INFECTION AND IMMUNITY, Jan. 1992, p. 136–142
0019-9567/92/010136-07$02.00/0

Vol. 60, No. 1

T-Cell-Dependent Immunity and Thrombocytopenia in Rats Infected with *Plasmodium chabaudi*

HERVÉ WATIER,[1]† CLAUDIE VERWAERDE,[1] IRÈNE LANDAU,[2] EKKEHARD WERNER,[1] JOSETTE FONTAINE,[1] ANDRÉ CAPRON,[1] AND CLAUDE AURIAULT[1]*

Centre d'Immunologie des Maladies Transmissibles et Allergiques (Pr. Capron), Institut National de la Santé et de la Recherche Médicale U167, Centre National de la Recherche Scientifique, URA 624, Institut Pasteur, 1 Bis Rue du Dr. Calmette, 59019 Lille Cédex,[1] and Laboratoire de Zoologie-Vers (Pr. Chabaud), Centre National de la Recherche Scientifique URA 114, Muséum National d'Histoire Naturelle, 75231 Paris Cédex 05,[2] France

Received 14 June 1991/Accepted 11 October 1991

Normal, splenectomized, and athymic Fischer rats were infected with *Plasmodium chabaudi*. In normal rat infections, acute-phase infection resolved rapidly and completely. In splenectomized rats, infection resulted in high parasitemia and ultimately death. In nude rats, parasite growth was reduced compared with normal rats, and a persistent parasitemia (between 20 and 45%) was observed for several months. Complete resolution of the infection was achieved after adoptive transfer of T lymphocytes, even when transfer occurred during the course of infection. These results indicated that an acquired, T-lymphocyte-dependent immunity was necessary for the complete recovery observed in normal rats. In normal rats, thrombocytopenia and splenomegaly occurred during infection. By contrast, in nude rats, both of these pathological manifestations were only observed after thymus grafting. Thrombocytopenia was also absent in the splenectomized animals. Despite an increase in platelet-associated immunoglobulin levels during the infection, thrombocytopenia was not transferred by injection of infected rat serum to normal recipients. It has been concluded that the nude rat infection can be regarded as a novel and useful model for studying the T-cell-dependent effector and pathological mechanisms and to investigate the anti-*P. chabaudi* immune response.

In 1991, despite all eradication strategies, malaria still remains a major preoccupation of health services worldwide. This human parasitic disease is caused by one of four species of plasmodia (*Plasmodium falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*). Of them, *P. falciparum* is the most frequent and is responsible for the most severe complications.

The immune response against *Plasmodium* spp. has not yet been completely elucidated. The most contradictory observation is that "immune" subjects in endemic areas are protected against the disease (premunition) while bloodstream forms of the parasite continue to multiply slowly, allowing transmission to the *Anopheles* vector (28). Immunity against human plasmodial species is widely recognized as being dependent on T lymphocytes (23). A proliferative T-lymphocyte response to *P. falciparum* antigens, accompanied by interleukin-2 and gamma interferon (IFN-γ) production, can be observed in patients exposed to the parasite (23).

Immunopathological events during malaria, particularly in "non-immune" subjects, could be generated by an overwhelming immune response. Excessive hemolysis, nephropathy, and cerebral malaria are mediated by humoral and/or cellular immune mechanisms (11, 47). Thrombocytopenia is a common pathological feature of malaria (48). Peripheral platelet hyperdestruction is involved, but disseminated intravascular coagulation is rare (24). Because of an increase in platelet-associated immunoglobulin G levels during infection (27), human malarial thrombocytopenia is considered to be of immunological origin.

For many years, rodent experimental infections with African rodent parasites have provided useful models for malaria studies (33). Observations made from murine models are often relevant to the human malarial pathology, cerebral malaria being one of the best illustrations (21). Studies of murine thrombocytopenia have confirmed previous observations in humans, notably, the relationship between the loss of platelets and the immunocompetence of the animals. Moreover, the likely role of antibodies under T-cell dependence has been demonstrated in the genesis of the thrombocytopenia (20).

In rodent models, protective immunity is also dependent on T lymphocytes (49), as demonstrated in rats infected with *P. berghei* (6, 46) and in mice infected with *P. yoelii* (12, 41), *P. chabaudi* (4, 9, 10, 22, 31, 32), or *P. vinckei* (10). The murine models of *P. chabaudi* malaria are widely used (7, 31, 33, 50) and have allowed a precise analysis of the role of $CD4^+$ T lymphocytes in the protective immunity (31, 32, 50). Because the naturally resistant rats could only be infected with rat-adapted strains of *P. chabaudi*, this host-parasite combination has been poorly studied (13, 34). In this study, the evolution and the pathological features occurring during infection of normal, splenectomized, and athymic nude rats with *P. chabaudi* were compared. To determine precisely the role of T lymphocytes in this model, nude rats were immunologically reconstituted.

MATERIALS AND METHODS

Animals. Experimental groups of male Fischer 344 rats (4 weeks old, 60 to 80 g), or male BALB/c mice (7 to 10 weeks old) were maintained on a standard diet under an 8.00

* Corresponding author.
† Present address: Laboratoire d'Immunologie (Pr. Bardos), Faculté de Médecine, 37032 Tours Cédex, France.

a.m.–8.00 p.m. artificial lighting regimen. Nude rats (homozygous *nu/nu* and heterozygous *nu/+*) with the Fischer background were produced from the line established in 1983 at the Institut Pasteur de Lille (38).

