Parasitology (1980), **80**, 171–182
With 4 figures in the text

The diagnosis of malaria infection using a solid-phase radioimmunoassay for the detection of malaria antigens.

Application to the detection of *Plasmodium berghei* infection in mice

L. MACKEY, L. PERRIN, E. LEEMANS *and* P. H. LAMBERT
WHO Immunology Research and Training Centre, Centre de Transfusion, Hôpital Cantonal, 1211 *Geneva* 4

(*Accepted* 30 *May* 1979)

SUMMARY

A method has been devised to show that malaria parasites can be detected serologically in infected blood with a high degree of sensitivity. Using a murine malaria model, parasites were demonstrated in a solid-phase radio-immunoassay which measured antibody-binding inhibition. Lysed red blood cells (r.b.c.) were incubated with labelled specific antibody and were then reacted in antigen-coated tubes. The degree of inhibition of antibody binding in the tubes correlated with the level of parasitaemia in the test blood. Using homologous antisera the test detected infection at a level of 1 parasite/million r.b.c. The specificity of the method was shown by comparison of antibody-binding inhibition in normal and infected r.b.c. and in r.b.c. from non-infected mice with induced reticulocytosis. The sensitivity was shown *in vitro* in tests of serially diluted blood of high parasitaemia and *in vivo* for the detection of early infection. The presence of antibody in the test blood did not significantly affect the sensitivity of parasite detection.

INTRODUCTION

The diagnosis of malaria infection depends on the recognition of parasites in stained blood films. This simple procedure is the ideal method and is entirely satisfactory in acute infections and in the investigation of individual cases. However, it may be time consuming and less reliable in large-scale field studies in endemic areas for the diagnosis of infection when parasitaemia is at low levels or during phases of the disease in which overt parasitaemia is absent at the time of testing. Several serological methods are available for the detection of malaria antibodies (Voller, 1976); however, the presence of antibody proves experience of malaria, but does not differentiate between present and past infection. Immunological methods have been used to study the antigens associated with malaria (Wilson, McGregor, Hall, Williams & Bartholomew, 1969; Wilson, 1974; Seitz, 1976). The antigens involved form a complex group which has not yet been fully characterized. They include integral antigens of the parasite itself, antigens expressed on the membrane of infected r.b.c. and soluble antigens found in the serum during infection. The aim of the present study was to find whether intra-erythro-

0031-1820/80/0079-0313 $01.00

cytic parasites can be detected serologically with a degree of sensitivity at least equal to that of microscopic examination of blood films. The need for a sensitive immunodiagnostic test for the identification of infected individuals in epidemiological studies, particularly in malaria control programmes, has been recognized by the WHO expert committee on malaria (WHO Expert Committee on Malaria, 1974).

MATERIALS AND METHODS

Parasites

Plasmodium berghei of the Anka Strain, kindly supplied by Dr Wéry, Antwerp, was used throughout this study.

Mice

Female mice of the OF/1 strain (Centre de Recherche et d'Elevage des Oncins, St. Germain sur l'Arbresle, France) aged 6–10 weeks were infected by intraperitoneal injection of 0·1 ml of infected blood (parasitaemia level 30–50 %) diluted 1/1000 in phosphate-buffered saline (PBS). Parasitaemia was monitored by examination of Wright-stained blood films made from a drop of tail blood. Approximately 1 week after infection, when the level of parasitaemia had reached at least 30 %, blood was taken by cardiac puncture under ether anaesthesia into heparinized tubes, after which the mice were sacrificed. After every 10th serial passage, blood from the 1st passage of the original strain stored in liquid nitrogen was used to begin a further series of 10 passages.

In 1 experiment, normal mice were bled 4 times at weekly intervals to induce reticulocytosis. On each occasion, 0·4 ml of blood was removed from the retro-orbital sinus under light ether anaesthesia.

Separation of parasites

Whole heparinized blood was mixed with an equal volume of a solution of 1 % saponin in 0·15 M NaCl (saponin–NaCl) for 1 min. The mixture was centrifuged at 200 ***g*** for 4 min to remove any intact blood cells. The supernatant fluid was recovered and centrifuged at 6000 ***g*** for 10 min. The supernatant fluid was discarded and the parasites resuspended and washed 3 times in PBS. Parasites recovered in this way were used both to immunize animals for the preparation of antiserum and for the preparation of antigen-coated tubes (see below).

