

- sults with parasites in group O red blood cells grown in FCS compared with human A+ serum.
9. K. Marsh, J. A. Sherwood, R. J. Howard, in preparation.
  10. The agglutination titers for the following isolates are shown in parentheses (acute, convalescent): Isolate 176 (<1/4, 1/256); isolate 179 (1/8, >1/512); isolate 188 (<1/4, >1/128); isolate 189 (<1/4, >1/64); isolate 198 (<1/4, >1/64); isolate 200 (<1/4, >1/64); isolate 235 (1/50, >1/1600).
  11. Infected erythrocytes were purified to >80 percent parasitemia by centrifugation on a Percoll gradient containing sorbitol [S. B. Aley, J. A. Sherwood, R. J. Howard, *J. Exp. Med.* **160**, 1584 (1984)]. The cells were incubated with an equal volume of undiluted serum GA4 for 30 minutes at 37°C, and washed three times in PBS, and antibody was eluted with isotonic glycine buffer at pH 3 [O. P. Revvig and K. Hannestad, *Vax. Sang.* **33**, 280 (1977)]. The eluate was dialyzed against RPMI 1640 medium, concentrated to half the original volume by negative

- pressure dialysis, and tested for agglutination at this concentration.
12. Adaptation of *P. falciparum* to culture in vitro often leads to loss of the capacity of infected cells to adhere to melanoma cells [I. J. Udeinya, P. M. Graves, R. Carter, M. Aikawa, L. H. Miller, *Exp. Parasitol.* **56**, 207 (1983)] or loss of expression of knobs on the erythrocyte membrane [S. G. Langreth *et al.*, *ibid.* **48**, 213 (1979)]. Loss of the capacity of *P. falciparum*-infected erythrocytes to be agglutinated by sera from immune adults has not been reported previously.
  13. K. N. Brown, I. N. Brown, P. I. Trigg, R. S. Phillips, L. A. Hills, *Exp. Parasitol.* **28**, 318 (1970); H. Lustig Shear, R. S. Nussenzweig, C. Bianco, *J. Exp. Med.* **149**, 1288 (1979); A. Celada, A. Cruchaud, L. H. Perrin, *J. Immunol. Methods* **63**, 263 (1983).
  14. J. Brown and M. E. Smalley, *Clin. Exp. Immunol.* **41**, 423 (1980).
  15. I. J. Udeinya, L. H. Miller, I. A. McGregor, J. B. Jensen, *Nature (London)* **303**, 429 (1983).

16. P. H. David, M. Hommel, L. H. Miller, I. J. Udeinya, L. D. Oligino, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 5075 (1983).
17. J. W. Barnwell, R. J. Howard, H. A. Coon, L. H. Miller, *Infect. Immun.* **40**, 985 (1983).
18. M. J. Dunn, *J. Clin. Invest.* **48**, 674 (1969); K. Tanabe, R. B. Mikkelsen, D. F. H. Wallach, *J. Cell Biol.* **93**, 680 (1982); H. Ginsburg, S. Kutner, M. Krugliak, Z. I. Cabantchick, *Mol. Biochem. Parasitol.* **14**, 313 (1985); B. C. Elford, J. D. Haynes, J. D. Chulay, R. J. M. Wilson, *ibid.* **16**, 43 (1985).
19. I. J. Udeinya, J. A. Schmidt, M. Aikawa, L. H. Miller, I. Green, *Science* **213**, 555 (1981).
20. J. H. Leech, J. W. Barnwell, L. H. Miller, R. J. Howard, *J. Exp. Med.* **159**, 1567 (1984); S. B. Aley, J. A. Sherwood, R. J. Howard, *ibid.* **160**, 1585 (1984).
21. S. B. Aley, J. A. Sherwood, K. Marsh, O. Eidelman, R. J. Howard, *Parasitology*, in press.
22. R. J. Howard and K. Marsh, in preparation.