Parasite. The rat-adapted 96V strain (13) of *P. chabaudi chabaudi* was maintained in rats by serial intraperitoneal blood passages of heparinized (Choay, Paris, France) and Hanks-Wallace (GIBCO, Grand Island, N.Y.)-diluted infected blood. For all experiments, except when indicated, 10^7 parasitized erythrocytes (PRBC) were injected. For infection of nude rats, parasitized blood was depleted of mononuclear cells by two successive centrifugations on a Ficoll gradient (Ficoll-Paque; Pharmacia, Uppsala, Sweden) and washed in Hanks-Wallace solution before injection. Depletion was controlled by leukocyte enumeration ($<10^3$/ml; 95% polymorphonuclear cells). Parasitemias were determined by microscopic examination of 300 erythrocytes on May-Grünwald-Giemsa-stained smears of tail blood. The percentage of parasitemia was calculated as the number of PRBC per 100 RBC. As *P. chabaudi* presents a synchronous erythrocytic cycle with night schizogony (30), parasitemias during the day consisted only of nondividing parasites and were stable.

Subinoculations to BALB/c mice. BALB/c mice developed a lethal infection when inoculated with *P. chabaudi* 96V, even for inocula as low as 10^2 PRBC. Survival time was shown to be a linear function of the decimal logarithm of the inoculum doses. To determine the number of PRBC in a rat blood sample with undetectable parasitemia, 200 μl of this sample was subinoculated into mice (five per experiment), the mean survival time was defined, and the number of PRBC was deduced from the standard curve.

Hematological parameters. Blood was collected from the retro-orbital sinus under ether anesthesia. Hemoglobin level and total leukocyte and platelet counts were determined by photometry and flow cytometry (Autolyzer 801 Contraves; Cunow, Cergy-Pontoise, France) on EDTA-anticoagulated blood. Differential nucleated cells counts (polymorphonuclear leukocytes, lymphocytes, monocytes, and erythroblasts) were performed on May-Grünwald-Giemsa-stained blood smears. Prothrombin time, partial thromboplastin time, and fibrinogen levels were measured on citrated platelet-poor plasma. Femoral bone marrow was collected from sacrificed rats, and thin smears were rapidly performed. The number of megakaryocytes in an area of 50 mm^2 was determined by microscopic examination. Spleens were then removed and kept for weight and size determinations.

Surgical procedures. Splenectomy was performed on 4-week-old rats under diazepam (Roche, Neuilly sur Seine, France) and ketamine (Rhône-Mérieux, Lyon, France) anesthesia. For thymus grafting, *nu/nu* rats (10 days old) were ether anesthetized and grafted subcutaneously with thymic tissue (two or three thymus grafts per animal) derived from neonatal, normal rat donors.

Adoptive transfer of T lymphocytes. T lymphocytes were prepared by the procedure described previously by Duquesne et al. (15). Briefly, spleen or lymph node mononuclear cells were fractionated on a nylon wool column. The T-lymphocyte-enriched cellular suspension was then washed and counted. Cells (2×10^7 per recipient) were injected intravenously 6 h before infection. Control rats received only the medium. In another experiment, splenic T lymphocytes were prepared from noninfected and *P. chabaudi*-infected (day 9 during the resolution phase of the infection) euthymic rats by the same procedure. Cells (2×10^7 per nude rat) were injected on day 16 of the infection (plateau phase).

Passive transfer of serum from infected rats. Blood was collected from *P. chabaudi*-infected rats (day 6 or 7 of the infection). Thrombocytopenia was verified by platelet enumeration (see above). Freshly collected, filtered (0.22-μm-pore-size filters) serum (200 μl or 2 ml) was intravenously injected in normal recipients, and platelet counts were performed at 12, 24, and 48 h after transfer. Serum (200 μl or 2 ml) from normal, age-matched, noninfected rats, prepared under the same conditions, was used as a control.

Detection of antiplatelet antibodies and platelet-associated immunoglobulins by enzyme-linked immunosorbent assay (ELISA). The technical procedure described by Rupin et al. (43) was modified as follows. For antiplatelet antibodies, platelets collected from normal, noninfected rats were washed in phosphate-buffered saline–9 mM EDTA and fixed with 1% paraformaldehyde (Sigma, La Verpillière, France). Platelets, 5×10^6, were coated in each well of a Nunclon Delta 63320 microtitration plate (Nunc, Roskilde, Denmark) by centrifugation. Sera from thrombocytopenic animals (day 6 or 7), diluted 1/20, were incubated for 1 h at 37°C. The presence of antiplatelet total antibodies was revealed by 1/5,000-diluted sheep anti-rat biotinylated immunoglobulin serum (Amersham International, Amersham, U.K.) and 1/1,000-diluted streptavidin-biotinylated horseradish peroxidase complex (Amersham). Antiplatelet immunoglobulin G antibodies were revealed by 1/5,000-diluted peroxidase anti-rat immunoglobulin G Fc-specific serum (Miles, Puteaux, France). For platelet-associated immunoglobulins, blood from infected rats was collected. Thrombocytopenia was controlled by platelet enumeration (see above). Each citrated, platelet-rich plasma was layered on an arabinogalactan gradient composed of 1 ml of 30% and 1 ml of 10% arabinogalactan (Sigma) diluted in 0.01 M Tris–3 mM EDTA–0.13 M NaCl–0.5% bovine serum albumin buffer, pH 6.65, and centrifuged for 1 h at $2,000 \times g$. Platelets without contaminating plasma proteins and erythrocytes were harvested at the 10/30% interface and washed in phosphate-buffered saline-EDTA. Thereafter, the procedure was identical to that described for antiplatelet antibody detection.

Statistics. Data are presented as means ± standard deviations (SD). The significance of the results was calculated by a modified Student's *t* test.

RESULTS

Normal Fischer rat infection. Groups of five rats each were infected with various inocula, ranging from 10^4 to 10^9 PRBC (Fig. 1). In all cases, parasitemias followed a bell-shaped curve. Even with the higher inoculum, mortality was never observed. Kinetics of parasitemia could be divided into three parts. (i) During the growth phase, the parasitemia varied as a function of both time and inoculum. A mathematical model was obtained from the experimental data (Fig. 2A): $p(t) = 10^{[c \cdot \log(I) + d]} \cdot t^{[e \cdot \log(I) + f]}$, where p is the percent parasitemia, t is the time in days, and I is the inoculum. c, d, e, and f were constants in a given experiment.

