the parasite or in the evaluation of the specific immune response of the patient; and also quantitatively, since they may serve as markers of the intensity and severity of the disease, or may indicate the efficiency of chemotherapy.

Polyclonal antibodies were initially used to quantitate circulating cathodic antigen (Carlier *et al.*, 1980b). The preparation of hybridomas which secrete monoclonal antibodies (Köhler & Milstein, 1975) permitted the large-scale production of monospecific reagents without the necessity of using purified antigenic preparations for the immunization. The first monoclonal antibodies against schistosomes were produced by Verwaerde *et al.* (1979), but these antibodies, although recognizing an epitope immunodominant in infected humans (Dissous, Prata & Capron, 1984), were not specific for circulating antigens. Abdel-Hafez *et al.* (1984) and Abdel-Hafez, Phillips and Zodda (1984) prepared mouse monoclonal antibodies which recognized different stages of schistosomes and could specifically detect circulating schistosome antigens, but these antibodies were mainly egg-specific and have not been evaluated in human schistosomiasis.

The present paper describes another strategy, based on the detection of adult worm-derived circulating antigens by monoclonal antibodies produced in the mouse and directed against the circulating cathodic (M) antigen.

MATERIALS AND METHODS

Parasites and animals. S. mansoni (Puerto Rican strain) was maintained by passage in *Biomphalaria glabrata* snails and golden hamsters. Male syngeneic BALB/c mice (Iffa Credo, France) were used for immunization.

Parasite antigens. Excretory/Secretory antigens (ESA) were obtained from adult *S. mansoni* worms incubated for 3 h in a water bath at 37°C according to Capron *et al.* (1968). This preparation previously shown to contain the circulating cathodic (M) antigen (Carlier *et al.*, 1978, 1980a) was used for immunization of mice. *Schistosoma mansoni* polysaccharide antigens were prepared by affinity chromatography on Con-A Sepharose (Capron *et al.* in Mott & Dixon, 1982). Trichloroacetic acid (TCA) soluble extract of *S. mansoni* was prepared as described by Carlier *et al.* (1980a). Urinary *S. mansoni* antigen was obtained from pooled bladder punctures of *S. mansoni* infected hamsters, using normal hamster urine as a control. The urine was centrifuged (2000 ***g***, 10 min), desalted by dialysis against distilled water and lyophilized. It was reconstituted in distilled water to its original volume or concentrated if necessary. Soluble egg antigen of *S. mansoni* was prepared according to the method of Boros and Warren (1970). Soluble antigenic extracts from schistosomes, *Fasciola hepatica*, *Dipetalonema viteae*, *Ascaris suum* and hydatid cyst fluid from *Echinococcus granulosus* were prepared as previously described (Capron *et al.*, 1968). All antigenic preparations were lyophilized. Antigen concentrations are reported as the dry weight of lyophilized materials resuspended in saline or serum.

Immunization of mice. BALB/c mice of 10–12 weeks of age were injected with 200 μl ESA (protein concentration: 0·5 mg/ml) in complete Freund's adjuvant both via the subcutaneous route and into their footpads. Two similar injections were performed at 15 and 25 days after the first immunization. Two mice, selected for their best precipitin response against adult *S. mansoni* antigen in immunodiffusion analysis were injected at day 40 intravenously with 20 μl of the same antigenic preparation (ESA) but without adjuvant. Four days later, the spleens were removed and the cell suspensions prepared for fusion.

Cell fusion and selection of hybrids. Cell fusion between splenic lymphocytes from BALB/c mice immunized with excretory/secretory *S. mansoni* antigens and the non-secreting myeloma cell line SP_2O was used. The hybridization technique was essentially similar to that described by Grzych *et al.*, (1982), using PEG as the fusion agent. Hybrid cell culture supernatants from each well were collected and screened by indirect immunofluorescence on cryostat sections of schistosomula and

adult worms of *S. mansoni*, as well as by a radioimmunoassay using soluble antigen (as described below). Cells were cloned by the limiting dilution technique. Positive clones were injected subcutaneously into BALB/c mice to induce solid tumours. After homogenization of these tumours, cells were injected into the peritoneal cavities of BALB/c mice and the ascitic fluids were collected 2 weeks later and stored at −20°C until use.

The purification of the monoclonal antibodies was carried out by using 33% ammonium sulfate precipitation and DEAE ion exchange chromatography.