Preparation of antibody to P. berghei

(*a*) *Preparation of antisera*

Antisera were raised in rabbits and in OF/1 mice. Inocula consisted of separated, washed parasites suspended in PBS and emulsified with an equal volume of Freund's incomplete adjuvant (FIA). The rabbits were immunized 6 times at 2 weekly intervals; on each occasion $5–7 \times 10^9$ parasites were used to prepare an inoculum of 2 ml which was injected at several subcutaneous sites. Blood was taken from ear veins 10 days after the final injection. The mice were immunized 4 times by intraperitoneal injection of a 0·1 ml inoculum containing $5–7 \times 10^8$

parasites which had been sonicated for 1 min before being emulsified with FIA. Blood was obtained from the retro-orbital sinus 1 week after the final injection. Antibody titres were measured by indirect immunofluorescence (IF).

Blood samples were allowed to clot at room temperature for 1 h and at 4 °C for 1 h. The serum obtained was stored at − 20 °C. Ammonium sulphate fractions of these sera were prepared using 48 % saturated ammonium sulphate in the case of rabbit sera and 33 % for mouse sera. These fractions were used to extract specific anti-*P. berghei* antibody.

(*b*) *Preparation and radio-isotope labelling of specific antibody*

Purified IgG was prepared from rabbit antiserum by chromatography from the ammonium sulphate fraction of the serum using a DEAE–cellulose (Whatman DE 23) column equilibrated with 0·01 M phosphate buffer, pH 8·2. IgG from normal serum was prepared in the same way.

Specific antibody to *P. berghei* was prepared from purified IgG in the case of the rabbit antiserum and from the ammonium sulphate fractions of the mouse antisera. Separated parasites were thoroughly washed with PBS and suspended in the IgG solution (concentration 2 mg/ml) or ammonium sulphate fraction (concentration 5–6 mg/ml) at a concentration of $2–5 \times 10^8$ parasites/ml. The suspension was mixed for 1 h at room temperature and at 37 °C for 30 min. It was then centrifuged at 6000 *g* for 10 min and the supernatant fluid discarded. The parasites were washed extensively with PBS until the optical density of the supernatant fluid was zero. Antibody was eluted from the parasites by suspending them in a volume of 0·12 M HCl–glycine buffer, pH 2·8, equal to the starting volume of IgG solution. The suspension was immediately centrifuged at 6000 *g* for 10 min and the supernatant fluid dialysed against saline overnight. The protein concentration was then adjusted to 1 mg/ml. The purified IgG from the rabbit antiserum was absorbed with normal mouse r.b.c. both before and after the preparation of specific antibody and after the labelling of the specific antibodies. For the absorption, the preparations were mixed with an equal volume of washed normal mouse r.b.c. and incubated at 37 °C for 1 h and at 4 °C for 30 min before centrifugation. The antibodies were labelled with ^{125}I (Radiochemical Centre Amersham, England) by the chloramine-T method (McConahey & Dixon, 1966). The specific activity of the radio-isotope labelled material was about 1·0 mCi/mg. The labelled IgG was stored in aliquots at − 70 °C.

Purified, unlabelled antibody remained stable for at least 6 months at − 70 °C. Labelled antibody was stable for up to 2 months.

Test for the detection of malaria antigens

(*a*) *Preparation of antigen-coated tubes*

Parasites from a pool taken from several different passages were separated and washed as described. Total parasites, including all blood forms, were counted in a haemocytometer and suspended in 1 ml of 0·1 M sodium carbonate/bicarbonate buffer, pH 9·5 and sonicated for 1 min. The sonicated parasites were then diluted with carbonate buffer to give a concentration equivalent to 15×10^6 whole para-

sites/ml. This was stored in aliquots at −70 °C. To coat tubes, the suspension was further diluted with carbonate buffer to $1{\cdot}5 \times 10^6$/ml. Disposable 4 ml vol. polypropylene tubes (Beckman) were coated with 200 μl of this suspension. The tubes were closed and incubated at 37 °C for 3 h, then stored at 4 °C for up to 2 weeks. Immediately before use the tubes were emptied and washed 3 times with PBS containing 0·05 % Tween 20 (PBS–Tween).

