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PARASITOLOGIC AND CLINICAL HUMAN RESPONSE TO IMMUNOGLOBULIN ADMINISTRATION IN FALCIPARUM MALARIA

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Abstract. The protective effect of African IgG antibodies against *Plasmodium falciparum* malaria was investigated by passive transfer in Thai patients. Sera from 333 African adults were collected in the Cote d'Ivoire and subjected to extensive screening. One hundred fifty-three samples were discarded for safety reasons, and IgG was extracted from those remaining under conditions allowing their use by the intravenous (iv) route.

Eight Thai patients with *P. falciparum* parasitemia were treated by iv inoculation of the IgG: six with a 100 mg/kg dose given over three days, one with a single 20 mg/kg dose, and one with a single 200 mg/kg dose. To ensure a safety margin of at least 48 hours, subjects were chosen among patients having a recrudescent parasitemia following quinine treatment failure at the RI level. At that stage, symptoms were mild or absent and parasitemia was low but increasing (range 4, 200–9,000/μl).

The IgG pool exerted a profound, stage-specific, but non-sterilizing effect on each of the strains tested, and proved to be safe. Asexual parasitemia decreased by a mean 728-fold (range 46–1,086), while gametocytes were unaffected. Clearance of parasites and symptoms was as fast or faster than with drugs, and was consistent in the eight patients treated, suggesting that target antigens were equally expressed in geographically remote isolates. In peripheral blood smears, no mature forms were seen at any time during the followup, which does not support the hypothesis that reversal of cytoadherence occurred.

After the disappearance of the transferred antibodies, recrudescent parasites from three patients were found to be susceptible to the same extent (mean decrease of 1, 310-fold) to the same IgG preparation, indicating that selection of parasites able to escape the effect of antibodies had not occurred. No adverse side-effects were detected during the followup, which lasted one year.

In 1961 and 1962, three historic experiments were conducted in 31 African children, which demonstrated that a protective effect against *Plasmodium falciparum* malaria could be achieved in most cases by passive transfer of immunoglobulins by the intramuscular (im) route.[1–3]

Nearly 30 years later, despite intensive research in immunity to blood stages, considerable progress in the knowledge of the antigens of this stage, and a wide array of biological assays, we still do not know which were the mechanisms underlying the observed clinical effect, nor which were the critical antibody specificities responsible for it. For that reason and for others related to the relevance of animal models of infection, we thought that complementary in vivo experiments had to be performed in order to establish an in vivo/in vitro comparative analysis using the tools developed since that time. In addition, improved means of purifying IgG have become available that now allow the safe use of the intravenous (iv) route for administration, eliminating the long delay and loss that occur with the im route. It also appeared valuable to determine whether or not a geographic restriction of protective specificities may exist, and in view of the fast increase in the prevalence of multi-drug resistant parasites, to investigate the therapeutic potential of fast-acting iv antibodies.

To ensure the safe conditions needed for such an experiment, it was performed in a well-equipped hospital close to an endemic area, and subjects were selected from cases of early recrudescence following drug therapy failure with low parasitemia and moderate or absent symptoms

in order to benefit from a two- to three-day margin of safety.

MATERIALS AND METHODS

Immunoglobulin preparation

Since no assay is universally recognized as being able to reflect acquired protection against malarial asexual blood stages, the selection of serum donors was based on epidemiologic and clinical backgrounds.

The African region was chosen because of the high and stable levels of transmission prevailing in rural areas. A total of 400–500 ml of blood were collected from each of 333 young adults (age range 19–23 years) in Cote d'Ivoire residing in two Agriculture Technical Colleges, but originating from surrounding villages. Only those who were born and had resided all their life in these villages were included in the study. Criteria for participation included a known history of repeated malaria attacks during childhood and adolescence, and the absence of any ongoing preventive means of protection, such as drug prophylaxis, insecticide use, or mosquito nets.

The number of infective mosquito bites in the area was estimated in previous surveys to be 0.5–3/day during the transmission season. Following centrifugation, plasma was frozen at −30°C and sent on dry ice in plastic pouches to France. To avoid contamination during handling by traces of serum remaining on the plastic pouches, each bag was sealed in polyethylene bags using gloves that were changed at each step. Labeled aliquots were submitted to screening for abnormal agglutinins, hemolysins, antibodies to syphilis, hepatitis B (HB) surface antigen, HBs and HBc antibodies, and human immunodeficiency virus (HIV) 1 and 2 antibodies by ELISA, immunofluorescence, and Western blots. Any positivity for one of the above parameters, including borderline or doubtful results (i.e., dissociation between results from duplicate wells), even when further assays did not confirm the initial doubt, led to exclusion of these samples. Serum samples from 155 donors were discarded.

The purification of IgG was performed under Good Manufacturing Practice (GMP) conditions by the Centre Regional de Transfusion Sanguine de Lille, following a process including separation of IgG by the Cohn ethanol method, and further treatment by a mild pepsin digestion at pH 4.[4]

To avoid possible contamination by mislabeling or identification mistakes, the plasma bags of the donors were pooled in groups of 10, handled separately, and all HBsAg and HIV serologic tests were repeated a second time on each of the pools before combining them and proceeding to the extraction steps. The final product was submitted to and satisfied all controls required by European and French regulations. In particular, it had a minimal content of aggregates (< 0.1%), and was stable without an increase in polymers and fragments, following incubation of a sample at 37°C for four weeks. The final product contained 0% albumin, 100% IgG at a concentration of 51 g/l (no other serum protein was detected), glycocol (2.5 g/l), saccharose (55 g/l), and lipid (0.21 g/l). It was lyophilized in vials containing 2.5 g of purified IgG, and stored at 4°C until use.