Determinatiion of antibody subclass and specificity. Ouchterlony and radioimmunoassay tests were carried out with the supernatants of hybrid cells and heavy chain-specific antisera to mouse IgM, IgG1, IgG2a, IgG3 and IgA, purchased from Bionetics Labs (MD, USA).

As some of the monoclonal antibodies were shown to immunoprecipitate schistosome antigens, their specificity was investigated using immunodiffusion in gel techniques, performed with hybrid cell supernatants, ascitic fluids or purified antibodies and varius parasite antigens.

Immunofluorescence test: Cryostat sections of *S. mansoni* schistosomula or adult worms were fixed in cold propanone (acetone) for 10 min. Hybrid cell supernatants were concentrated three times by lyophilization and placed (50 μl) on the worm sections for 30 min. After three washings in 0·01 M phosphate buffered saline (PBS), pH 7·2, fluorescein-labelled antimouse immunoglobulins (Miles-Yeda, Rehovot, Israel) diluted 1:40 in PBS were added for 30 min. Positive (immune mouse serum) and negative (normal mouse serum or myeloma cell culture supernatants) controls were included in each experiment.

Radiolabelling of the monoclonal antibody. The purified monoclonal antibodies were radioiodinated by the chloramine T technique (Hunter & Greenwood, 1962), modified as follows: 200 μg of purified antibodies were mixed with 0·2 mCi ^{125}I-sodium iodide (Amersham, Les Ulis, France) and chloramine T at 4°C and the iodation was stopped after 2 min by addition of sodium metabisulfite. The radiolabelled antibody was recovered by gel-filtration through PD 10 columns (Pharmacia, Uppsala, Sweden). The average specific activity was 1 mCi/mg. To check the immunological properties of the labelled monoclonal, radioimmunoelectrophoresis was used as a quality control. Immunoelectrophoresis was performed as described by Capron *et al.* (1968), but by using a mixture of unlabelled polyclonal and radiolabelled monoclonal antibodies (5×10^6 cf/min/well). The plates were then extensively washed, dried and autoradiographied with an X-OMAT AR Film (Eastman Kodak, Rochester, NY, USA).

Radioimmunoprecipitation PEG assay (RIPEGA). The assay was performed as described by Santoro *et al.* (1978). Briefly, different concentrations of urine from schistosome-infected hamsters were dissolved in normal human or mouse serum diluted 1:5 (v:v) in 0·1 M borate buffer, pH 8·4. Equal volumes (200 μl) of urine samples and radioiodinated monoclonal antibody (10^4 ct/min) were mixed. After incubation for 1 h at 37°C, followed by a 4 h incubation at room temperature, 3 ml of 8% (w/v) PEG in borate buffer were added and the samples were incubated overnight at 25°C. After centrifugation (1500 *g*, 20 min at 4°C) the precipitate was washed in 8% PEG and the radioactivity was measured in a Gamma counter. All tests were done in duplicate. Results were expressed as percentage of radioactivity present in the precipitate.

Two-site immunoradiometric assay. Flexible polyvinyl plates (Falcon, Becton Dickinson, Oxford, UK) were coated with 100 μl mouse monoclonal antibody (10 μg/ml) diluted in 0·05 M carbonate/bicarbonate buffer pH 9·6 by incubation for 3 h at room temperature and further 12 h at 4°C. Unbound monoclonal antibody was removed by eight extensive washings of the microplates in 0·01 M PBS pH 7·6 containing 0·2% Tween (PBS/Tween). Aliquots of 100 μl of the samples diluted 1:2 (v/v) in PBS were added and incubated for 3 h at room temperature. After eight washings in PBS/Tween, 100 μl of radioiodinated monoclonal antibody (10^5 ct/min/well) were incubated 3 h at room temperature. The unbound radioactive antibody was aspirated and the wells were washed five times with 200 μl of PBS/Tween. The radioactivity of each well was measured in a Gamma counter.

Serum and urine samples. *S. mansoni* infected hamsters or mice were bled by retroorbital puncture. Infected patient sera and urines as well as control samples from uninfected humans were obtained from endemic and non-endemic areas from Brazil and Kenya respectively. Serum and urine samples were stored at −20°C. Rheumatoid factor and circulating immune complex (^{125}I-C1q-binding test) levels were measured to assess their influence in the radioimmunoassay results.