(*b*) *Labelled antibody*

On the day of the test, an aliquot of ^{125}I-labelled antibody prepared from rabbit or mouse antiserum to *P. berghei* was thawed and diluted to 1 ml with PBS containing 10 % (w/v) human serum albumin (PBS–HSA), then centrifuged at 18000 *g* for 10 min at 4 °C to remove aggregates. The antibody was diluted with PBS and used at a concentration of 2–4 μg/ml. Normal, labelled rabbit and mouse IgG were prepared in the same way. The radioactivity was measured in a gamma counter (Biogamma, Beckman).

(*c*) *Test and control red blood cells*

Red blood cells were separated from heparinized whole mouse blood of known parasitaemia and washed 3 times with PBS. An equal volume of saponin–NaCl solution was added and mixed. From this mixture, a series of dilutions was prepared either in PBS or in a solution containing 10 % normal lysed mouse r.b.c., prepared as follows. Normal, washed mouse r.b.c. were lysed with an equal volume of saponin–NaCl solution and were then diluted 1 in 5 with PBS–Tween. A control series of lysed, normal mouse r.b.c. was prepared by diluting the r.b.c.–saponin mixture with PBS–Tween.

(*d*) *Test procedure*

The test preparations of r.b.c. were incubated with labelled specific antibody to *P. berghei*. After this, the supernatant fluid of the r.b.c.–antibody mixture was reacted in tubes coated with parasite antigens. The amount of radioactivity fixed in the tubes was compared with that of control tubes reacted with the antibody alone. From the results, the inhibition of antibody binding by the test preparations was calculated. The method was applied to diluted r.b.c. from blood of high parasitaemia and to the diagnosis of early infection *in vivo*.

To 200 μl vols of the r.b.c. preparations at each dilution to be tested were added 200 μl of diluted, labelled antibody. Control tubes contained 200 μl of PBS–HSA in place of r.b.c. suspensions. In certain experiments, a second control was included, containing 200 μl of labelled normal IgG and 200 μl of PBS–HSA. The tubes were incubated at 37 °C for 1 h, then centrifuged at 6000 *g* for 5 min. From the supernatant fluid, 150 μl was taken into each of 2 antigen-coated tubes and incubated at room temperature for 3 h. The tubes were then washed 3 times with PBS–Tween and the radioactivity counted. The results were expressed as the percentage inhibition of radioactivity in the test preparations compared to that of the antibody–PBS control tubes.

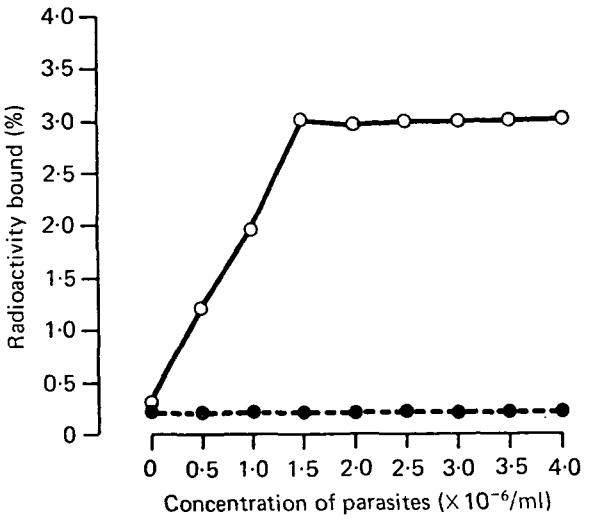

Fig. 1. Binding of radio-isotope labelled antibody (○—○) to *Plasmodium berghei* and of radio-isotope labelled normal immunoglobulin (●---●) in tubes coated with parasite antigens prepared from suspensions of parasites at different concentrations.