30 August 1985; accepted 3 October 1985

## Rat Resistance to Schistosomiasis: Platelet-Mediated Cytotoxicity Induced by C-Reactive Protein

DANIEL BOUT\*, MICHEL JOSEPH, MICHEL PONTET, HAN VORNG, DIDIER DESLÉE, ANDRÉ CAPRON

In rats infected with the parasite *Schistosoma mansoni*, the concentration of C-reactive protein in the serum increases after the lung stage of infection and is at its highest at the time of terminal worm rejection. The peak of platelet-mediated cytotoxicity induced by infected serum that has been heated (and is free of immunoglobulin E) as well as the time course for the development of platelet cytotoxic activity in infected rats was found to be correlated with the concentration of C-reactive protein. Rat and human platelets treated with homologous serum obtained during an acute phase of inflammation or with purified C-reactive protein were able to kill the immature forms of the worm in vitro. Platelets treated with C-reactive protein were furthermore capable of conferring significant protection against schistosomiasis in transfer experiments. Collectively these data indicate that a system that includes C-reactive protein and platelets participates in the natural resistance of the rat to schistosomal infection.

SCHISTOSOMIASIS AFFECTS AN ESTIMATED 200 to 300 million people. Vaccination appears to be the most favorable approach to controlling the disease, but before a vaccine can be developed there must be a greater understanding of the nature of the protective immune responses in humans and experimental animals (1). The mouse and rat have been widely used as experimental models for schistosomiasis; both animals show some degree of resistance to reinfection. The mouse, described as a permissive host, allows schistosomes to complete their life cycle to full maturity during a primary infection. In this case, resistance to reinfection is directly proportional to the degree of granulomatous inflammation that occurs in response to eggs trapped in the liver (2). Inflammation is associated, for example, with a sharp and long-lasting increase in serum amyloid P-component (SAP), the major acute-phase reactant in the mouse (3). In contrast, the rat, termed a nonpermissive host, demonstrates a natural resistance to schistosomiasis

which is manifested as a 95 percent terminal rejection of immature worms at 4 weeks of a primary infection (4). No clear and definite explanation has been provided to account for this phenomenon. To determine whether inflammation plays a role in natural resistance of the rat to schistosomiasis, and possibly in resistance to reinfection, we studied the development and the potential role of serum C-reactive protein (CRP), the homolog of murine SAP, during the course of infection.

Rat CRP closely resembles human CRP in its amino acid composition, in having five subunits per molecule, and in its electron microscopic appearance as a pentameric annular disk (5). CRP is a major acute-phase reactant in most species and has the property of calcium-dependent binding to the phosphoryl choline and to phosphoryl choline-containing substances. Among the characteristics that differentiate rat CRP from that of other species is that rat CRP is unable to activate homologous complement. CRP is synthesized by hepatocytes and is probably

under the influence of humoral mediators such as interleukin-1 (6), which is actively synthesized by monocytes and macrophages when they are stimulated by microorganisms, by microbial products, and by activated T lymphocytes or their lymphokine products (5). CRP has been shown to interact with cells, noticeably with platelets (7), and to affect their cellular functions. The functions of CRP are poorly known (5). Several biological activities have been measured in vitro with purified CRP (5) that are consistent with a role in host defense, but the difficulty lies in extending them to good models of host resistance in vivo.

As shown in Fig. 1A, Fischer rats developed a marked and prolonged elevation of the serum concentration of CRP as measured by immunoelectroassay (9), with a peak at day 28 after infection with *Schistosoma mansoni*. The high levels persisted almost until day 41.