Table 1. Immunological properties of monoclonal antibodies against *S. mansoni* excretory/secretory/antigen

Isotype		Structure recognized (IFT)*
IgG3	κ	Adult caecum and somatic antigen
IgM	κ	Adult tegument
IgG1	κ	Adult tegument
IgM	κ	Adult somatic antigen
IgM	κ	Adult somatic antigen
IgM	κ	Adult caecum

* Indirect fluorescence on cryostat reactions of adult *S. mansoni.*

RESULTS

Production of monoclonal antibodies.
Various monoclonal antibodies were obtained by hybridization of lymphoid cells from ESA-immunized mice and SP_2O cells. Of these, several were shown to recognize an antigen with the properties attributed to the cathodic (M) antigen (Table 1), namely a positive fluorescence reaction on the caecum of adult worms (Fig. 1). Various antibody (sub)classes were found in the hybrid supernatants. Among them, a monoclonal named 40:B1 of the IgG3 κ-subclass was selected.

Species and genus specificity of the monoclonal antibody
An important characteristic of the monoclonal antibody 40:B1 is its capacity to immunoprecipitate

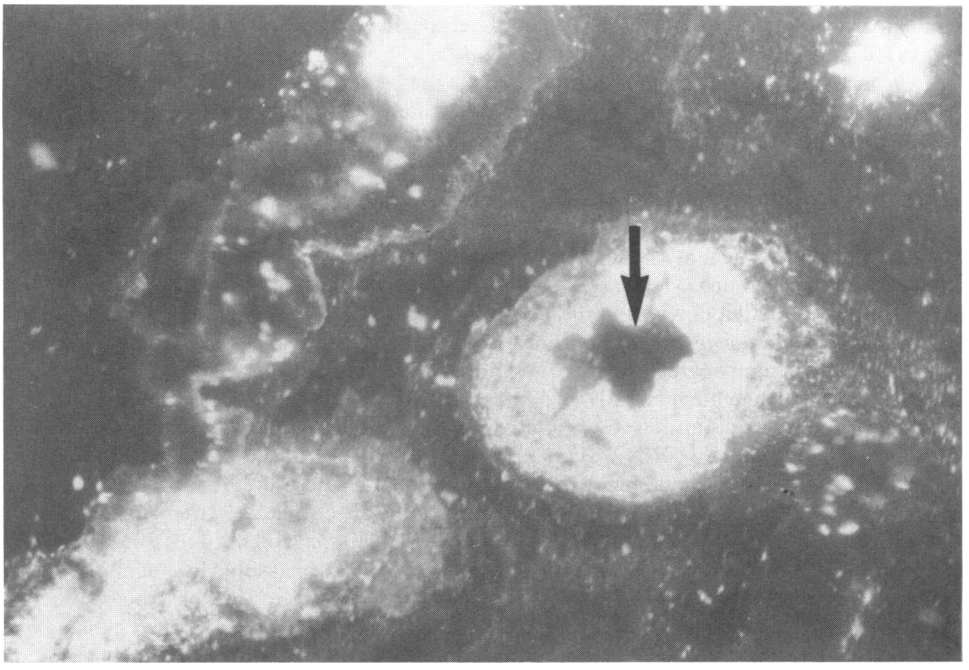

Fig. 1. Indirect fluorescence of 40:B1 monoclonal antibody. Cryostat sections of adult worms were incubated with 40:B1 monoclonal, then with fluorescein-labelled anti-mouse Ig. Staining of the worm caecum is observed.