Subjects

The study was designed to provide a sufficient time safety margin that would allow the administration of drug therapy in case the experimental procedure was ineffective. For that reason, subjects were selected from among a group of quinine treatment failures at the RI level (the RI level of resistance corresponds to a recrudescence of parasites, following their initial disappearance, in the absence of reinfection by mosquitoes). Thus, IgG treatment could be initiated at an early stage after the appearance of recrudescent parasites. At this stage, when parasitemia starts to increase again, symptoms are usually mild or absent, which allows a safety margin of at least 48 hr under close medical supervision. Within this group of recrudescences, the criteria for entry into the study were parasitemia between 5,000 and 10,000/μl, absence of symptoms, or alternatively fever < 38.5°C without any other accompanying symptom, absence of alterations of liver, cardiac, pulmonary or kidney functions, absence of other detectable infections, absence of glucose-6-phosphate dehydrogenase deficiency, Hb > 8 g/l, and informed consent of the children's parents. This protocol, as well as the safety conditions used for IgG preparation, was thoroughly reviewed and approved by the Bangkok hospitals ethical committee. Progress in establishing the safety of human IgG preparations for clinical use, extensive clinical experience with such preparations, and experience with IgG ther-

TABLE 1

*Regimens used and parasite counts in 11 treatments performed with African IgG in 8 Thai patients**

Patient no.	IgG regimen (mg/kg)	Parasitemia per μl	Fever clearance (hours)	Maximal parasite reduction	
				Fold (parasitemia)	Hours
First treatment					
1	20/30/50	6,200	NR	775 (8)	33
2	20/30/50	5,067	83	101 (50)	104
3	20/30/50	7,089	76	886 (8)	77
4	20/30/50	4,189	92	46 (90)	113
5	20/30/50	4,333	43	393 (11)	73
6	20/30/50	8,689	NR	1,086 (8)	67
Mean		5,927	73	547 (29)	78
7	200	5,911	12	656 (9)	55
8	20	8,067	52	161 (50)	68
Second treatment					
8	100/100	17,111	16	1,711 (10)	70
1	100/100	13,200	16	1,200 (11)	82
3	100/100	8,167	35	1,020 (8)	120
Mean		12,826	22	1,310 (10)	90†
Mean of 11 treatments		8,002	47	728 (24)	78

* NR = not relevant: no fever at start of therapy or during follow-up.
† A 903-fold decrease occurred in a mean 69 hours in these 3 treatments.

apy for malaria in the 1960s were all critical in making the decision to perform a limited, well-controlled trial for research purposes.

Eight children were included in the study. They originated from Prachinburi province, 150 km northeast of Bangkok, and were admitted to the Hospital for Tropical Diseases in Bangkok, where the initial quinine treatment was given, the patients were followed up, and the recrudescence was observed. In many instances, the mothers had reported the occurrence of at least one malaria attack in the past. Five of these participants were 5–9 years old, and their weights ranged from 16 to 23 kg; the other three were 10–12 years old and weighed from 22 to 25 kg. Nurses and physicians were assigned the specific and constant supervision of these patients night and day.

During the recrudescence of parasitemia following quinine failure, subjects had no symptoms, except for occasional and brief peaks of fever (on average once in three days). At the time of IgG administration, the *P. falciparum* asexual parasitemia ranged from 4, 200 to 9,000/μl. Gametocytes were present in four subjects. In vitro assays demonstrated various degrees of resistance to chloroquine and quinine in each of the eight isolates. Antibody titers to asexual blood stage antigens as determined by immunofluorescence were initially relatively high (range 1: 1, 600–1:51,000 on day 0), and some increased during the followup period. Blood parasite counts were assessed on thin and thick blood films prepared hourly for the first four hr, then every six hr for the first four days, and twice a day thereafter. In addition, in two cases blood films were taken 30 min after the start of the infusion. These slides were screened in duplicate, and parasites were counted over 10,000 red blood cells (RBC) on thin smears and over 1,000 white blood cells (WBC) on thick smears by two independent microscopists.

RESULTS

The first six patients were treated with a total dose of 100 mg/kg over a three-day period (day 0: 20 mg/kg, day1: 30 mg/kg, and day 2: 50 mg/kg). Intravenous inoculations of the African Ig (over a four-hr period in 5% glucose) were well tolerated and did not induce any detectable general or localized reaction. There was no modification of pulse, blood pressure, respiration, or EKG tracing. Body temperature, which was in the range of 37–38°C with occasional peaks at 38.5–39°C before treatment, decreased in a mean 73-hr time period (Table 1) to reach normal values in four patients (range 36.5–37.5°C), and remained in the 36.5–38°C range in the two other patients. The spleen was slightly enlarged in seven patients before treatment. It showed a moderate increase (mean 4 cm) within 48 hr in four

of seven patients, and a mild and transient enlargement of the liver was also noted in four patients.