RESULTS

Lysed r.b.c. from mice infected with *P. berghei* were used as a source of antigen in experiments designed to detect intra-erythrocytic parasites by radio-immunoassay. Labelled antibodies prepared from rabbit and mouse antisera to *P. berghei* were used to measure the specific binding of radio-isotope labelled antibody in tubes coated with parasite antigens, following incubation of the antibody with the test r.b.c.

To find the optimal concentration of parasite antigen for the preparation of antigen-coated tubes, the binding of radio-isotope labelled antibody and of labelled normal IgG were tested in tubes coated with sonicated parasites prepared from parasite suspensions ranging in concentration from 10^5 to 4×10^6/ml. The results are shown in Fig. 1. There was increasing binding of antibody at parasite concentrations up to $1{\cdot}5 \times 10^6$/ml, at which there was 3·0% (approximately 15000 c.p.m.) binding of antibody and 0·2% (approximately 1000 c.p.m.) binding of normal IgG. At higher concentrations, there was no further increase in antibody binding and the non-specific binding of normal IgG remained constant. Tubes coated with antigen from sonicated parasites at a concentration of $1{\cdot}5 \times 10^6$/ml were used in the following experiments.

An experiment was carried out to detect parasite antigens in serial dilutions of r.b.c. suspensions from infected blood of 30% parasitaemia. To control the effect of concentration of r.b.c. in influencing non-specific antibody adsorption, the test r.b.c. were diluted in PBS and in PBS containing 10% normal r.b.c. The infected r.b.c. were also compared with normal r.b.c. diluted in PBS. The antibody used was purified from rabbit antiserum to *P. berghei*. The results are shown in Fig. 2. There was a significantly greater degree of antibody binding inhibition when the

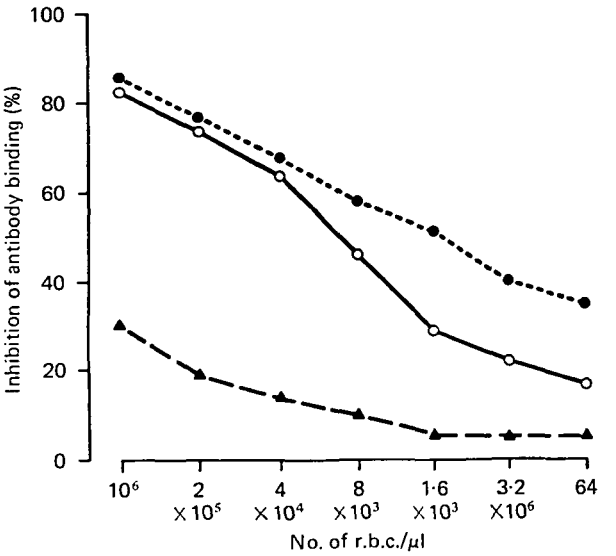

Fig. 2. Antibody-binding inhibition test carried out on r.b.c. from blood of 30% parasitaemia from mice infected with *Plasmodium berghei* and on normal mouse r.b.c. in serial 5-fold dilutions of PBS-Tween (▲---▲). The infected r.b.c. were diluted in PBS containing 10% normal r.b.c. (●---●) and in PBS-Tween (○—○). The antibody was purified from rabbit antiserum to *P. berghei*. The concentration of r.b.c. refers only to that of the test r.b.c. and does not include normal r.b.c. used as diluent.

test r.b.c. were diluted with normal r.b.c. The sensitivity of the test was limited in this experiment by the degree of inhibition shown by normal r.b.c.

The fixation of antibody by normal mouse r.b.c. was due, at least in part, to contamination of the inoculum used to immunize the rabbits by fragments of mouse r.b.c. membrane, leading to the production of antibodies which could not be completely absorbed. To minimize the fixation of antibody by normal mouse r.b.c. experiments were carried out using antibody purified from antiserum raised in OF/1 mice immunized with *P. berghei*. The results of these experiments are illustrated in Fig. 3. The fixation of antibody from mouse antiserum by normal r.b.c. was less than that found with rabbit antibody, thus giving a greater degree of sensitivity of antigen detection. At the lowest concentration of test r.b.c. which gave a significant difference from the control, the test preparation contained 12·8 r.b.c./μl and 3·8 parasites/μl. This is equivalent to a level of detection of approximately 1 parasite/million r.b.c. in whole blood.