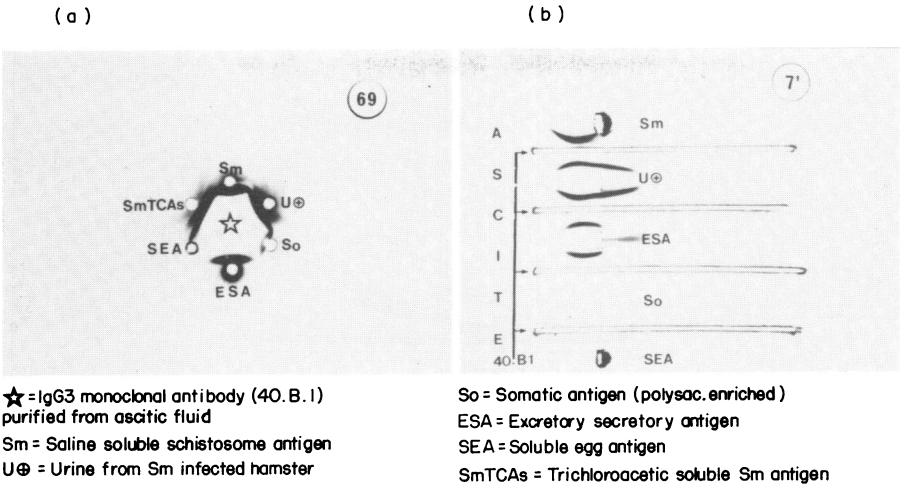

Fig. 2. Specificity of 40:B1 monoclonal antibody as studied by immunodiffusion in gel against various parasite antigens:
(a) Identity reaction with adult *S. mansoni* saline extract (top well) (Sm), TCA-soluble extract (Sm TCAs) and urine of *S. mansoni*-infected hamsters (U^+) shown by the 40:B1 monoclonal antibody. A precipitin line is also found with excretory/secretory antigens of adult worm (ESA) but not with soluble egg antigen (SEA).
(b) Immunoelectrophoresis of the 40:B1 monoclonal antibody (ascitic fluid) against adult *S. mansoni* antigen (Sm), urine from infected hamster (U^+), excretory/secretory antigen (ESA), showing the cathodic position of the precipitin line. No reaction is found with SEA. Contrary to Fig. 2a, no precipitation is observed with somatic antigen (So), which was used in immunoelectrophoresis at lower concentration than in the Ouchterlony test of Fig. 2a.

soluble schistosome antigens. By the Ouchterlony technique, a genus and adult stage specificity for schistosomes was found. A reaction of identity was indeed obtained between schistosome antigen in urine from infected hamsters and different preparations of adult schistosomes. As shown both by Ouchterlony test (Fig. 2a) or by immunoelectrophoresis (Fig. 2b), no precipitation was obtained with soluble egg antigen, confirming therefore the anti-M specificity of the monoclonal antibody. None of the antigenic extracts from other parasites reacted with the 40:B1 antibody (not shown).

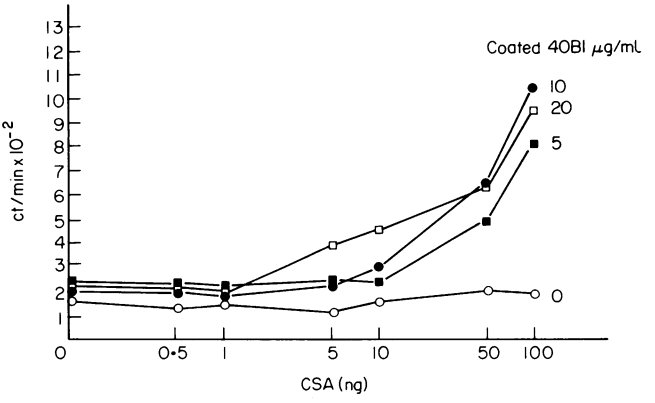

Fig. 3. Two-site immunoradiometric assay using labelled 40:B1 monoclonal antibody. Polystyrene microplates were coated with the 40:monoclonal antibody at the indicated concentration. A standard curve was made by adding the indicated amount of infected hamster urine (lyophilized) as a source of antigen (CSA) resuspended in normal human serum. A concentration of 10 μg/ml monoclonal antibody was chosen for coating in subsequent assays.

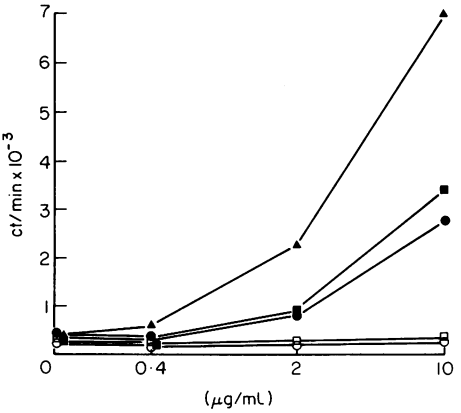

Fig. 4. Specificity of the two-site immunoradiometric assay using the 40:B1 monoclonal. Normal human serum was supplemented with the indicated concentrations of parasite antigens. Only antigenic preparations from adult worms of *S. mansoni* (▲) or *S. bovis* (■) gave positive results in the assay. *S. mansoni* schistosomulum antigen could not be detected (□), as well as extracts from other parasites (*Fasciola hepatica*, *Dipetalonema viteae*, *Ascaris suum* and *Echinococcus granulosus*) (○). (●) *S. mansoni*-infected hamster (urine).