The role of CRP in protection against schistosomiasis was investigated as follows. A rat serum was obtained 24 hours after the subcutaneous injection of turpentine (0.5 ml per 100 g of body weight), which is known to increase the serum concentration of acute phase proteins. This serum was unable to kill the immature worms (schistosomula) in vitro either alone or in association with macrophages or eosinophils. However, platelets from normal rats incubated with 10 percent serum from turpentine-injected rats, but not with 10 or 20 percent serum from normal rats, were larvicidal in vitro (Table 1). Furthermore, the

D. Bout, M. Joseph, N. Vorng, D. Deslée, and A. Capron, Centre d'Immunologie et de Biologie Parasitaire, Institut Pasteur, 59019 Lille Cédex, France.  
M. Pontet, Département de Biochimie, U.E.R. Biomédicale des Saints-Pères, 45 Rue des Saints-Pères, 75270 Paris Cedex, 06, France.

\*Send correspondence to Department of Immunology, Montreal General Hospital Research Institute, 1650 Cedar Avenue, Montreal, Quebec, H3G 1A4 Canada.

platelets obtained from two rats that had been injected 4 hours previously with 2 ml of such a serum expressed high cytotoxic activity against schistosomula ( $76.5 \pm 3.5$  percent; mean  $\pm$  standard error) when compared to controls ( $14 \pm 5$  percent), suggesting an in vivo activation of platelets. Involvement of CRP was studied by using platelets activated by CRP purified (8) from the serum of turpentine-injected rats. The results showed that cytotoxicity in vitro was

CRP dose-dependent with a plateau occurring at 250 ng/ml and that CRP-depleted serum was no more efficient (Table 1). We also tested CRP purified from either normal (two lots) or turpentine-treated (three lots) rat sera. At a concentration of 5  $\mu$ g/ml, as measured by immunoelectroassay and by Lowry analysis, these sera showed a larvicidal activity of  $24.4 \pm 2$  percent (two duplicates) and  $75.9 \pm 5.1$  (three duplicates), respectively. This suggested that

during an inflammatory state, CRP could be selectively produced in a form able to more efficiently activate platelets to kill schistosomula. These data could be related to the recent demonstration of neo-CRP, which is antigenically different from native CRP (10).

Pure interleukin-1 (11), which could have possibly contaminated the CRP preparation and is known to be functionally active at very low concentrations, was tested. No platelet cytotoxicity could be evoked (Table 1).

Human platelets incubated with CRP-rich human serum from a patient with rheumatoid arthritis or with CRP purified from the pooled sera of patients with a history of inflammatory conditions also expressed cytotoxic activity against schistosomula in vitro (Table 1). Moreover, the CRP-like molecule from the horseshoe crab (*Limulus polyphemus*) (12) could activate human and rat platelets to be cytotoxic (Table 1). In these same cell systems, experiments performed with the use of Boyden chambers demonstrated that a direct contact of platelets with schistosomula was not essential to the larvicidal effect, as had been demonstrated previously with immunoglobulin E (IgE)-treated platelets (13) and lymphokine-activated mouse macrophages (14).

With regard to the kinetics of the CRP response during experimental schistosomiasis, we studied the schistosomicidal capacity of rat serum from infected animals that was collected at various times after infection and tested with platelets from normal rats. High cytotoxicity was shown as early as 13 days after infection and until day 62 (Fig. 1B). Interaction of platelets with infected rat serum was demonstrated previously during the 30- to 50-day period of infection (13) when IgE antibodies are produced at their maximum level. The cytotoxic effect was ascribed to IgE antibodies in this system with a maximum at day 42. Because CRP function is unaffected by heating for 3 hours at 56°C, at which temperature IgE antibodies are destroyed, we tried to distinguish between the possible role of CRP and IgE using native or heated serum collected from rats in the course of infection. The CRP concentrations did not change in the heated serum. Native and heated serum mediated the same levels of cytotoxicity from day 13 to day 31, suggesting a predominant role of CRP in this early phase of infection. However, unlike the native serum, the heated serum was no longer effective after 41 days, confirming our previous observations of the IgE dependency of platelet activation during the later period.