In the preceding experiment, parasites were detected with a high degree of sensitivity in serial dilutions of blood of high parasitaemia. To find whether the method would be equally sensitive for the detection of early infection when parasitaemia is low, blood samples were examined at short time intervals after infection. A group of 24 mice were each inoculated with 0·1 ml of infected blood containing 10^6 parasites. Three mice were sacrificed and blood was collected at 6, 23, 30, 46, 56, 70, 96 and 144 h after infection. The parasitaemia and antibody-

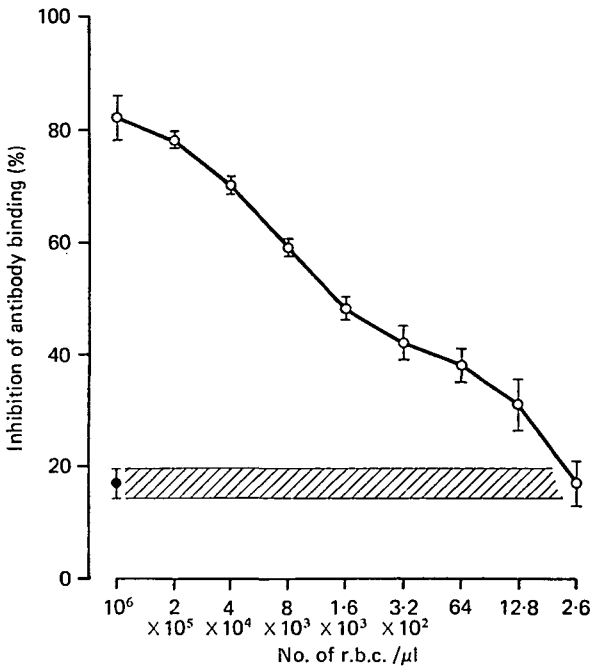

Fig. 3. Antibody-binding inhibition test carried out on diluted r.b.c. from blood of 30% parasitaemia from mice infected with *Plasmodium berghei* and on normal mouse r.b.c. at a concentration of $10^6/\mu l$. The antibody was purified from mouse antiserum to *P. berghei*. The mean inhibition ± 2 S.D. is shown for each concentration of infected r.b.c. The hatched area includes the mean ± 2 S.D. for normal r.b.c. at $10^6/\mu l$. Six samples were tested at each concentration of r.b.c.

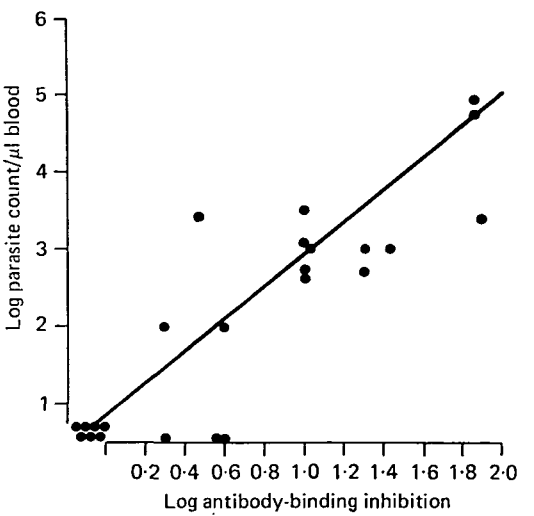

Fig. 4. Correlation of parasite counts and antibody-binding inhibition in mice during the early stages of *Plasmodium berghei* infection. Red blood cells were tested at a concentration of $10^6/\mu l$ and the results corrected in relation to control values for normal r.b.c. r, Coefficient of linear correlation = 0·7507; R, Spearman coefficient of correlation = 0·8154; $P < 0{\cdot}001$.

Table 1. *Antibody-binding inhibition in different preparations of* Plasmodium berghei-*infected red blood cells*

Concentration of r.b.c./μl	Antibody-binding inhibition (%)* A	B	C	D	E
1×10^6	88	86	83	73	48
4×10^4	72	76	75	61	7
$3{\cdot}2 \times 10^2$	12	11	15	11	0

A, Whole blood; B, unwashed r.b.c.; C, washed r.b.c.; D, whole r.b.c. incubated with hyperimmune serum; E, r.b.c. lysed and incubated with hyperimmune serum.