As a control of specificity after radiolabelling, ^{125}I-labelled monoclonal 40:B1 antibody was able to precipitate the cathodic (M) antigen after mixing with monospecific polyclonal antibodies. By radioimmunoelectrophoresis, a single cathodic precipitation line was revealed by autoradiography, indicating the immunoreaction of the labelled monoclonal in the immunoprecipitate (not shown).

Radioimmunoassay of circulating Schistosome antigen

The radioimmunoprecipitation PEG assay (RIPEGA) was first used to evaluate the capacity of 40:B1 monoclonal antibody to detect circulating schistosome antigen (CSA) in infected patient sera. The PEG concentration of 8% was found optimal to precipitate the immune complexes without precipitating free mouse IgG3. Various concentrations of urinary *S. mansoni* antigen or ESA (0 to 10 μg/ml) (dry weight of the lyophilized materials) were mixed with normal human serum, and the detection limit was found to be 50 ng of antigen. Although the RIPEGA could detect circulating schistosome antigen in infected patient sera, false positive results were found in endemic controls, and sera from uninfected humans with high levels of rheumatoid factor yielded significant PEG-precipitates not shown. Alternative techniques were thus investigated.

Two-site immunoradiometric assay

Polyvinyl flexible plates coated with several concentrations of the 40:B1 monoclonal antibody were used as the solid phase. As shown in Fig. 3, an antibody concentration of 10 μg/ml appeared optimal for coating the microplate wells. By using infected hamster urine or ESA as a standard for (crude) circulating antigen, a detection limit of between 5 and 10 ng (dry weight) was found. The applicability of the two-site immunoradiometric assay for the detection of circulating schistosome antigen was shown by using serial dilutions of infected human serum, with a progressive decrease in the bound radioactivity.

Conversely, immunoprecipitation of patient sera containing circulating schistosome antigen by an excess of (unlabelled) 40:B1 monoclonal before the assay led predictably to a progressive decrease in the bound radioactivity with the amount of cold monoclonal used, and unrelated monoclonal antibodies had no such reducing effect (data not shown).

The genus specificity of the 40:B1 monoclonal antibody was confirmed using the immunoradiometric assay. As shown in Fig. 4, no other parasite extract could indeed react in the assay at concentration up to 10 μg/ml. Not all schistosome antigens could even be detected in the immunoradiometric assay, and the adult stage specificity already found by immunoprecipitation was confirmed using the more sensitive immunoradiometric assay.

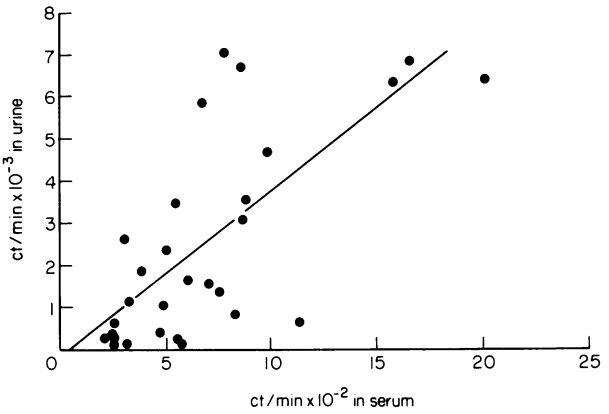

Fig. 5. Results with two-site immunoradiometric assay of urine or serum samples from the same *S. mansoni*-infected patients. The calculated regression line $y=3{\cdot}67x-21{\cdot}83$ is shown. A positive correlation is found between circulating antigen level in serum and urine ($r=0{\cdot}69$, $P<0{\cdot}01$).

Studies to detect the presence of circulating schistosome antigen

Studies on application of the immunoradiometric assay to the immunodiagnosis of schistosome infection are detailed in the accompanying report (Feldmeier *et al.*, 1986).

Low binding was obtained using the immunoradiometric assay with sera from uninfected humans, even those containing high levels of rheumatoid factors.

The reproducibility was estimated by repetitive measurements of serum or urine samples containing low, medium or high levels of circulating schistosome antigens. Within assay variation was 8·5%, inter-assay variation was 12·3% ($n=10$).