The parasite growth rate (Fig. 2B) could represent a mathematical parameter to evaluate the level of the host defenses. This growth was not exponential, but in a given group of rats and when compared for all inoculation doses, there was little variation of the growth rate since this rate consisted of between one-fourth and one-half of the expected number of merozoites produced during the schizog-

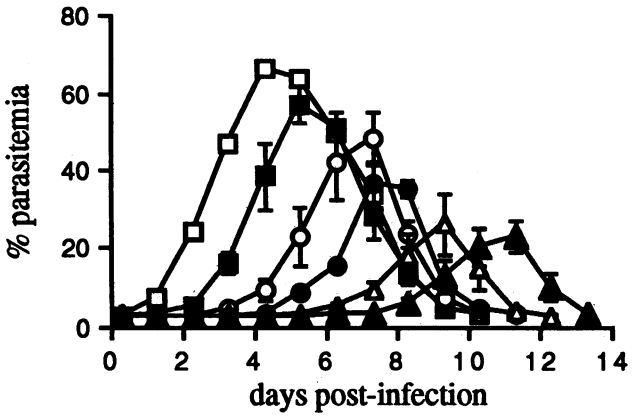

FIG. 1. Kinetics of *P. chabaudi* infection in the normal Fischer rats with different inocula. Groups of five rats each received 10^8 (■), 10^7 (○), 10^6 (●), 10^5 (△), and 10^4 (▲) PRBC, and one rat was inoculated with 10^9 PRBC (□). Parasitemias (means and SD) were determined daily. This experiment is representative of two experiments.

ony. These results suggested that host defenses did not vary significantly during this phase in a given animal and could be modulated by the number of parasites inoculated.

(ii) The timing and importance of the peak of parasitemia appeared as linear functions of the decimal logarithm of the inoculum (data not shown).

(iii) The resolution phase was characterized by a progressive and rapid decrease of the parasitemia and the appearance of crisis forms. On days 9 and 10 of an infection with 10^8 PRBC, up to 60% of the total parasite stages observed were gametocytes, with an increase in the absolute number of circulating gametocytes (from 50×10^6/ml on day 7 to 125×10^6/ml on day 9) indicating a neogametocytogenesis. No parasites could be detected on blood smears after day 12 (with a 10^8 PRBC inoculum). This complete parasite elimination was confirmed by subinoculation experiments in BALB/c mice, a model in which *P. chabaudi* infection is lethal, even with a 10^2 PRBC inoculum (data not shown). Immunity to reinfection could be demonstrated since no parasitized cells could be found after a challenged infection with 10^8 PRBC of the same strain of parasite 4 weeks after recovery.

Hematological changes during infection in normal rats. Minimal hemoglobinemia, maximal leukopenia, and thrombocytopenia (Table 1) were observed at the peak of parasitemia. An intense erythroblastic regeneration appeared in the following days. Lymphopenia represented the major part of the leukopenia (82% diminution). A moderate thrombocytopenia, never accompanied by hemorrhagic signs, followed the parasitemia. With a higher inoculum (10^8 PRBC), the peak of parasitemia occurred 2 days before the maximum decrease in platelet levels, indicating the necessity of a latency period for the generation of this thrombocytopenia (data not shown). Megakaryocyte density in bone marrow increased during the infection (from 227 ± 20/50 mm^2 on day 0 to 290 ± 17/50 mm^2 on day 7 of infection). Fibrinogen levels increased slightly from 2.12 ± 0.22 g/liter on day 0 to 2.80 ± 0.37 g/liter on day 7 of infection. Prothrombin times (12.7 ± 0.4 to 12.9 ± 0.4 s) and partial thromboplastin times (17.9 ± 0.4 to 18.8 ± 3.1 s) were unchanged. These data excluded a disseminated intravascular coagulation in the genesis of this peripheral thrombocytopenia. The volume of the spleen increased during the infection, with a maximal splenomegaly on day 9 (sixfold increase).

***P. chabaudi* infection in splenectomized rats.** Young rats were splenectomized just before the infection, and the course of infection was compared with that of nonsplenectomized rats. Parasite growth was more rapid, hemoglobinemia fell to under 3 g/liter, and 100% lethality was observed (Fig. 3). In spite of the thrombocytosis due to splenectomy, no significant decrease in platelet counts was observed during the infection (Table 2).

***P. chabaudi* infection in athymic nude rats.** Infection of nude rats (*nu/nu*) appeared to be completely different from that observed in euthymic controls (Fig. 4). Initial parasite growth rate was dramatically reduced (Fig. 4A). The maximum parasitemia appeared later and corresponded to the beginning of a plateau phase instead of a real peak. Then, parasitemia plateaued at 20 to 45% over several months (Fig. 4B), suggesting an equilibrium between parasite multiplication and host resistance. No mortality occurred before day 100. A chronic anemia was observed with significant erythroblastic regeneration (2×10^9 erythroblasts per liter). Splenomegaly did not appear at the peak of parasitemia as in