The asexual parasitemias decreased by a mean 543-fold and remained at low levels for 7–12 days (Figure 1 and Table 1). The average time needed to reach the minimum parasitic load was 78 hr (range 33–113). A major decrease in parasitemia (> 100-fold) was observed after the first 20-mg injection in two patients, and occurred in the remaining patients only after the second 30-mg injection (two patients) or after the third 50-mg injection. In one case, parasitemia reached 0 (undetectable levels) for three days (and in two other cases with concomitant *P. vivax* parasites; see below). When present, gametocyte counts fluctuated during the study (Figure 1), but there was no decrease related to IgG injection and in general, no correlation with asexual parasite counts ($r = 0.3$, $P = 0.1$).

In view of the satisfactory tolerance and results observed, two additional cases were treated with a single dose, one at a dosage of 20 mg/kg, which we found had a major effect in two previous patients, and one at a dosage of 200 mg/kg, which is similar to the dose used in the experiment in the 1960s. At 20 mg/kg, tolerance was good, clearance of fever occurred in 52 hr, and parasitemia diminished in two steps at 20 and 68 hr, but the lowest parasitemia reached was still higher than in patients receiving 100 mg/kg (Table 1 and Figure 2). Both the spleen and the liver showed moderate enlargement (1.5 cm at day 0 to 3 cm at day 1).

In the patient given a 200 mg/kg dose over four hr, fever and parasitemia decreased within a time frame similar to other patients. However, parasitemia decreased in two steps, an initial reduction (160-fold) within two hr after the start of infusion and the second within 48 hr (Table

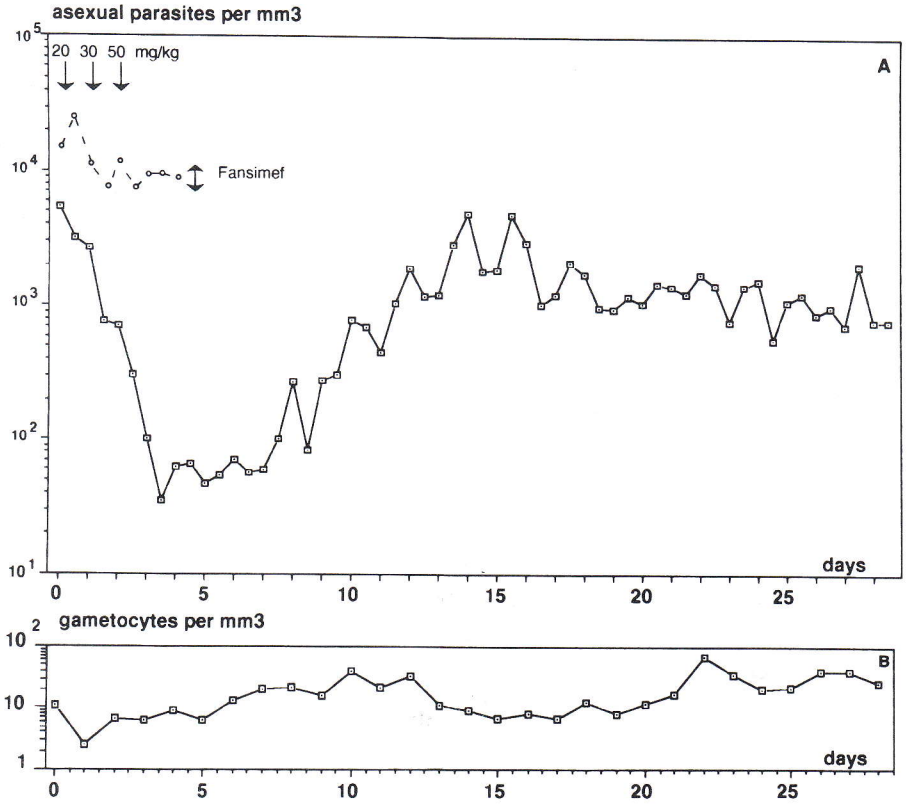

FIGURE 1. Geometric mean of the number of *Plasmodium falciparum* asexual blood forms **(A)** and gametocytes **(B)** in six patients receiving 100 mg/kg of the African IgG pool over three days, and in one patient receiving control IgG **(dotted line).** In the followup, data from patients receiving either a second IgG treatment or Fansimef® (after days 15 and day 22, respectively) were excluded from the mean after the day of treatment.