Circulating schistosome antigen was detected in serum and urine of infected humans, and a significant correlation was found between serum and urine concentrations (Fig. 5). A correlation was also found between circulating antigen levels and *S. mansoni* egg counts in stools ($r=0{\cdot}65$, $P<0{\cdot}001$).

DISCUSSION

These studies indicate that an assay using the 40:B1 monoclonal antibody provides a suitable test for detection of the adult schistosome-derived M antigen in the serum or urine of infected hosts.

Two stages of the life cycle of schistosomes, the egg and the adult worm, present lasting antigenic stimuli to their host, and are a source of persistent antigenemia in the circulation. Other immunoassays using polyclonal or monoclonal antibodies have allowed mainly the detection of circulating schistosome egg-derived antigens, in correlation with egg output. These assays cross-reacted with cercarial antigens but adult worm antigen was less efficiently detected (Abdel-Hafez *et al.*, 1983). An alternative approach is to assay antigens released by the adult worm in the circulation of infected animals or humans. Among these excretory/secretory products, the circulating cathodic (M) antigen was selected because of its presence not only in serum, but also in urine from infected hosts (Carlier *et al.*, 1975; Deelder *et al.*, 1976).

Indeed, most of the circulating antigens induce antibody responses in active infection, and the level of free schistosome antigen, i.e. in excess of molecules bound by antibodies, is low in serum. Furthermore, circulating antigen-antibody complexes introduce an additional complication in the assay system, as there is a competition between the antibody reagent of the test and antibodies in host serum, together with variable *in vitro* association-dissociation of complexed antigen depending on the antigen:antibody ratio of the immune complexes and the respective affinities of the reagent and host antibodies. Such interference are expected not to occur, at least with *S. mansoni*, with urine

samples where free antigen can be detected. Mice were immunized with excretory/secretory products from adult *S. mansoni*, which contains the cathodic (M) antigen (Carlier *et al.*, 1978; 1980a). These animals were used as spleen cell donors for the hybridization. Of the monoclonal antibodies produced against schistosomes, two were found specific for the gut, *i.e.* the demonstrated source of the M antigen (Carlier *et al.*, 1980a). The 40:B1 monoclonal was found suitable for measurement of the circulating cathodic (M) antigen. The IgG3 subclass of this antibody can be related to the polysaccharidic nature of the immunogen (Carlier *et al.*, 1980a) since polysaccharides induce a preferential antibody response of this isotype in mice (Perlmutter *et al.*, 1978).

Both by immunoprecipitation and by radioimmunoassay, the 40:B1 monoclonal antibody was found specific for the adult stage of schistosomes, with no recognition of schistosomulum or soluble egg antigens. Although binding homologous *S. mansoni* antigen more efficiently in the radioimmunoassay, the monoclonal was cross-reactive with *S. bovis* which is closely related to *S. haematobium*, but not with other trematode or cestode antigens, and appeared therefore specific for the genus *Schistosoma*. This cross-reactivity among schistosomes cannot allow in principle a precise identification of the present infection in patients from endemic areas where both *S. mansoni* and *S. haematobium* are found, but in fact, circulating cathodic (M) antigen levels appeared to be correlated with *S. mansoni* burden, but not with *S. haematobium*, as shown in the accompanying paper. It should be noted that the circulating anodic antigen of adult schistosomes is also cross-reactive (Qiuan & Deelder, 1983). Cross-reactivity is found as well with monoclonal antibodies to schistosome egg antigens (Abdel-Hafez *et al.*, 1984).

Two types of radioimmunoassays were investigated with the ^{125}I-labelled monoclonal antibody. The RIPEGA allowed the measurement of circulating cathodic (M) antigen in the serum from infected individuals with a detection limit of 50 ng/ml. Despite the adult schistosome specificity of the labelled antibody RIPEGA was found unsuitable for direct immunodiagnosis, because of the false positive results obtained with sera from uninfected patients containing high levels of rheumatoid factor. However, after absorption on IgG-coated dishes, the RIPEGA could detect circulating cathodic (M) antigen in patient sera without rheumatoid factor interference (Feldmeier *et al.*, 1986).