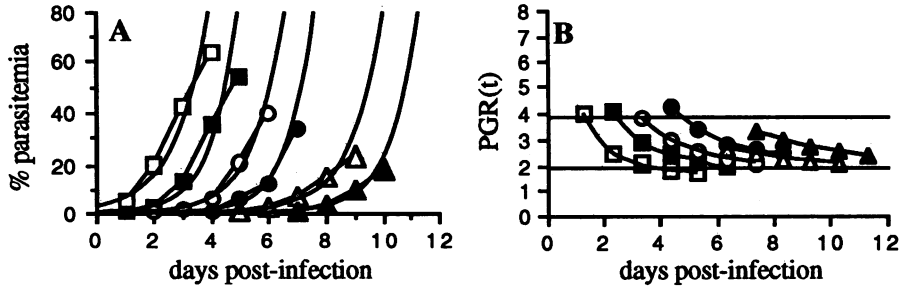

FIG. 2. Initial parasite growth: mathematical model (A) and determination of the parasite growth rate (PGR) (B). All curves from Fig. 1 (and see legend to Fig. 1 for details, including symbols) could be fit as a power function (A): $p(t) = a \cdot t^b$ with a good correlation coefficient ($r \geq 0.99$). Because $\log(a)$ is a linear function of $\log(I)$ [$\log(a) = c \cdot \log(I) + d$; $r = 0.98$] and b is a linear function of $\log(I)$ [$b = e \cdot \log(I) + f$; $r = 0.98$], we deduced the following mathematical model: $p = 10^{[c \cdot \log(I) + d]} \cdot t^{[e \cdot \log(I) + f]}$. From this equation, the parasite growth rate could be theoretically determined as $PGR(t) = [p(t + 1)]/p(t) = [1 + (1/t)]^b$ and graphically represented in panel B. The PGR values, quite similar to those experimentally determined (horizontal lines, mean value ± 1 SD), presented slight variations in each group and between all groups.

TABLE 1. Hematological parameters during *P. chabaudi* infection in normal rats

Day	Mean ± SD (n = 5)						
	Parasitemia (%)	Hemoglobin (g/liter)	Erythroblasts, 10^6 (per liter)	Leukocytes, 10^6 (per liter)	Lymphocytes, 10^6 (per liter)	Platelets, 10^9 (per liter)	Spleen wt, 10^{-1} (g)
0	0	14.15 ± 0.15	122 ± 44	7,600 ± 790	6,500 ± 700	872 ± 18	35 ± 3
6	29.5 ± 11	7.60 ± 1.60	346 ± 48	1,430 ± 20	1,175 ± 25	370 ± 42	152 ± 5
9	3.2 ± 2	8.45 ± 1.05	587 ± 123	1,875 ± 75	1,325 ± 175	459 ± 37	225 ± 6
12	0	11.70 ±0.35	159 ± 99	2,250 ± 50	1,660 ± 50	771 ± 19	173 ± 10

infected euthymic rats but was evident on days 70 (fivefold increase) and 120 (sixfold). Histological examination of the spleens revealed an intense splenic erythropoiesis, contrary to the splenic lymphoid reaction observed at the peak in infected normal rats (data not shown).

Immunological reconstitution of nude rats. Adoptive transfer to nude rats with normal rat, nylon wool-enriched T lymphocytes the same day (Fig. 5A) or 16 days after the infection (Fig. 5B) resulted in complete resolution of infection. Nevertheless, the transfer of T lymphocytes on day 0 did not modify the parasite growth rate (Fig. 5A). No difference was observed in the infection characteristics whatever the origin (spleen or lymph node) of the transferred cells. Resolution of infection could be achieved with both normal and specific (from normal rats with a resolved *P. chabaudi* infection, on day 9) T lymphocytes (Fig. 5B). The T-cell dependence of the resolution of the infection was definitely confirmed by thymus grafting in nude rats during the neonate period. This reconstitution had no effect on the parasite growth rate but induced a slight but significant ($P < 0.005$) thrombocytopenia most likely related to the low parasitemia (Table 3). Splenomegaly was also evident as early as the time of maximal parasitemia and at the same level as in infected *nu*/+ rats (Table 3).

Studies on the mechanisms of thrombocytopenia. The evidence for the thymodependency of this peripheral thrombocytopenia prompted us to investigate the mechanisms of its genesis. Passive transfer of sera from infected (on day 6 or 7) thrombocytopenic rats was unable to induce a thrombocytopenia in normal recipients, even when 2 ml was injected per animal. No antiplatelet antibodies could be detected by ELISA in the serum of thrombocytopenic rats (data not shown). A small increase in platelet-associated immunoglobulin levels in infected thrombocytopenic rats could be detected by ELISA (Table 4).

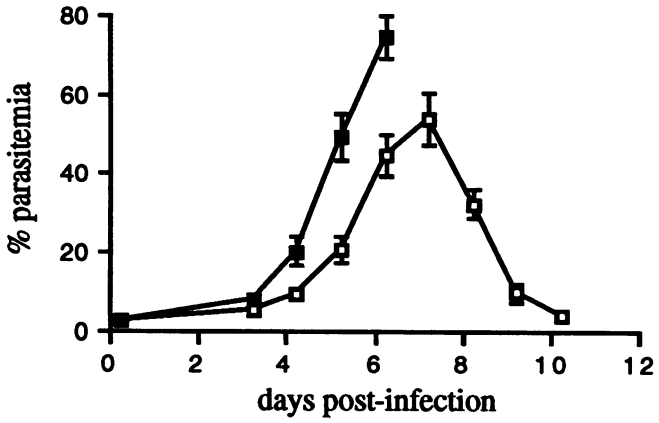

FIG. 3. Growth kinetics of *P. chabaudi* during infection in splenectomized rats. Five rats were splenectomized and infected 6 h later with 10^7 PRBC. Parasitemias (mean values and SD) of this group of rats (■) were compared with those of nonsplenectomized rats infected with the same inoculum (□). On day 7 all splenectomized rats died. This is a representative experiment of two experiments.

DISCUSSION

In this report, we describe a novel experimental model of malaria based on the infection of Fischer rats of different immunological status with *P. chabaudi*. The nude rat infection, with its original characteristics, is a convenient model to study the T-cell-dependent effector and pathological mechanisms occurring during the course of a blood stage infection.