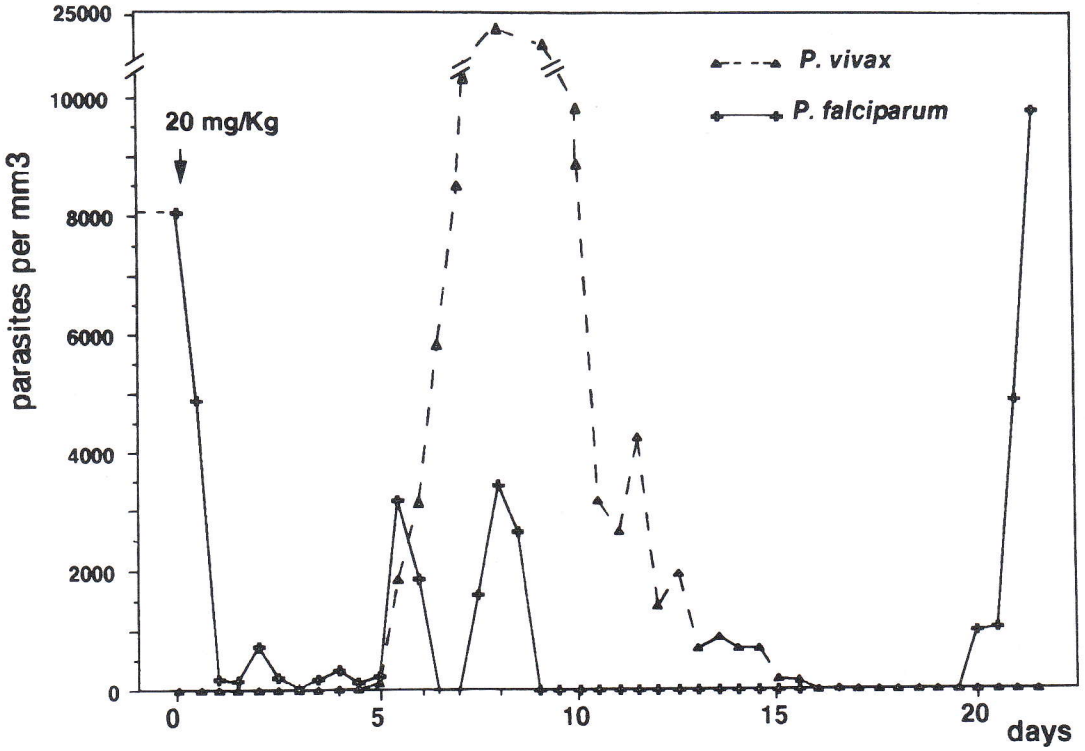

FIGURE 2. *Plasmodium falciparum* and *P. vivax* parasitemia in patient 8 after treatment with a single 20 mg/kg dose of IgG.

1 and Figure 3). The spleen reaction was intense but transient. Spleen size, starting at 1 cm, reached 6 cm in 10 hr, then decreased over the next 12 hr. The increase in liver size observed was moderate (1.5–2 cm). No other abnormal changes were observed over the next few days.

The kinetics of the transferred African IgG were evaluated indirectly by monitoring subjects for antibody levels to several antigens, namely HBs antigen, and the malarial antigens LSA,[5] RESA,[6] and FIRA.[7] A fast decrease was noted and low titers were progressively reached within two weeks (Figure 4). Consistent with this estimation, parasitemia started to increase again in the eight treated patients by days 9–12. In two cases, the increased level of parasitemia and the reoccurrence of symptoms required the initiation of radical drug treatment (on day 22). In the others, parasitemia fluctuated at rather high levels without symptoms for several days (Figure 1).

In three of the cases in whom parasitemia reached levels higher than 8,000/μl with a parallel increase in fever up to 38.5–40°C, African IgG injection was repeated (on days 15, 28, and 29, respectively). This was performed in order to determine if the first injection may have selected for a parasite population with different antigenic features. Thus, a relatively high dose of 100 mg/kg/day for two days was chosen.

Fever was cleared in an average time of 22 hr. Parasitemia started to decrease after the first injection in two cases and after the second injection in the third case (Figure 5); it reached low levels in an average time of 69 hr, with a further decrease at 96 hr, and the mean reduction of parasitic load was 1, 310-fold (Table 1 and Figure 6). In contrast with previous treatments, no spleen or liver reaction was noted.

Among the 11 treatments given, a close correlation was found between the level of the initial parasitemia and the degree of reduction of the parasite count ($r = 0.85$, $P < 0.01$) (Figure 7). This result is presumably related to the mode of action of antibodies, which appear to act by cooperating with blood monocytes,[8] and to the fact that the triggering agent of IgG-armed monocytes seems to be the parasite itself (H. Bouharoun-Tayoun and P. Druilhe, unpublished data).