The immunochemical characterization of the M antigen revealed the occurrence of several antigenic moieties with different molecular weights, all recognized by monospecific antisera. The polymeric nature of the M antigen and its immunoprecipitation by the 40 B1 monoclonal suggested the presence of repetitive epitopes, which is suitable for the two-site immunoradiometric assay (Addison & Hales, 1971). By using the 40:B1 monoclonal both as the solid phase and labelled antibody, the M antigen could indeed be detected in excretory/secretory products from *S. mansoni* as well as in the serum and urine from infected hosts. The detection limit was estimated as between 5–10 ng of lyophilized adult *S. mansoni* excretory/secretory products, and a seven-fold difference in binding was observed between control samples and samples supplemented with an excess of this antigen.

Studies with the immunoradiometric assay for immunodiagnosis of schistosome infection are detailed in a accompanying report (Feldmeier *et al.*, 1986), which indicate that the 40:B1 monoclonal represents a sensitive, rapid and technically feasible test to detect the presence of adult schistosome-derived circulating antigen in schistosomiasis mansoni.

Interestingly, a significant correlation ($r = 0{\cdot}69$) was found between results of the immunoradiometric assay of serum and urine from the same infected individuals. This might be of value for clinical or epidemiological studies, especially in endemic areas where collection of blood samples is often difficult.

Thus, in summary, the two-site immunoradiometric assay using the 40:B1 anti-schistosome monoclonal antibody appears to be a sensitive and feasible technique to detect adult schistosome derived M antigen in serum and urine and to quantify active infection in schistosomiasisis patients. Its application to immunodiagnosis of schistosomiasis is presently being evaluated in a collaborative programme with the International Atomic Energy Agency.

This work was supported by the International Atomic Energy Agency (3230/R2/TC). Serum and urine samples from endemic areas were kindly supplied by Professor Prata. The assistance of Mrs M. F. Massard and C. Colson is appreciated.

REFERENCES

ABDEL-HAFEZ, S.K., PHILLIPS, S.M. & ZODDA, D.M. (1984) *Schistosoma mansoni*: Detection and characterization of schistosome derived antigens by inhibition enzyme-linked immunosorbent assay (ELISA) utilizing monoclonal antibodies. *Exp. Parasitol.* **55,** 219.

ADDISON, C.M. & HALES, C.N. (1971) Two-site assay of human growth hormone. *Horm. Metab. Res.* **3,** 59.

ANDRADE, Z.A. & SADIGURSKY, M. (1978) Immunofluorescence studies of schistosome structures which share determinants with circulating schistosome antigens. *Trans. Roy. Soc. trop. med. Hyg.* **72,** 316.

BERGGREN, W.L. & WELLER, T.H. (1967) Immunoelectrophoretic demonstration of a specific circulating antigen in animals infected with *Schistosoma mansoni*. *Am. J. trop. med. Hyg.* **16,** 606.

BOROS, D.L. & WARREN, K.S. (1970) Delayed hypersensitivity type granuloma formation and dermal reaction induced and elicited by soluble factor reaction isolated from *Schistosoma mansoni* eggs. *J. exp. Med.* **132,** 488.

CAPRON, A., BIGUET, J., VERNES, A. & AFCHAIN, D. (1968) Structure antigénique des helminthes. Aspects immunologiques des relations hôte-parasite. *Path. Biol.* **16,** 121.

CARLIER, Y., BOUT, D., BINA, J.C., CAMUS, D., FIGUEIREDO, J.F.M. & CAPRON, A. (1975) Immunological studies in human schistosomiasis. I. Parasitic antigen in urine. *Am. J. trop. med. Hyg.* **24,** 949.

CARLIER, Y., BOUT, D. & CAPRON, A. (1978) Further studies on the circulating 'M' antigen in human and experimental *Schistosoma mansoni* infections. *Ann. Immunol. (Paris)* **129C,** 811.

CARLIER, Y., BOUT, D., STRECKER, G., DEBRAY, H. & CAPRON, A. (1980a) Purification, immunochemical and biologic characterization of the schistosoma circulating 'M' antigen. *J. Immunol.* **124,** 2442.

CARLIER, Y., NZEYIMANA, H., BOUT, D. & CAPRON, A. (1980b) Evaluation of circulating antigens by a sandwich radioimmunoassay and of antibodies and immune complexes, in *Schistosoma mansoni* infected parturients and their newborn children. *Am. J. trop. med. Hyg.* **29,** 74.

DEELDER, A.M., KLAPPE, H.T.M., VAN DEN AARDWEG, G.J.M.J. & VAN MEERBEKE, E.H.E.M. (1976) *Schistosoma mansoni*: demonstration of two circulating antigens in infected hamsters. *Exp. Parasitol.* **40,** 189.