* Red blood cells tested at a concentration of $10^6/\mu$l and the results corrected in relation to control values for normal r.b.c.

Table 2. *Reticulocyte counts and antibody-binding inhibition in mice bled at weekly intervals*

Day	Reticulocytes (%)	Antibody-binding inhibition (%)*
1	$1{\cdot}4 \pm 0{\cdot}4$	$12{\cdot}1 \pm 2{\cdot}3$
8	$4{\cdot}5 \pm 2{\cdot}0$	$13{\cdot}7 \pm 3{\cdot}4$
15	$5{\cdot}5 \pm 2{\cdot}4$	$12{\cdot}8 \pm 2{\cdot}8$
22	$9{\cdot}2 \pm 2{\cdot}5$	$13{\cdot}6 \pm 3{\cdot}1$

* Red blood cells tested at a concentration of $10^6/\mu$l.

binding inhibition were estimated on individual samples from each animal. In the first 48 h, parasites were detected microscopically in 2 of 12 mice. In both of these and in a further 3 which were negative microscopically, there was significant antibody-binding inhibition. In the later samples, there was increasing positivity of the test results. As shown in Fig. 4, there was a highly significant degree of correlation between the parasite counts and the measurements of antibody-binding inhibition ($R = 0{\cdot}8154 : P < 0{\cdot}001$).

The preceding experiments were carried out using r.b.c. washed 3 times to remove free antibody before testing. To find whether the presence of antibody in the test blood may interfere with binding to the labelled antibody and hence affect the sensitivity of the assay, an experiment was carried out to compare the level of parasite detection in the presence and absence of antibody in the intial test preparations. The samples tested included washed and unwashed r.b.c., whole blood containing antibody and intact and lysed r.b.c. exposed to hyperimmune serum. Blood was taken from infected mice on day 8, at which time parasitaemia was 40% and the antibody titre measured by IF was 1:80. A sample of washed r.b.c. was incubated with an equal volume of hyperimmune serum of IF titre 1:2500 for 1 h at 37 °C, then washed 3 times in PBS before lysis. A second sample of washed r.b.c. was lysed and then incubated with an equal volume of hyperimmune serum. The hyperimmune serum used was the same mouse antiserum to *P. berghei* from which the purified antibody was prepared for use in the assay. The test was carried out on these samples and on washed and unwashed

r.b.c. and on whole blood lysed without further treatment. The results are shown in Table 1. The level of antibody-binding inhibition did not differ significantly in the various r.b.c. preparations except when hyperimmune serum was reacted with parasites released from lysed r.b.c. before testing.

Since reticulocytosis develops during the course of *P. berghei* malaria and becomes a marked feature in the late stages of infection, the possibility that the presence of large numbers of reticulocytes may have been responsible for non-specific adsorption of antibody (Lustig, Nussenzweig & Nussenzweig, 1977) in the present experiments was tested in non-infected mice with induced reticulocytosis. Five normal mice aged 8–10 weeks were bled at weekly intervals, i.e. on days 1, 8, 15 and 22 of the experiment and the samples were examined individually. As shown in Table 2, the reticulocyte counts rose to approximately 10% and there was no increase in antibody-binding inhibition.

To test the species specificity of the anti-*P. berghei* antibody preparation used for parasite detection, washed r.b.c. from 39 Gambian children with acute *P. falciparum* infection and known parasitaemia were assayed using the same procedure as described for the murine system but using normal human r.b.c. controls. In 31 of the 39 cases (80%) there was significant antibody-binding inhibition. The sensitivity of parasite detection in this heterologous system was much lower than in the murine system, with the limit of detection equivalent to approximately 200 parasites/million r.b.c. Further experiments on the detection of *P. falciparum* infection are in progress.