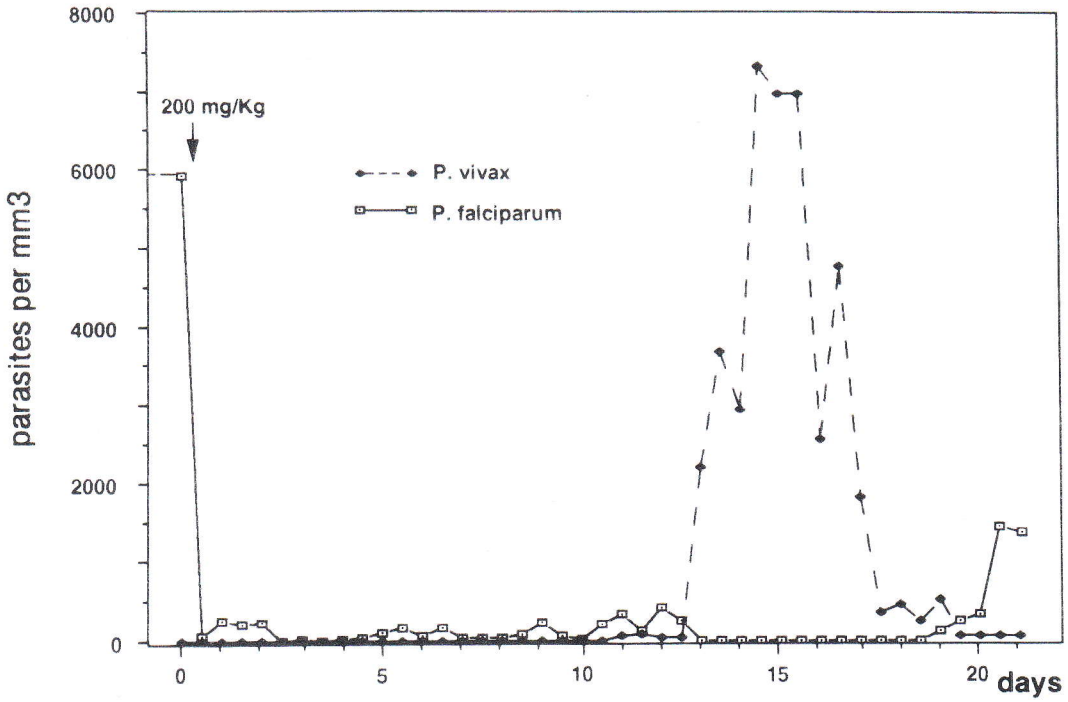

FIGURE 3. *Plasmodium falciparum* and *P. vivax* parasitemia in patient 7 after treatment with a single 200 mg/kg dose of IgG.

In the followup of these 11 treatments in eight patients, the only parasites that could be seen were young ring forms and gametocytes. In the smears taken at 30 min, every hour for the first 4 hr, and in subsequently taken smears, no mature forms could be detected at any time by two microscopists. In addition, we did not see parasites resembling the so-called "crisis forms" described by Taliaferro and Canon during recovery in infected monkeys,[9] and frequently reported, but only in in vitro conditions, with *P. falciparum*. However, a large proportion of morphologically altered, elongated, and distorted rings with dense and large Maurer dots were seen only for a very brief period of time in the slides taken just before a major decrease of parasite counts occurred in six patients, but were not seen in subsequently prepared slides. Since similar observations were made, though only in a small percentage of parasites in one patient receiving control IgG (see below), the meaning of this observation remains debatable.

All of the few parasites that remained at the end of the initial decrease had normal morphologic features and, when cultured, grew readily and at a fast rate (i.e., in patient 7, parasitemia on day 2 was 1 parasite per 500,000 RBC and in vitro, a parasitemia of 0.03% was reached after 3 days of culture).

A transient decrease in WBC counts was noted in all patients, except one who already had low counts at the start of the study. This was due primarily to a decrease of approximately 40% in the number of polymorphonuclear cells, without a major or consistent change in the total numbers of lymphocytes or monocytes. The decrease of parasitemia was not associated with any change in hematocrit, RBC counts, or platelet counts. No modification of any biochemical parameters in blood and urine samples occurred during the treatment or the followup period. In particular, blood creatinine levels remained normal and albuminuria was not seen in these patients. Haptoglobin and complement fractions (C3, C4) levels remained within normal ranges.

In two cases, *P. vivax* parasites that were undetectable in the first few days appeared during the followup (Figures 2 and 3). Their increase in numbers correlated closely, especially in one case, with the progressive decrease and disappearance

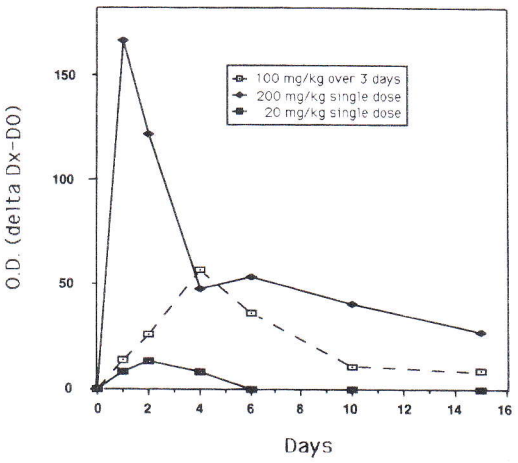

FIGURE 4. Evaluation of antibody titers to a *Plasmodium falciparum* liver-stage antigen in patients receiving 200 mg/kg, 20 mg/kg, or 100 mg/kg doses of IgG (each plot is the mean of four patients). Antibody levels were determined by a solid-phase enzyme-linked immunosorbent assay with a 41-amino acid synthetic peptide containing a B cell epitope of the liver stage antigen and sera diluted 1:100. Results shown are the differences between the initial values measured on day 0 samples and values in samples collected at various times during the followup. O.D. = optical density.

of *P. falciparum*. Following the spontaneous disappearance of *P. vivax* in one patient and after chloroquine treatment in the other patient, *P. falciparum* parasites (which were resistant to chloroquine) reappeared. In contrast, with IgG treatment, which clearly had no impact upon sexual blood stages, all *P. falciparum* asexual and sexual forms became totally but transiently undetectable during the *P. vivax* parasitemia.

All patients were treated with Fansimef® (a combination of sulfadoxine-pyrimethamine and mefloquine) before being discharged from the hospital. They were followed up at home; examinations performed one year later did not detect any abnormalities. In particular, results of screening for HIV antibodies and HBs antigens were negative.