The nude rat model first allowed a better understanding of nonlethal infections in euthymic rats. The spleen seems to play a major role in the innate immunity observed since the parasite growth was notably increased after splenectomy of normal rats, leading to the deaths of the animals. This worsening of the infection after splenectomy was described previously in other experimental models (28). Moreover, initial adaptation of *P. chabaudi* strains to rats (a refractory host) succeeded only after splenectomy (13, 34). Parasite growth in nude rats was dramatically reduced compared with that in normal rats. This decrease was not related to the T-lymphocyte deficiencies since neither adoptive transfer nor thymus grafting modified parasite growth. Since macrophage and natural killer activities are increased in athymic rats (16) and macrophages have been described as being major effector cells in malaria (11), the immunity observed in nude rats could be due to the increased activity of phagocytes. By determining the parasite growth rate in euthymic rats, a mathematical model of this innate immunity could be deduced. Weak variations of this rate were observed in a given animal during the growth phase. This rate was found to be constant independently of the inoculum, suggesting that natural host defenses were modulated by the parasite burden.

Adoptive transfer of T lymphocytes and thymus grafting in

TABLE 2. Platelet counts in splenectomized rats on day 6 of *P. chabaudi* infection

Rats	Mean ± SD (n = 5)		
	Infected		Noninfected
	Parasitemia (%)	Platelets, 10^9 (per liter)	Platelets, 10^9 (per liter)
Nonsplenectomized	43.0 ± 3.0	208 ± 43	687 ± 39
Splenectomized	68.5 ± 3.5	816 ± 130	984 ± 56

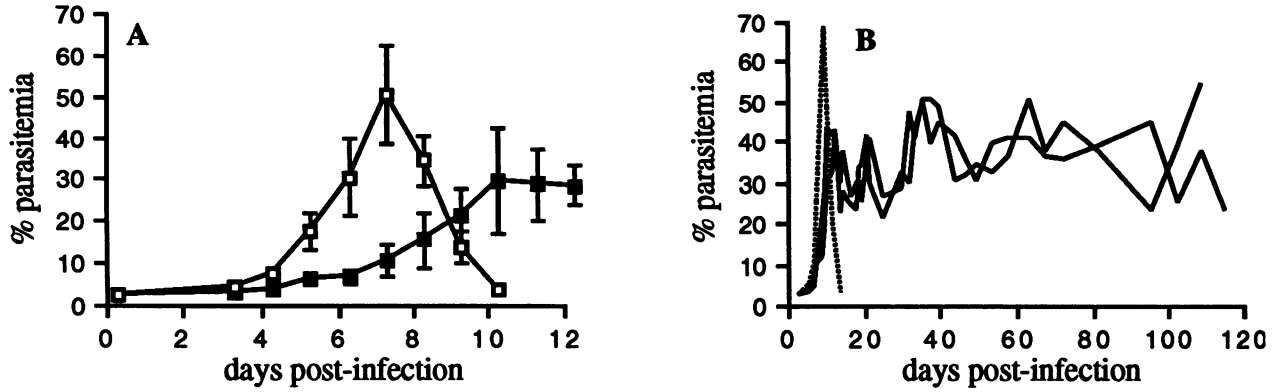

FIG. 4. Kinetics of *P. chabaudi* infection in Fischer nude rats. (A) Kinetics during a short period. Four *nu/nu* (■) and five *nu/+* rats (□) were infected with a 10^7-PRBC mononuclear cell-depleted inoculum. Parasitemias were determined daily. This experiment is representative of four experiments. (B) Kinetics during a long period. Parasitemias of two *nu/nu* rats (solid curves) and five control *nu/+* rats (mean parasitemia, dashed curve) infected as for panel A were determined. Parasitemias were monitored regularly during 4 months. On day 120, one of the two *nu/nu* rats was still alive.

athymic rats clearly showed that the resolution phase is T cell dependent. Moreover, complete parasite elimination could also be achieved by transfer of T lymphocytes (either normal or specific) during an infection in nude rats. The important role of T lymphocytes in malarial immunity (49, 50) was particularly demonstrated in nude mice infected with different plasmodial species (4, 9, 41). Malaria in nude mice displays a chronic evolution with high parasitemia over several weeks (10, 12, 22, 51), as does *P. chabaudi* infection in $CD4^+$ T-cell-deprived mice (31) and in SCID mice (32). The effector mechanisms which operate in vivo remain largely unknown. The persistence of such a long plateau phase in nude rats is uncommon among the rodent experimental infections and allowed some immunomanipulation in the course of the chronic infection. This model could be useful to identify precisely the different lymphocyte subsets able to clear the parasites.

The nude rat model also appeared useful in investigating the thymodependency of the pathology, particularly, thrombocytopenia. Thymodependency of the splenomegaly has been known for a long time to occur in rodent models (reviewed in reference 26). Thrombocytopenia became evident in nude rats only after thymus grafting. Neither a serum thrombocytopenic factor nor antiplatelet antibodies could be detected in the infected normal rats. Such an absence of a serum thrombocytopenic factor has been described for simian malaria (45). In mice infected with *P. berghei*, thrombocytopenia is mediated by $CD4^+$ T lymphocytes and, in contrast with our results, can be passively transferred by serum (20). The increase in platelet-associated immunoglobulin levels during the rat infection did not argue for or against some precise mechanisms of platelet destruction; such increases have been observed in nonimmune thrombocytopenias and could signal peripheral platelet hyperdestruction (18).