Table 1. Effect of CRP on ability of rat and human normal platelets to kill schistosomula in vitro. Blood platelets were obtained from rats or humans as described (13), incubated for 2 hours with 10 percent tested sera or with purified CRP in 10 percent normal serum, and used at physiological concentration in flat-bottomed microplates at 37°C in 5 percent CO<sub>2</sub> with 10 percent normal serum. Fifty schistosomula in EMEM were added and percentage of dead larvae was evaluated after 24 hours. Each test was made in duplicate (mean  $\pm$  standard error). CRP's were purified from turpentine-injected rat sera and from human patient sera by affinity chromatography on phosphoethanolamine substituted Sepharose beads (8). Contaminants were removed by DEAE anion exchange and finally purification was by filtration on Sephacryl S-300 gel. These purified CRP's were dialyzed against 10 mM citrate buffer, pH 7.4, and EMEM before use. PAGE-SDS of purified rat CRP showed only two bands corresponding to purified rat CRP as already described (9). When tested by bidimensional immunoelectrophoresis using rabbit IgG raised against rat serum (8), this purified CRP showed only one peak. CRP-depleted, turpentine-injected rat serum was prepared by affinity chromatography on phosphoethanolamine-Sepharose (8) and used at the same concentration as the starting serum.

Treatment	Dead schistosomula (%)*	
	With platelets	Without platelets
<i>Rat model</i>		
Normal rat serum (CRP 0.48 mg/ml)	14.0 $\pm$ 1	8.5 $\pm$ 0.5
Turpentine-injected rat serum (CRP 1.28 mg/ml)	81.0 $\pm$ 4	13.5 $\pm$ 0.5
Purified rat CRP		
0.01 $\mu$ g/ml	11.0 $\pm$ 1	
0.05 $\mu$ g/ml	31.0 $\pm$ 5	
0.25 $\mu$ g/ml	62.0 $\pm$ 5	
0.75 $\mu$ g/ml	58.0 $\pm$ 1	
10.00 $\mu$ g/ml	67.5 $\pm$ 1.5	
250.00 $\mu$ g/ml	69.5 $\pm$ 8.5	3.5 $\pm$ 0.5
CRP-depleted serum from turpentine-injected rats	20.5 $\pm$ 1	6.0 $\pm$ 1
<i>L. polyphemus</i> CRP (500 $\mu$ g/ml)	73.0 $\pm$ 0	8.5 $\pm$ 0.5
Interleukin-1 (5 U/ml)	18.0 $\pm$ 3	15.0 $\pm$ 2
<i>Human model</i>		
Normal human serum (CRP 0.5 $\mu$ g/ml)	3.5 $\pm$ 0.5	5.0 $\pm$ 1.1
Serum from patient with rheumatoid arthritis (CRP 15 $\mu$ g/ml)	72.5 $\pm$ 2.5	7.0 $\pm$ 1.6
Purified human CRP (500 $\mu$ g/ml)	90.5 $\pm$ 0.5	8.0 $\pm$ 3.2
<i>L. polyphemus</i> CRP (500 $\mu$ g/ml)	77.5 $\pm$ 0.5	6.0 $\pm$ 3

\* $\pm$  Standard error.

Table 2. Protection against schistosomiasis induced by the passive transfer of treated platelets. Male Fischer rats (250 g) were injected with  $1.5 \times 10^9$  platelets 1 hour after infection by percutaneous exposure to 500 (first experiment) or 1000 (second experiment) *S. mansoni* cercariae. The platelets had been incubated for 4 hours with 10 percent acute-phase serum or with protein at a final concentration of 1 mg/ml and washed. Liver perfusion for worm enumeration was performed 21 days later. SE, standard error.

Treatment of platelets	Number of rats	Total worm (mean $\pm$ SE)	Protection (%)	P*
<i>First experiment</i>				
Normal platelets	4	93 $\pm$ 16.3		
Platelets treated with acute-phase serum	5	44.4 $\pm$ 7.8	52.3	<0.005
CRP-treated platelets	5	17 $\pm$ 12.6	81.8	<0.05
<i>Second experiment</i>				
Lactoferrin-treated platelets	3	248 $\pm$ 1.15		
CRP-treated platelets	5	123.6 $\pm$ 4.4	50.2	<0.001

\*Student's *t*-test.