For obvious ethical reasons, we could not include in our study a control group receiving normal IgG, since IgG from non-immune donors had been previously shown to be non-protective.[1] However, some type of control was still available from 32 patients of the same series of quinine RI failures who showed the same recrudescence, but who did not receive IgG. In these patients, who originated from the same area, two types of patterns of evolution were observed. The first showed that recrudescent parasites were detected, and thereafter increased in number rather steadily up to levels ranging from 1,000 to 30,000/μl. In this group, the occurrence of clinical symptoms obliged us to commence treatment with another drug. Upon treatment with Fansimef®, about half of the patients had a parasitemia that still increased the next day, up to a maximum of 53,000/μl, which was cleared in 72–144 hr (mean 96 hr). The second pattern showed parasitemia increasing up to 1,000–11,000/μl, but which remained thereafter at high levels with daily fluctuations, with only occasional peaks of fever and no other symptomatology that would lead to drug treatment. All were treated before being discharged. None of these cases showed a profound and sustained decrease in parasitemia before drug treatment, which is in contrast to the results seen in the IgG-treated group.

Since the effect of immune IgG proved to be relatively fast, one control trial with non-immune IgG and short-term followup was thought to be ethically acceptable and was performed in one patient after completion of the eight-patient study. Initial parasitemia was 13,000/μl and the body temperature peaked once at 38.8°C, but other symptoms were absent. Following iv inoculations of 100 mg/kg of control IgG from healthy French blood donors over a three-day period (20, 30, and 50 mg/kg, respectively), parasitemia still fluctuated between 8,000 and 12,000/μl up to day 4 (Figure 1). Fansimef® was given to this patient before discharge.

DISCUSSION

When transferred to malarious Thai patients, the African adult IgG pool used proved to exert a consistent, but non-sterilizing effect on each strain tested; this effect was stage-specific and probably species-specific. These results broadly confirm the conclusions in previous studies performed from 1961 to 1963[1–3] and provide new information.

The effect we observed was consistent among the 11 treatments at three different doses in eight patients. In contrast, two failures occurred among the first series of 12 Gambian children treated in 1961,[1] four partial failures (i.e., early recrudescence) occurred among the second series of nine East African children,[3] and no effect was

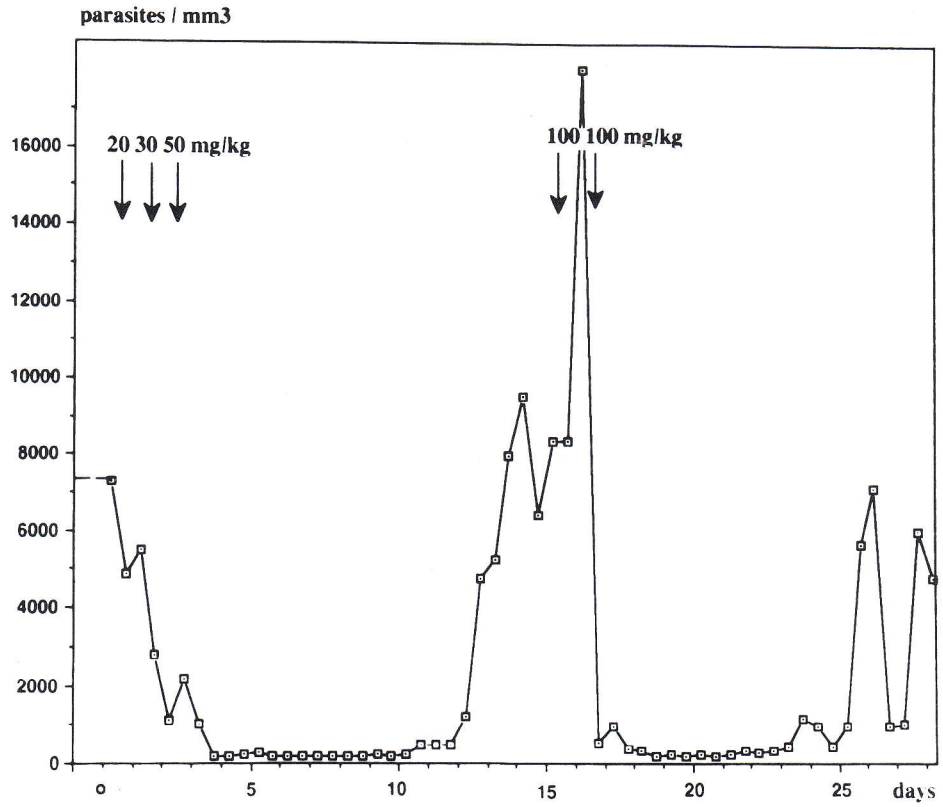

FIGURE 5. *Plasmodium falciparum* asexual parasitemia in patient 3 after the first and second IgG treatments.

seen in four African patients receiving antibodies from Papua New Guinea.[10] Although the investigators of these studies indicated that this could possibly be attributed to a concomitant bacterial or viral infection, alteration of Ig after storage, or to an insufficient dose of Ig or to antigenic polymorphism, these results left some doubt about the consistency of the antibody mediated effect, particularly in the geographically remote strains. In contrast, our data show that antibodies developed after 20 years of exposure to the parasites can confer a protection effective on all strains tested. Obviously, the fact that the antibodies originate from Africa and the parasites from Asia strongly reinforces this point, especially since either inconclusive[11] or less convincing [12] data were obtained (i.e., only a delay in patency) when this question was addressed in the chimpanzee or the *Aotus* monkey, respectively.