Cellular mechanisms were probably involved in the rat malarial thrombocytopenia, as is often suspected in immune thrombocytopenias (2). Spleen cells could play a role since thrombocytopenia was absent in splenectomized rats. Cellular interactions among platelets, macrophages, and *P. falciparum* PRBC have been described for human malaria (35). CD36 antigen (platelet glycoprotein IV) on macrophages and platelets (36), thrombospondin secreted by macrophages and platelets (44), and TRAP (thrombospondin-

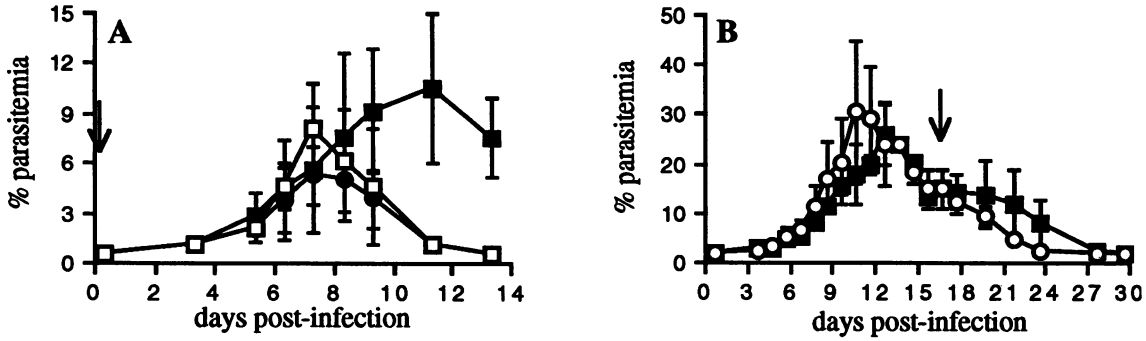

FIG. 5. Adoptive transfer of T lymphocytes in *P. chabaudi*-infected nude rats. (A) Three groups of three *nu/nu* rats each were given intravenously either 2×10^7 spleen (□) or lymph node (●) normal T lymphocytes or medium only (■). Six hours later, all rats were intraperitoneally infected with a 10^7-PRBC mononuclear cell-depleted inoculum. Parasitemias (mean values and SD) were determined daily. This experiment is representative of three experiments. (B) Four *nu/nu* rats were infected with a 10^7-PRBC mononuclear cell-depleted inoculum. On day 16, 2×10^7 normal (■) or specific (○) T lymphocytes (from euthymic rats with a resolved *P. chabaudi* infection, on day 9) were adoptively transferred to rats (two rats each). Arrows indicate the days of transfer.

TABLE 3. Platelet counts and spleen weight in *nu/+*, *nu/nu*, and thymus-grafted *nu/nu* rats at the maximal parasitemia

Rats	Mean ± SD (n = 3)		
	Parasitemia (%)	Platelets, 10^9 (per liter)[a]	Spleen wt, 10^{-1} (g)
nu/+	0	720 ± 41	36 ± 2
Infected *nu/+*[b]	41.5 ± 7.5	296 ± 23	143 ± 7
nu/nu	0	736 ± 17	60 ± 5
Infected *nu/nu*[c]	33.5 ± 5.5	710 ± 35*	93 ± 4
Infected and grafted *nu/nu*[c]	15.0 ± 3.0	548 ± 26*	207 ± 12

[a] *$P < 0.005$ between the two values.
[b] On day 7 of infection.
[c] On day 10 of infection.

related anonymous protein) on parasite asexual blood stages (42) are probably some of the interacting molecules. CD36 has been described as the thrombospondin receptor (1), and TRAP is highly homologous to a repetitive region of thrombospondin involved in cellular attachment (39, 40). Such adhesive interactions have not yet been described for rodents, with the exception of the sequestration of *P. chabaudi* PRBC in the microvasculature by adhesion to endothelial cells (14). Activation of platelets, inducing in particular a thrombospondin secretion (17), and subsequently their destruction, could be a consequence of these adhesive interactions (25, 35).

It is also interesting to consider the role of cytokines in the activation of platelets and macrophages during T-cell-dependent thrombocytopenia. Gamma interferon, tissue necrosis factor alpha, and IL-6 are able to induce the effector functions of platelets towards *Schistosoma mansoni* larvae (3, 37). The activation of platelets by cytokines, which are produced in large amounts during malarial infection (11), could further induce their destruction. Thrombocytopenia is rarely observed after gamma interferon treatment in humans (29), and tissue necrosis factor alpha does not mediate thrombocytopenia in murine malaria (20). By contrast, IL-6-transgenic mice develop a syndrome resembling Castelman disease which is characterized by an immune thrombocytopenia (5). IL-6 is a thrombopoietin and induces a thrombocytosis in vivo. Nevertheless, in some circumstances, a slight thrombocytopenia has been observed in mice treated with IL-6 (8). It could be possible that an intense and prolonged secretion of IL-6, such as that described for malaria (19), induces a thrombocytopenia rather than a thrombocytosis. Further studies with our rat model may illuminate the cellular mechanisms involved and the possible role of IL-6 in platelet hyperdestruction.

Among the malarial experimental models, *P. chabaudi* rat malaria could take an original place. The nude rat infection is a promising model for the study of the T-cell-dependent mechanisms of immunity and immunopathology. It makes possible well-defined immunological interventions, such as the injection of cytokines, monoclonal antibodies, or T-cell clones, during the plateau phase. Moreover, pharmacological studies are also possible during this phase.

TABLE 4. Platelet-associated immunoglobulin levels in normal rats

Rats	Mean optical density ± SD (n = 4)		
	Expt 1, anti-total immunoglobulin	Expt 2, anti-total immunoglobulin	Expt 3, anti-immunoglobulin G
Normal	0.126 ± 0.005	0.175 ± 0.002	0.413 ± 0.008
Infected	0.237 ± 0.013	0.678 ± 0.077	0.648 ± 0.042

ACKNOWLEDGMENTS

We thank Pierre Billaut, Marc Bouquerel, Marc Témoingt, Myriam Boulanger, Eric Busière, Brigitte Ingold, and Elisabeth Rideau for excellent technical assistance. We are grateful to Yvonne Bouxom for her contribution to the histological work. We thank Anne-Sophie Lermoyer, Yves Gruel, and Bénédicte Delahousse for their hematological collaboration. We thank Daniel Lagersie, Jean-Paul Dessaint, and Daniel Camus for their helpful discussions and David Williams for his useful comments.

This work was supported by the Institut National de la Santé (INSERM) (U167) and the Centre National de la Recherche Scientifique (CNRS) (URA 624).