DEELDER, A.M., KORNELIS, D., VAN MARCK, E.A.E., EVELEIGH, P.C. & EGMOND, J.G. (1980) *Schistosoma mansoni*: characterization of two circulating polysaccharide antigens and the immunological response to these antigens in mouse, hamsters and human infection. *Exp. Parasitol.* **50,** 16.

DESSAINT, J.P. & CAPRON, A. (1982) Immunoassays of antibodies and circulating antigens in schistosomiasis. *Clinics in Immunology*, **2,** 655.

DISSOUS, C., PRATA, A. & CAPRON, A. (1984) Human antibody response to *Schistosoma mansoni* surface antigens defined by protection monoclonal antibodies. *J. Infect. Dis.* **149,** 227.

FELDMEIER, H., NOGUEIRA-QUEIROZ, J.A., PEIXOTO-QUEIROZ, M.A., DOEHRING, E., DESSAINT, J.P., DE ALENCAR, J.E., DAFALLA, A.A. & CAPRON, A. (1986) Detection and quantification of circulating antigen in schistosomiasis by monoclonal antibody II. The quantification of circulating antigens in human schistosomiasis mansoni and haematobium: relationship to intensity of infection and disease status. *Clin. exp. Immunol.* **65,** 232.

GOLD, R., ROSEN, F.S. & WELLER, T.H. (1969) A specific circulating antigen in hamsters infected with *Schistosoma mansoni*: detection of antigens in serum and urine and correlation between antigenic concentration and worm burden. *Am. J. trop. med. Hyg.* **18,** 545.

GRZYCH, J.M., CAPRON, M., BAZIN, H. & CAPRON, A. (1982) *In vitro* and *in vivo* effector function of rat IgG2a monoclonal anti-*S. mansoni* antibodies. *J. Immunol.* **129,** 2739.

HUNTER, W.M. & GREENWOOD, F.C. (1962) Preparation of iodine ^{131}labelled human growth hormone of high specific activity. *Nature*, **194,** 495.

KÖHLER, G. & MILSTEIN, C. (1975) Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature*, **256,** 495.

MOTT, K.E. & DIXON, H. (1982) Collaborative study on antigens for immunodiagnosis of schistosomiasis. *Bull. WHO.* **60,** 729.

NASH, T.E. (1974) Localization of the circulating antigen within the gut of *Schistosoma mansoni*. *Am. J. trop. med. Hyg.* **23,** 1085.

NASH, T.E. (1982) Factors that modulate the clearance and ultimate fate of a specific schistosome antigen (GASP) in schistosome infections. *J. Immunol.* **128,** 1608.

NASH, T.E. (1983) Fate and mechanisms of clearance of PSAP, a schistosome antigen, in schistosomiasis. *J. Immunol.* **131,** 2520.

OKABE, K. & TANAKA, T. (1961) Urine precipitation reaction for *Schistosomiasis japonica*. *Kurume Med. J.* **8,** 24.

PERLMUTTER, R.M., HANSBURG, D., BRILES, D.E., NICOLOTTI, R.A. & DAVIE, J.M. (1978) Subclass restriction of murine anti-carbohydrate antibodies. *J. Immunol.* **121,** 566.

QIAN, Z.L. & DEELDER, A.M. (1983) *Schistosoma japonicum*: immunological characterization and detection of circulating polysaccharide antigens from adult worms. *Exp. Parasitol.* **55,** 168.

SANTORO, S., BOROJEVIC, R., BOUT, D., TACHON, P., BINA, C. & CAPRON, A. (1977) Mother-child relationship in human schistosomiasis mansoni. I. Parastic antigens and antibodies in milk. *Am. J. trop. med. Hyg.* **26,** 1164.

SANTORO, F., CAPRON, M., JOSEPH, M., ROUSSEAUX-PREVOST, R. & CAPRON, A. (1978) Circulating antigens and immune complexes in *Schistosoma mansoni* infected rats. Characterization by radioimmunoprecipitation PEG assay (RIPEGA). *Clin. exp. Immunol.* **32,** 435.

VERWAERDE, C., GRZYCH, J.M., BAZIN, H., CAPRON, M. & CAPRON, A. (1979) Production d'anticorps monoclonaux anti- *Schistosoma mansoni*. Etude préliminaire de leur activité biologique. *C.R. Acad. Sci.* **289,** 275.