Asexual parasite counts decreased but gametocytes were apparently unaffected, which is consistent with the results of the studies in the 1960s. As expected, the iv route enabled us to obtain a much faster effect. Symptoms were cleared in 12–92 hr (average 47) and the maximum parasite decrease occurred in 33–120 hr (average 78). In the historic studies where IgG was injected im, a route that results in a loss of material and a 24–48 hr delay in the peak blood concentration, the decrease in both body temperature and parasitemia was progressive over 7–9 days. In fact, the fast rate of events observed using iv Ig treatment is consistent with that of fast-acting drugs,[13] and this rapidity could probably in itself be deleterious. This assumption is derived from the results recorded using a single 200 mg/kg dose, in which nearly 2×10^{10} parasites were removed from the peripheral blood within 2 hr. In our opinion, this could predict that severe adverse reactions could eventually be observed if higher parasitemias were treated by large doses of protective IgG. However, for all other regimens where lower daily doses were used, the IgG treatment proved to be very safe.

It may appear surprising, as discussed earlier

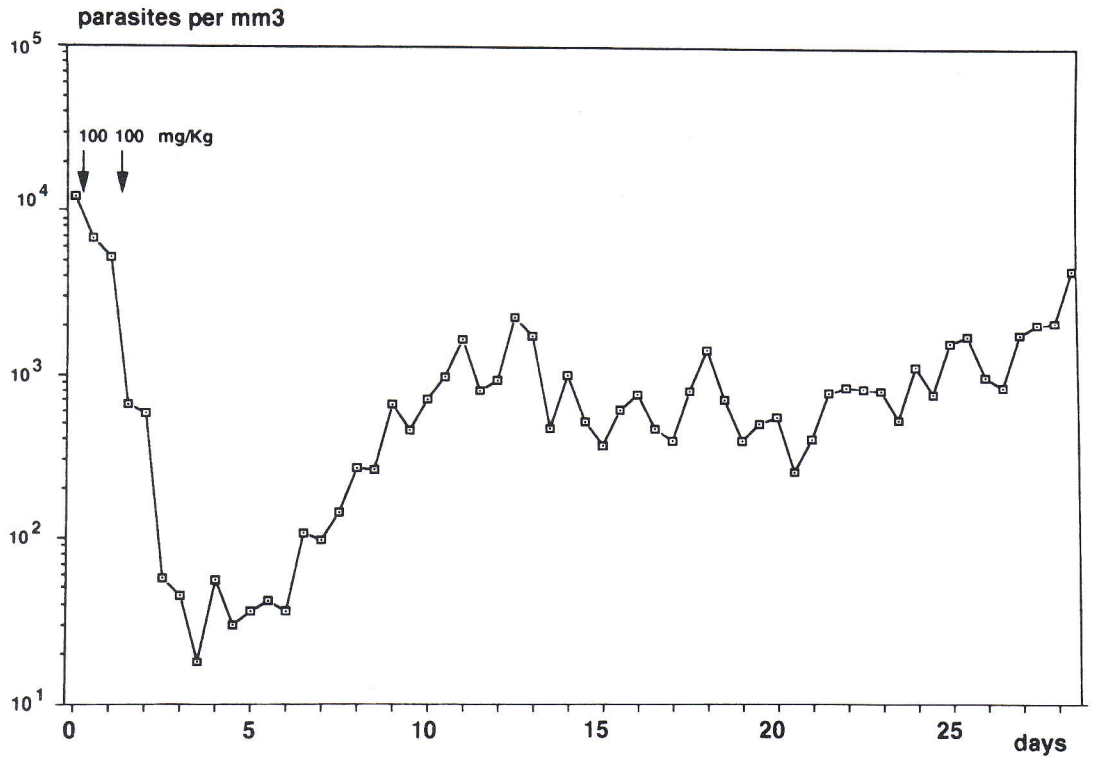

FIGURE 6. Recrudescent parasitemia (geometric mean of *Plasmodium falciparum* asexual blood forms) in three patients after a second IgG treatment (200 mg/kg over two days). The second treatment with IgG was given to patients 1, 3, and 8, respectively, on days 15, 28, and 29 after the first IgG dose. The zero point indicates the day when the second IgG treatment was administered.

by McGregor,[14] that an antibody able to clear 99–99.9% of the parasites in the subjects was unable to clear the few remaining parasites. One explanation generally proposed is that Ig may select parasites, and those parasites are able to escape the effect of Ig. However, one should recall that most African adults who have reached a state of premunition remain able to support low-grade parasitemias for years, if they continue to be exposed. Thus, with naturally acquired im-