REFERENCES

1. **Asch, A. S., J. Barnwell, R. L. Silverstein, and R. L. Nachman.** 1987. Isolation of the thrombospondin membrane receptor. J. Clin. Invest. **79**:1054–1061.
2. **Aster, R. H., and T. J. Kunicki.** 1989. Platelet immunology: historical perspective and future directions, p. 3–5. *In* T. J. Kunicki and J. N. George (ed.), Platelet immunobiology, molecular and clinical aspects. J. B. Lippincott Co., Philadelphia.
3. **Auriault, C., M. Damonneville, V. Pancré, and A. Capron.** 1990. Effector functions of platelets in parasitic diseases and their regulation by cytokines. Diagn. Microbiol. Infect. Dis. **13**:423–427.
4. **Brake, D. A., C. A. Long, and W. P. Weidanz.** 1988. Adoptive protection against *Plasmodium chabaudi adami* malaria in athymic nude mice by a cloned T cell line. J. Immunol. **140**:1989–1993.
5. **Brandt, S. J., D. M. Bodine, C. E. Dunbar, and A. W. Nienhuis.** 1990. Dysregulated interleukin 6 expression produces a syndrome resembling Castelman's disease in mice. J. Clin. Invest. **86**:592–599.
6. **Brown, I. N., A. C. Allison, and R. B. Taylor.** 1968. *Plasmodium berghei* infections in thymectomized rats. Nature (London) **219**:292–293.
7. **Brown, K. N.** 1990. The parasitology of malaria and the study of protective immunity. Immunol. Lett. **25**:97–100.
8. **Carrington, P. A., R. J. Hill, P. E. Stenberg, J. Levin, L. Corash, J. Schreurs, G. Baker, and F. C. Levin.** 1991. Multiple in vivo effects of interleukin-3 and interleukin-6 on murine megakaryocytopoiesis. Blood **77**:34–41.
9. **Cavacini, L. A., C. A. Long, and W. P. Weidanz.** 1986. T-cell immunity in murine malaria: adoptive transfer of resistance to *Plasmodium chabaudi adami* in nude mice with splenic T cells. Infect. Immun. **52**:637–643.
10. **Cavacini, L. A., L. A. Parke, and W. P. Weidanz.** 1990. Resolution of acute malarial infections by T-cell-dependent non-antibody-mediated mechanisms of immunity. Infect. Immun. **58**:2946–2950.
11. **Clark, I. A.** 1987. Cell-mediated immunity in protection and pathology of malaria. Parasitol. Today **3**:300–305.
12. **Clark, I. A., and A. C. Allison.** 1974. *Babesia microti* and *Plasmodium berghei yoelii* infections in nude mice. Nature (London) **252**:328–329.
13. **Coombs, G. H., and W. E. Gutteridge.** 1975. Growth *in vitro* and metabolism of *Plasmodium vinckei chabaudi*. J. Protozool. **22**:555–560.
14. **Cox, J., S. Semoff, and M. Hommel.** 1987. *Plasmodium chabaudi*: a rodent malaria model for in-vivo and in-vitro cytoadherence of malaria parasites in the absence of knobs. Parasite Immunol. **9**:543–561.
15. **Duquesne, V., C. Auriault, F. Darcy, J.-P. Decavel, and A. Capron.** 1990. Protection of nude rats against *Toxoplasma* infection by excreted-secreted antigen-specific helper T cells. Infect. Immun. **58**:2120–2126.
16. **Festing, M. F. W.** 1981. Athymic nude rats, p. 267–283. *In* M. E.