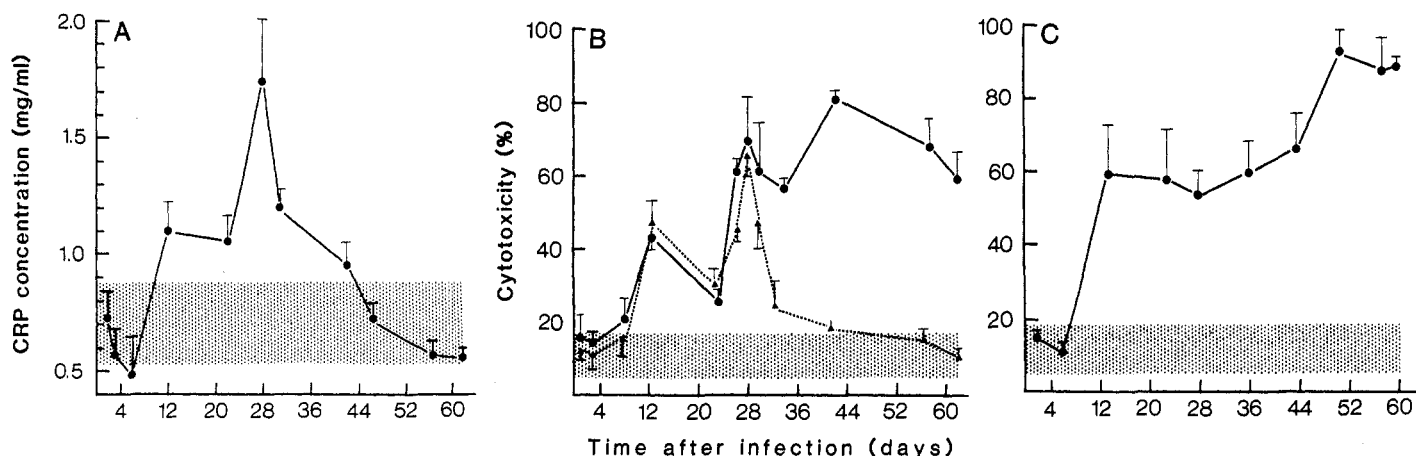


Fig. 1. (A) Changes in serum CRP concentration in Fischer rats after percutaneous infection by 500 cercariae of *S. mansoni* (worm enumeration by liver perfusion of five rats at day 21 gave  $104 \pm 6.8$  worms). Each point represents the arithmetic mean with standard deviation (SD), for five animals. The range of the controls (five normal rats at day 0, day 7, and day 31) shown in the shaded area is calculated as the mean  $\pm 1$  SD in order to be consistent with the results described for the experimental groups. (B) Killing properties of normal rat platelets activated by serum of rats during the course of schistosomiasis. The test was performed as described in Table 1. The solid

line corresponds to untreated serum and the dashed line to heated (3 hours at  $56^\circ\text{C}$ ) serum. Each point represents the percentage of cytotoxicity ( $\pm$  SD) of serum from three different animals tested in duplicate. The range of the controls (three normal rats at day 0, day 7, and day 31) shown in the shaded area is calculated as the mean  $\pm 1$  SD. (C) Time course for the development of the cytotoxic effect of platelets from infected rats. Each point represents the percentage of cytotoxicity ( $\pm$  SD) of three different animals tested in duplicate. The range of the controls (three normal rats at day 23, day 36, day 43, and day 50) shown in the shaded area is calculated as the mean  $\pm 1$  SD.