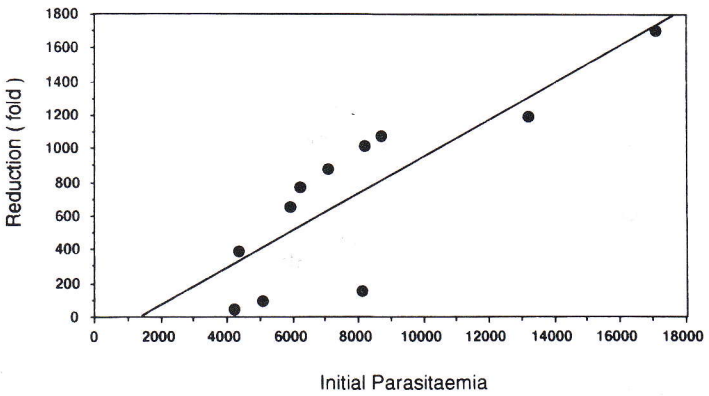

FIGURE 7. Correlation between the parasitic load decrease and the initial levels of parasitemia at the time of treatment.

munity being of a non-sterilizing type, it appears logical to us that its mediator, when passively transferred, can at best only achieve a similar state. Indeed, in vitro cultivation demonstrated that the parasites seen on the slides in very small numbers (only 1 or 2 per slide after a 20 min examination) were still viable, and in vivo produced an increase in parasitemia soon afterwards. Thus, passively transferred immunity had only a partial and transient effect.

These results are difficult to compare with those of previous studies, where several cases were followed as outpatients, and no data were available after day 10. In our series, parasitemia started to increase from days 9–13 onward, at about the time when the levels of the transferred antibodies had markedly decreased. The rate of clearance of IgG was not measured, but only indirectly evaluated by ELISA titers. However, the short half-life observed by this procedure is consistent with previous studies that showed a striking increase (seven-fold) in both synthesis and catabolism of IgG in malaria-infected subjects compared with drug-protected Africans or healthy Europeans.[1] Several other mechanisms may have participated in the clearance of the antibodies, such as the development of anti-allotypes by the subjects, or the consumption of critical antibody by target parasite antigens.

The molecular characterization of many malarial antigens has produced increasingly clear evidence for a tremendous diversity and strain polymorphism of the proteins of the parasite.[15] Moreover, at the level of erythrocyte-associated antigens, immune pressure was found capable of inducing the expression of antigenic variants among cloned parasites.[16] It is, in this context, essential to stress that the recrudescent parasites we observed were still susceptible to the same antibodies in all these cases. Although this antibody preparation was highly polyclonal, this allows us to rule out the induction or selection by the injected antibodies of a particular parasite population with a distinct antigenic phenotype. In addition, the recrudescences coincided well with the disappearance of detectable amounts of the transferred antibodies and occurred earlier at the 20 mg/kg dose than at the 200 mg/kg dose. It is certainly not possible to decide from the parasitologic and clinical data presented herein whether the consistency of the effect observed with a single IgG preparation resulted from the presence in immune subjects of a given antibody specificity directed to non-polymorphic antigens, or from the presence of a wide range of strain-specific antibodies able to act on variant antigens, even from geographically remote parasites. These results do show that passively acquired antibody mimicked the state of partial resistance called premunition and conferred resistance to all parasite isolates tested.

In falciparum malaria, sequestration of maturing rings, trophozoites, and schizonts takes place in deep-seated capillaries of most organs. Therefore, the peripheral blood parasitemia is usually much lower than the total parasitemia. Much emphasis has recently been placed on the study of the sequestration phenomenon and the associated mediating molecules.[17] Based on monkey malaria models and in vitro studies, it has been proposed that the reversal of cytoadherence by antibodies would be a major mechanism mediating protective immunity in *P. falciparum*-infected humans. Our results do not support this view. It still may be argued that reversal of cytoadherence occurred, but was not detected. This, in our opinion, is highly unlikely since no maturing ring (i.e., pigmented) or segmenter was seen at any time among the 11 treatments performed, while in monkey experiments, numerous circulating schizonts could be readily seen.[18, 19] Finally, circulating schizonts were also undetected in the three previous trials conducted in the 1960s, thus bringing to 42 the number of patients in whom this phenomenon was not observed.

Plasmodium vivax parasites, which were undetected during the month preceding the start of therapy, emerged rather early at the time when *P. falciparum* parasite levels decreased. There was a mutual exclusion of the two species, since when *P. vivax* disappeared spontaneously in one case and following chloroquine administration in the other case, *P. falciparum* reappeared.

Similar exclusion of one parasite species by another has been previously documented in laboratory experiments.[20] African IgG has been reported to be at least partially effective on *P. malariae* parasites in three patients.[1, 2] No data is available for *P. ovale*. Since the prevalence of *P. vivax* is extremely low in Africa, one may assume that our donors were not immunized against this species. Thus, the emergence of *P. vivax* can be taken as an indication that the protective effect of antibodies was species-specific, and that cer-

tainly *P. falciparum* and possibly *P. ovale* do not induce cross-protection against *P. vivax*.

With the precise conditions we used (i.e., early recrudescence after therapeutic failure, a relatively low parasitemia, and an extended dose schedule), the IgG treatment had a fast and consistent effect, and above all, was safe. However, in our opinion, its large scale use should not be encouraged, and at least for some time, should be limited only for research purposes, such as investigating the basis of protective immunity. Nevertheless, this experiment allowed us to better define the conditions permitting the design of limited, well-controlled assays of protective IgG in humans. As such, it opens the future possibility of assessing the protective effect of defined antibody specificities, and therefore provides one means to designate in the natural model the target antigens of protective mechanisms.

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