DISCUSSION

Our experiments demonstrate that intra-erythrocytic malaria parasites can be demonstrated serologically with a high degree of sensitivity. Using a murine malaria model, infection was detected in a solid phase radio-immunoassay which measures inhibition of specific antibody binding in parasite antigen-coated tubes following incubation of lysed, infected r.b.c. with radio-isotope labelled, specific antibody. Since the purpose of the study was to detect intact parasites rather than soluble malaria-associated antigens, the antibody used in the experiments was purified by adsorption to intact parasites washed free of soluble antigen and was therefore more specific for antigens on the parasite membrane.

Other available serological methods used in malaria diagnosis for the measurement of antibody levels give valuable information concerning the frequency and transmission of malaria and form an essential part of malaria control programmes (Voller, 1976). However, methods which measure antibody titre may indicate recent past experience of malaria, but cannot prove current infection. In the present experiments, parasitized r.b.c. were detected, thereby demonstrating active infection. The method used in this study is not proposed as a definitive immunodiagnostic test, but as a demonstration of the principle that the immunodiagnosis of malaria infection is practicable.

The specificity of the test was shown by comparison of antibody-binding inhibition by normal and parasitized r.b.c. Compared to normal r.b.c. at a concentration of $10^6/\mu l$, parasitized r.b.c. gave a significantly greater degree of inhibition at a

concentration of 12·8 r.b.c. (3·8 parasites)/μl. It has been shown that immunoglobulins in malaria-infected mice bind to the membranes of both infected and non-infected reticulocytes and that membrane-bound immunoglobulin is found in greater quantity on non-infected reticulocytes than on mature r.b.c. (Lustig *et al.* 1977). The possibility that reticulocytes in infected blood may have adsorbed antibody non-specifically was therefore tested in non-infected mice with induced reticulocytosis. Although the reticulocyte count reached approximately 10%, a level found in advanced *P. berghei* infection in association with high parasitaemia, there was no increase in antibody-binding inhibition.

A high degree of sensitivity was shown using homologous antisera where antibody-binding inhibition was significant at a level of 1 parasite/million r.b.c. This level of parasite detection at least equals that obtained by examination of thick blood films by microscopy. The sensitivity was demonstrated both in experiments in which serial dilutions of r.b.c. from blood of high parasitaemia were tested and in the detection of infection *in vivo*. In the earliest stages of infection, more positive results were found by radio-immunoassay than by microscopy and there was a high degree of correlation between the parasite counts and the degree of antibody-binding inhibition.

In order to test infected r.b.c. under as constant conditions as possible, in most experiments they were separated and washed 3 times before lysing with saponin. However, since results using lysed whole blood or washed, infected r.b.c. incubated with hyperimmune serum before lysis were the same as those obtained with washed r.b.c., the sensitivity of parasite detection was not influenced by the intial presence of antibody in the test preparations. The method can, therefore, be applied to whole blood or to untreated r.b.c. The specificity of the antibody used in the assay was further shown by the reduction of antibody-binding inhibition when lysed, infected r.b.c. were exposed to hyperimmune serum before testing.

The antibody used to react with infected r.b.c. was directed against membrane antigens of all the blood forms of *P. berghei*. This was shown by immunofluorescence carried out on suspensions of freshly isolated parasites from lysed, infected r.b.c. In these experiments, it was an advantage to use an antibody preparation capable of reacting with, and therefore detecting, all blood forms of the parasite. Using this antibody, there was a high degree of correlation between the number of parasitized r.b.c. in the test preparation and the degree of inhibition of antibody binding. However, since the antigens and corresponding antibodies in the test system have not been defined more closely and the antigens present in malaria may vary between individuals and in different phases of the disease, our method should be regarded as semi-quantitative.

While the test detected parasites with a high degree of sensitivity in infected mice, the effect of treatment is not known. It is possible that residual antigen present immediately after treatment may register in the test. However, any such effect is likely to be short-lived, since the antibody used is mainly directed to membrane antigens of the intact parasite. The sensitivity of detection of soluble antigen was found to be low with these reagents. June, Contreras, Perrin, Lambert & Miescher (1979) found that soluble antigen detectable by counter-immuno-

electrophoresis disappeared rapidly following treatment in *P. berghei*-infected mice and was absent in most cases 1 day after a single injection of chloroquine.