Gershwin and B. Merchant (ed.), Immunologic defects in laboratory animals, vol. 1. Plenum Press, New York.
17. **Frazier, W. A.** 1987. Thrombospondin: a modular adhesive glycoprotein of platelets and nucleated cells. J. Cell Biol. **105**:625–632.
18. **George, J. N.** 1990. Platelet immunoglobulin G: its significance for the evaluation of thrombocytopenia and for understanding the origin of α-granule proteins. Blood **76**:859–870.
19. **Grau, G. E., K. Frei, P.-F. Piguet, A. Fontana, H. Heremans, A. Billiau, P. Vassalli, and P.-H. Lambert.** 1990. Interleukin 6 production in experimental cerebral malaria: modulation by anticytokine antibodies and possible role in hypergammaglobulinemia. J. Exp. Med. **172**:1505–1508.
20. **Grau, G. E., P.-F. Piguet, D. Gretener, C. Vesin, and P.-H. Lambert.** 1988. Immunopathology of thrombocytopenia in experimental malaria. Immunology **65**:501–506.
21. **Grau, G. E., P.-F. Piguet, P. Vassalli, and P.-H. Lambert.** 1989. Tumor-necrosis factor and other cytokines in cerebral malaria: experimental and clinical data. Immunol. Rev. **112**:49–70.
22. **Grun, J. L., and W. P. Weidanz.** 1981. Immunity to *Plasmodium chabaudi adami* in the B cell deficient mouse. Nature (London) **290**:143–145.
23. **Ho, M., and H. K. Webster.** 1989. Immunology of human malaria. A cellular perspective. Parasite Immunol. **11**:105–116.
24. **Horstmann, R. D., M. Dietrich, U. Bienzle, and H. Rasche.** 1981. Malaria-induced thrombocytopenia. Blut **42**:157–164.
25. **Inyang, A. L., O. Sodeinde, D. T. Okpako, and E. M. Essien.** 1987. Platelet reactions after interaction with cultured *Plasmodium falciparum* infected erythrocytes. Br. J. Haematol. **66**: 375–378.
26. **Jayawardena, A. N.** 1981. Immune responses in malaria, p. 85–136. *In* J. M. Mansfield (ed.), Parasitic diseases, vol. 1. Immunology. Marcel Dekker, New York.
27. **Kelton, J. G., J. Keystone, J. Moore, G. Denomme, E. Tozman, M. Glynn, P. B. Neame, J. Gauldie, and J. Jensen.** 1983. Immune-mediated thrombocytopenia of malaria. J. Clin. Invest. **71**:832–836.
28. **Kreier, J. P., and T. J. Green.** 1980. The vertebrate host's immune response to *Plasmodia*, p. 111–162. *In* J. P. Kreier (ed.), Malaria, vol. 3. Immunology and immunization. Academic Press, Inc., New York.
29. **Kurzrock, R., J. R. Quesada, M. Talpaz, E. M. Hersh, J. M. Reuben, S. A. Sherwin, and J. U. Gutterman.** 1986. Phase I study of multiple dose intramuscularly administered recombinant gamma interferon. J. Clin. Oncol. **4**:1101–1109.
30. **Landau, I., and Y. Boulard.** 1978. Life cycles and morphology, p. 53–84. *In* R. Killick-Kendrick and W. Peters (ed.), Rodent malaria. Academic Press, Inc. (London), Ltd., London.
31. **Langhorne, J., S. J. Meding, K. Eichmann, and S. S. Gillard.** 1989. The response of CD4+ T cells to *Plasmodium chabaudi chabaudi*. Immunol. Rev. **112**:71–94.
32. **Meding, S. J., and J. Langhorne.** 1991. CD4+ T cells and B cells are necessary for the transfer of protective immunity to *Plasmodium chabaudi chabaudi*. Eur. J. Immunol. **21**:1433–1438.
33. **Mons, B., and R. E. Sinden.** 1990. Laboratory models for research *in vivo* and *in vitro* on malaria parasites of mammals: current status. Parasitol. Today **6**:3–7.
34. **Musoke, A. J., and H. W. Cox.** 1977. Experimental infection with *Plasmodium chabaudi* in rats. Observation on adaptation and the immune response to infection. J. Parasitol. **63**:464–470.
35. **Ockenhouse, C. F., C. Magowan, and J. D. Chulay.** 1989. Activation of monocytes and platelets by monoclonal antibodies or malaria-infected erythrocytes binding to the CD36 surface receptor in vitro. J. Clin. Invest. **84**:468–475.
36. **Ockenhouse, C. F., N. N. Tandon, C. Magowan, G. A. Jamieson, and J. D. Chulay.** 1989. Identification of a platelet membrane glycoprotein as a falciparum malaria sequestration receptor. Science **243**:1469–1471.
37. **Pancré, V., D. Monté, A. Delanoye, A. Capron, and C. Auriault.** 1990. Interleukin-6 is the main mediator of the interaction between monocytes and platelets in the killing of *Schistosoma mansoni*. Eur. Cytokine Net. **1**:15–19.
38. **Phillips, S. M., A. G. Bentley, G. Linette, B. L. Doughty, and M. Capron.** 1983. The immunologic response of congenitally athymic rats to *Schistosoma mansoni* infection. I. *In vivo* studies of resistance. J. Immunol. **131**:1466–1474.
39. **Prater, C. A., J. Plotkin, D. Jaye, and W. A. Frazier.** 1991. The properdin-like type I repeats of human thrombospondin contain a cell attachment site. J. Cell Biol. **112**:1031–1040.
40. **Rich, K. A., F. W. George IV, J. L. Law, and W. J. Martin.** 1990. Cell-adhesive motif in region II of malarial circumsporozoite protein. Science **249**:1574–1577.
41. **Roberts, D. W., R. G. Rank, W. P. Weidanz, and J. F. Finerty.** 1977. Prevention of recrudescent malaria in nude mice by thymic grafting or by treatment with hyperimmune serum. Infect. Immun. **16**:821–826.
42. **Robson, K. J. H., J. R. S. Hall, M. W. Jennings, T. J. R. Harris, K. Marsh, C. Newbold, V. E. Tate, and D. Weatherall.** 1988. A highly conserved amino-acid sequence in thrombospondin, properdin and in proteins from sporozoites and blood stages of a human malaria parasite. Nature (London) **335**:79–82.
43. **Rupin, A., Y. Gruel, P. Poumier-Gaschard, M. Chassaigne, J. Leroy, and P. Bardos.** 1990. Thrombocytopenia in systemic lupus erythematosus: association with antiplatelet and anticardiolipin antibodies. Clin. Immunol. Immunopathol. **55**:418–426.
44. **Sherwood, J. A., D. D. Roberts, S. L. Spitalnik, J. W. Lawler, L. H. Miller, and R. J. Howard.** 1990. Falciparum malaria parasitized erythrocytes bind to a carboxy-terminal thrombospondin fragment and not the amino-terminal heparin-binding region. Mol. Biochem. Parasitol. **40**:173–182.
45. **Shulman, N. R., F. A. Neva, J. N. Sheagren, and C. J. Canfield.** 1970. Malaria: host-defense mechanisms and complications. Ann. Intern. Med. **73**:295–306.
46. **Stechschulte, D. J.** 1969. *Plasmodium berghei* infection in thymectomized rats. Proc. Soc. Exp. Biol. Med. **131**:748–752.
47. **Warrell, D. A.** 1987. Pathophysiology of severe falciparum malaria in man. Parasitology **94**:S53–S76.
48. **Warrell, D. A., M. E. Molyneux, and P. F. Beales.** 1990. Severe and complicated malaria. Trans. R. Soc. Trop. Med. Hyg. **84**(Suppl. 2):1–65.
49. **Weidanz, W. P., and C. A. Long.** 1988. The role of T cells in immunity to malaria. Prog. Allergy **41**:215–252.
50. **Weidanz, W. P., J. Melancon-Kaplan, and L. A. Cavacini.** 1990. Cell mediated immunity to the asexual blood stages of malarial parasites: animal models. Immunol. Lett. **25**:87–96.
51. **Weinbaum, F. I., C. B. Evans, and R. E. Tigelaar.** 1976. Immunity to *Plasmodium berghei yoelii* in mice. I. The course of infection in T cell and B cell deficient mice. J. Immunol. **117**:1999–2005.