The relevance of these observations in vivo was assessed first by testing in vitro the larvicidal activity of platelets from infected rats at different times after exposure to cercariae, then by transfer of CRP-treated platelets to rats at the time of infection. Platelets from infected rats were larvicidal as early as 14 days after infection (Fig. 1C) at which time no antibody against schistosomes could be detected (15). The passive transfer of platelets treated with serum from turpentine-injected rats, as well as the transfer of purified CRP-treated platelets into normal syngeneic recipients on the day of a challenge infection, also led to a high degree of protection with, respectively, a 52.3 and 81.8 percent reduction in the total number of worms in the liver 3 weeks after infection compared to control rats injected with the same number of untreated platelets (Table 2). In another transfer experiment, a 50.2 percent protection was observed in rats injected with CRP-treated platelets compared to control rats injected with platelets treated with lactoferrin (as an unrelated protein) (Table 2). Phosphoryl choline-containing molecules have been identified at the membrane surface of schistosomula and are available for CRP binding.

We also demonstrated that radiolabeled CRP could be removed by unlabeled CRP from the surface of platelets previously incubated with  $^{125}\text{I}$ -labeled CRP in a dose-dependent fashion. By using a fluorescent antibody cell sorter, we showed that  $17.6 \pm 2.8$  percent of platelets ( $10^5 \times$

$8.86 \pm 0.35$  per microliter) obtained from five normal rats and only  $4 \pm 0.7$  percent of platelets obtained from four rats at 24 hours after turpentine injection ( $10^5 \times 4.32 \pm 0.12$  per microliter) were CRP positive. These observations suggest that CRP-positive platelets could be selectively sequestered at the site of inflammation.

The mechanism by which the mediator, or mediators, of CRP synthesis is produced during the course of rat schistosomiasis as well as the mechanism by which platelets are cytotoxic have still to be defined. In our system in vitro, the platelets were not lysed (lactate dehydrogenase was not found in culture medium) and did not appear to act through oxygen metabolites as judged by chemiluminescence assays.

Our results indicate that CRP is involved in schistosome killing by platelets in the rat model. The first decrease in worm load in vivo, which occurred at or shortly after the lung stage (10 to 15 days) (16), coincided with an increase in serum CRP, with the development of cytotoxicity against schistosomes by serum from infected rats in the presence of platelets from normal, uninfected rats, and with the larvicidal activity of platelets from infected rats in the presence of normal serum in vitro. Since schistosomes recovered from the liver at 21 days of infection were still susceptible to killing by CRP-activated platelets, and since the peaks of serum CRP and platelet-mediated cytotoxicity by heated, IgE-free serum from infected rats

coincided with the final worm rejection at 28 days of infection, it is possible that the CRP-platelet mechanism may also operate at the later stages of worm rejection. Serum from rats inoculated with irradiated cercariae and consequently protected against challenge with normal cercariae at 28 days (17) was also able to induce platelet-mediated cytotoxicity against schistosomula in vitro. This was demonstrated with serum taken from rats at 13 and 20 days, but not at 6 days, after infection with irradiated cercariae.

Recent studies have demonstrated a correlation between granuloma formation and the development of acquired immunity in the murine host during schistosomiasis, and it is conceivable that the inflammatory response, which is induced by antigenic egg products and is immunological in nature (2), destroys immature schistosomes by a mechanism similar to that observed in the rat model. In addition to enhancing our understanding of nonspecific effector mechanisms involved in resistance against parasites, these observations provide new insights into the interaction of platelets and CRP in inflammatory reactions.

#### REFERENCES

1. M. A. Smith and J. A. Clegg, *Science* 227, 535 (1985).
2. S. R. Smithers and M. J. Doenhoff, in *Immunology of Parasitic Infections*, S. Cohen and K. Warren, Eds. (Blackwell, Oxford, 1982), pp. 527-607.
3. M. B. Pepys, M. Baltz, K. Gomer, A. J. S. Davies, M. Doenhoff, *Nature (London)* 278, 259 (1979).
4. L. S. Ritchie, S. Garson, W. B. Knight, *J. Parasitol.* 49, 571 (1963).