The procedure used for the preparation of specific antibody represents only a partial purification in which the concentration of antibody to parasite membrane antigens is enriched by adsorption and elution of the immunoglobulin or ammonium sulphate fractions of the antiserum. Factors determining the amount of fixation in the antigen-coated tubes include the concentration of antibody in the original serum, the amount of antigen available for adsorption and possibly the acid elution, which may not fully remove antibody of highest avidity and which may damage the parasite membrane leading to release of antigen into the eluate. However, 3% fixation gave satisfactory antibody activity in the conditions of the test.

Antigens found in extracts of parasitized r.b.c. and in plasma during *P. falciparum* infection have been classified according to their resistance to heating at different temperatures (Wilson, *et al.* 1969). On the basis of labelled amino acid incorporation, some of the antigens concerned appear to be of host origin (Wilson, 1974). In *P. berghei* infection, antigens have been found in the serum which could not be shown in parasite extracts (Seitz, 1976). A selective expression of malaria antigen has been shown on the membrane of infected reticulocytes rather than on mature oxyphilic cells in *P. berghei* infection (Poels, Van Niekerk & Franken, 1978). It is not known whether these membrane antigens are the same as the soluble, circulating antigens. In our experiments, the antibody used for parasite detection reacted with membrane antigens of the parasite but did not react with the membranes of intact, infected r.b.c. The test gave negative results when intact infected r.b.c. were used in place of lysed r.b.c.

The results obtained in the murine model demonstrate that the immunodiagnosis of malaria infection is practicable and that a high degree of specificity and sensitivity can be obtained. The experimental techniques described here provide a basis from which an immunodiagnostic test could be developed for use in human malaria. With the establishment of *in vitro* methods for the culture of *P. falciparum* (Trager & Jensen, 1976; Jensen & Trager, 1977), the availability of the necessary reagents can now be envisaged.

This work was supported by WHO, the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases, the Swiss National Foundation (Grant no. 3.847.0.77).

REFERENCES

Jensen, J. W. & Trager, W. (1977). *Plasmodium falciparum* in culture: use of outdated erythrocytes and description of the candle jar method. *Journal of Parasitology* **63**, 883–6.

June, C. H., Contreras, C. E., Perrin, L. C., Lambert, P. H. & Miescher, P. A. (1979). Circulating and tissue-bound immune complex formation in murine malaria. *Journal of Immunology* **122**, 2154–61.

Lustig, H. J., Nussenzweig, V. & Nussenzweig, R. S. (1977). Erythrocyte membrane-associated immunoglobulins during malaria infection of mice. *Journal of Immunology* **119**, 210–16.

McConahey, P. H. & Dixon, F. J. (1966). A method for trace iodination of proteins for immunologic studies. *International Archives of Allergy and Applied Immunology* **29**, 186–9.

Poels, L. G., Van Niekerk, C. C. & Franken, M. A. M. (1978). Plasmodial antigens exposed on the surface of infected reticulocytes: their role in induction of protective immunity in mice. *Israel Journal of Medical Sciences* **14**, 575–81.

Seitz, H. M. (1976). The *Plasmodium berghei* infection in isogenic F1 (C57Bl × DBA) mice. II. Antibodies and antigens in the serum. *Zeitschrift für Tropenmedizin Parasitologie* **27**, 33–43.

Trager, W. & Jensen, J. B. (1976). Human malaria parasites in continuous culture. *Science* **193**, 674–5.

Voller, A. (1976). Serodiagnosis of malaria. In *Immunology of Parasitic Infections* (ed. S. Cohen and E. Sadun), pp. 107–119. Oxford: Blackwell Scientific Publications.

World Health Organization (1974). Expert Committee on Malaria. *WHO Technical Report Series*, No. 16.

Wilson, R. J. M., McGregor, I. A., Hall, P., Williams, K. & Bartholomew, R. (1969). Antigens associated with *Plasmodium falciparum* infections in man. *Lancet*, ii, 201–5.

Wilson, R. J. M. (1974). The production of antigens by *Plasmodium falciparum in vitro*. *International Journal for Parasitology* **4**, 537–47.

Printed in Great Britain