20 May 1985; accepted 3 October 1985

5. M. B. Pepys and M. L. Baltz, *Adv. Immunol.* **34**, 141 (1983).
6. S. S. MacIntyre, D. Schultz, I. Kushner, *Ann. N.Y. Acad. Sci.* **389**, 76 (1982).
7. B. A. Fiedel, R. M. Simpson, H. Gewurz, *ibid.*, p. 263.
8. M. Pontet, M. D'asnières, D. Gache, J. Escaig, R. Engler, *Biochim. Biophys. Acta* **671**, 202 (1981).
9. F. C. de Beer *et al.*, *Immunology* **45**, 55 (1982).
10. L. A. Potempa, K. M. Pleskach, R. P. T. Potempa, H. Gewurz, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **44**, 1190 (1985).
11. Ultrapure human interleukin-1 was provided by Genzyme, Boston.
12. F. A. Robey and T. Y. Liu, *J. Biol. Chem.* **256**, 969 (1981).
13. M. Joseph, C. Auriault, A. Capron, H. Vorng, P. Viens, *Nature (London)* **303**, 810 (1983).
14. D. T. Bout, M. Joseph, J. R. David, A. R. Capron, *J. Immunol.* **127**, 1 (1981).
15. A. Capron, J. P. Dessaint, M. Capron, M. Joseph, G. Torpier, *Immunol. Rev.* **61**, 41 (1982).
16. P. M. Knopf, B. L. Mangold, G. J. Makari, *Parasitology* **86**, 37 (1983).
17. M. J. Ford, Q. D. Bickle, M. G. Taylor, *ibid.* **89**, 327 (1984).
18. We thank J. P. Kusnier for the fluorescent antibody cell sorter analysis and M. Ollivier for technical help. We also thank M. B. Pepys and M. Baltz for quantification of rat serum CRP and helpful discussions, and R. F. Mortensen for review of the manuscript.

20 May 1985; accepted 3 October 1985

## Effect of Antibodies to Recombinant and Synthetic Peptides on *P. falciparum* Sporozoites in Vitro

D. MAZIER, S. MELLOUK, R. L. BEAUDOIN, B. TEXIER, P. DRUILHE, W. HOCKMEYER, J. TROSPER, C. PAUL, Y. CHAROENVIT, J. YOUNG, F. MILTGEN, L. CHEDID, J. P. CHIGOT, B. GALLEY, O. BRANDICOURT, M. GENTILINI

Antibodies were raised in mice immunized with several recombinant and synthetic peptides of the circumsporozoite protein of *Plasmodium falciparum*. The antibodies were evaluated for protective activity in a human hepatocyte culture system. They exerted their protective effect against the parasite at three points: sporozoite attachment to the hepatocyte surface, entry, and subsequent intracellular development. Inhibition of attachment and entry were found to be related to the antibody titer against the authentic circumsporozoite protein on the sporozoite surface, especially when peptides were administered with alum or complete Freund's adjuvant. Even when invasion was not totally inhibited, the presence of abnormal trophozoites and a frequent inhibition of schizont development in long-term cultures suggested continued activity of antibodies at the intracellular level after sporozoite penetration had been completed.

**D**ESPITE PROMISING RESULTS FROM earlier vaccination experiments against human malaria involving irradiated sporozoites (1, 2), progress on a purified sporozoite vaccine suitable for use in humans has been delayed for lack of the required immunogens. Recently, however, synthetic peptides and recombinant DNA circumsporozoite (CS) protein derivatives were produced and characterized (3, 4), and both are now available for experimentation. In the present study, we took advantage of a recently described system for cultivation of the liver stages of *Plasmodium falciparum* (5) to study the ant sporozoite activity of antibodies raised against synthetic peptides and recombinant CS protein products of this species.

Human hepatocytes obtained from liver biopsies (6) were seeded at a concentration of  $10^5$  cells per chamber in eight-chamber plastic Lab-Tek slides (Miles) and cultured for 24 to 48 hours before sporozoite inoculation. Sporozoites of *P. falciparum* were obtained from *Anopheles stephensi* and

*Anopheles freeborni* mosquitoes after feeding on gametocytes from cultures of the NF54 strain through an artificial membrane. Salivary glands were aseptically dissected and, after removal of medium from the culture chambers, sporozoites were added in 50  $\mu$ l of medium containing test or control serum. This medium was replaced 3 hours later by medium without antibody, and thereafter was changed daily.

Sera from groups of five mice immunized with recombinant or synthetic peptides were pooled for testing (3, 4). The animals had received two injections at a 4-week interval of one of the following recombinant protein constructs of the repeat region of the CS protein: R16tet32 (R16), R32tet32 (R32), or R48tet32 (R48) administered with complete Freund's adjuvant (CFA), alum, murabutide (7), or phosphate-buffered saline (PBS) or two injections of one of the following synthetic peptides administered with CFA: 8- or 16-amino acid peptide sequences of the repeat region of the CS protein or of region I or region II, which are

conserved amino acid sequences occurring outside the repeat regions in the CS proteins of both *P. falciparum* and *Plasmodium knowlesi*. Controls consisted of normal mouse serum, serum from mice injected with alum only, and monoclonal antibodies recognizing either the CS protein of *P. falciparum* or that of *Plasmodium vivax*. Monoclonal antibodies were purified from ascitic fluids and titrated at least  $1:10^5$  against the homologous sporozoites. The level of antibody reactivity against the immunizing peptides was measured by the enzyme-linked immunosorbent assay (ELISA) (3, 4), while the reactivity with the authentic CS protein on the surface of the sporozoite was determined by the immunofluorescent antibody test (IFAT).

Both qualitative and quantitative experiments were performed. In the qualitative experiments, five pairs of salivary glands dissected in a 1:20 dilution of test or control sera were added to each chamber. In quantitative experiments, 25  $\mu$ l of a 1:5 dilution of the serum was added to each chamber. Salivary glands were homogenized with culture medium in a tissue homogenizer, sporozoites counted, and an equal number added to each chamber in a volume of 25  $\mu$ l. Depending on the number available,  $1 \times 10^4$  to  $5 \times 10^4$  sporozoites per chamber were used to inoculate each of the cultures. In any one experiment, the size of the inoculum was the same for all chambers, including controls. Unless otherwise stated, all quantitative experiments were carried out in a final serum dilution of 1:10.

Cultures were examined with a fluorescent assay (5). The number of sporozoites that invaded a liver cell and developed into a liver form was determined for each culture chamber 24 hours after the inoculum was added to the chamber. This delay was required to identify unequivocally intracellular parasites. In preliminary experiments, test cultures were trypsinized at 24 hours to remove parasites attached to the cell surface. Examination of these cultures confirmed that the remaining forms were indeed intracellular. In short-term cultures (24 to 48

D. Mazier, S. Mellouk, B. Texier, P. Druilhe, B. Galley, O. Brandicourt, M. Gentilini, Département de Parasitologie, Groupe Hospitalier Pitie-Salpêtrière, 75013 Paris, France.

R. L. Beaudoin, J. Trospier, C. Paul, Y. Charoenvit, Malaria Branch, Naval Medical Research Institute, Bethesda, MD 20814.

W. Hockmeyer, Department of Immunology, Walter Reed Army Institute of Research, Washington, DC 20307.

J. Young, Department of Molecular Genetics, Smith Kline & French Laboratories, King of Prussia, PA 19101.

F. Miltgen, Muséum National d'Histoire Naturelle, Laboratoire de Zoologie-Vers, 75005 Paris, France.

L. Chedid, Service Immunothérapie Expérimentale, Institut Pasteur, 75015 Paris, France.

J. P. Chigot, Service de Chirurgie, Groupe Hospitalier Pitie-Salpêtrière.