

# Antibodies that Protect Humans against *Plasmodium falciparum* Blood Stages Do Not on their Own Inhibit Parasite Growth and Invasion In Vitro, but Act in Cooperation with Monocytes

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## Summary

IgG extracted from the sera of African adults immune to malaria were injected intravenously into eight *Plasmodium falciparum*-infected nonimmune Thai patients. Clinical and parasitological improvement was reproducibly obtained in each case. After the disappearance of the transferred Ig, recrudescence parasites were equally susceptible to the same Ig preparation. High levels of antibodies to most parasite proteins were detected by Western blots in the receivers' sera (taken before transfer) as in the donors' Ig, thus indicating that the difference was qualitative rather than quantitative between donors and receivers. In vitro, the clinically effective Ig had no detectable inhibitory effect on either penetration or intra-erythrocytic development of the parasite. On the contrary, they sometimes increased parasite growth. In contrast, these IgG, as the receivers' Ig collected 4 d after transfer, but not those collected before transfer, proved able to exert an antibody-dependent cellular inhibitory (ADCI) effect in cooperation with normal blood monocytes. Results were consistent among the seven isolates studied in vitro, as with the recrudescence parasites. Thus, the results obtained in the ADCI assay correlate closely with clinical and parasitological observations.

In regions where malaria is hyperendemic, it has been observed that the acute disease is mainly a feature of childhood. Though adults can still be infected, they reach after several years a state of relative resistance. This incomplete, nonsterilizing immunity, where low grade parasitemia can be observed and symptoms are usually absent, has been called premunition (1).

The effector mechanisms mediating premunition against *Plasmodium falciparum* blood stages are far from being well known. Due to the lack of MHC molecules on RBC, it can be a priori expected that cytotoxic lymphocytes have little or no role to play. Conversely, the in vivo effect in humans of passively transferred African adults' IgG strongly suggests that antibodies of IgG class are an important component of acquired immunity (2-4).

Relatively few studies have addressed the question of how these antibodies mediate protection. From these studies, despite conflicting data, it is nowadays generally accepted that antibodies inhibit merozoite penetration into erythrocytes. However, our former studies did not support the hypothesis that IgG from immune African adults may have a significant blocking effect on merozoite entry, but rather, suggested that they act by cooperating with leukocytes (5, 6). Thus, the

mode of action of antibodies remains controversial. One important consequence of this situation is an absence of a universally accepted assay relevant to protection, and therefore, a lack of reliable means to select for vaccine candidates by in vitro methods.

For this and other reasons, particularly the poorly known relevance of models, we thought that additional immunological investigations in man were required. A recent experiment of passive transfer of IgG in humans gave us the unique opportunity to perform in parallel an in vitro study using parasite isolates and antibodies the in vivo interactions of which were well defined by clinical and parasitological observations.

## Materials and Methods

*In Vivo Transfer of Immune IgG.* A set-up allowing us to perform in vivo studies under ethically acceptable conditions was designed. A pool of immune African globulins (PIAG)<sup>1</sup> was prepared from immune individuals. Since there is no universally accepted

<sup>1</sup> Abbreviations used in this paper: ADCI, antibody-dependent cellular inhibition; IFA, indirect fluorescent antibody; NSE, nonspecific esterase; PIAG, pool of immune African globulins; RESA, ring-infected erythrocyte surface antigen.

assay able to reflect acquired protection, premunition was defined and donors were selected on clinical and epidemiological grounds. All were adults living in rural areas where malaria is holoendemic, who experienced numerous malaria attacks in childhood and were free of symptoms and heavy parasitic loads. The detailed procedure of serum sampling in Africa, safety control studies, IgG extraction, and Ig administration in *P. falciparum*-infected patients is described in detail elsewhere (Sabchareun, A., T. Burnouf, D. Ouattara, P. Attanah, H. Bouharoun, P. Chantavanich, C. Foucault, T. Chongsuphajaisiddhi, and P. Druilhe, manuscript submitted for publication). Briefly, sera from 333 African adults were collected in a rural part of the Ivory Coast and submitted to extensive screening using all methods available in the present state of the art. One to three distinct assays were used to screen for abnormal hemagglutinins, hemolysins, antibodies to syphilis, HBs antigen, anti-HBs and c antibodies, and HIV-1 and -2 antibodies. 153 of the sera were discarded for safety reasons (positivity or doubtful results in one or several of the above tests). To avoid contamination by possibly mislabeled samples, these controls were repeated in subpools of 10 sera, which were finally mixed together. IgG pure enough for intravenous use was prepared under good manufacturing practice conditions by an industrial group (Biotransfusion CRTS, Lille, France), using the Cohn extraction procedure followed by partial pepsine treatment at pH 4, a method that does not alter the Fc fragment of IgG (7).

To ensure a safety margin of at least 48 h, allowing to switch to drug therapy, receivers of IgG were chosen among patients having a recrudescence parasitemia after drug treatment failure of a primarily acute case. In this situation, where parasitemia is progressively increasing but relatively low, symptoms are still relatively mild or absent, though they would progressively appear or increase in the next few days. Indeed, an increase in symptoms occurred in the group of 40 patients who were in the same situation of recrudescence after quinine therapy failure, and they served as controls. A total of eight Thai patients with *P. falciparum* parasitemia (5–10,000/mm<sup>3</sup>), were treated in the Hospital for Tropical Disease (Bangkok, Thailand), by slow intravenous inoculation of the African IgG pool, one with a 20-mg/kg single dose, six at 100-mg/kg total dose spread over 3 d (day 0, 20 mg/kg; day 1, 30 mg/kg; day 2, 50 mg/kg), and one with a 200-mg/kg single dose. In three cases, late recrudescence parasites that appeared upon follow-up were treated with a total dose of 200 mg/kg over 2 d (100 mg/kg/d). The patients remained under close medical supervision in the hospital, received Fansimef (an antimalarial drug made of a combination of sulfadoxine-pyrimethamine and mefloquine) before being discharged, and were followed up at home for 1 yr.

**IgG Preparations Used In Vitro.** Samples of PIAG prepared as described above were obtained before and after the partial pepsine treatment. In addition, we studied IgG from the following sera: (a) five individual immune african adults; (b) eight Thai patients treated with PIAG, using samples obtained on day 0 before IgG was transferred and on day 4 after initiating treatment; (c) six *P. falciparum* primary attack cases in French travellers, who had high antibody titers but presumably had no clinical protection against reinfection; (d) five healthy French blood donors with no history of malaria as normal controls.

IgG was extracted from the above additional set of sera by ion exchange chromatography on DEAE-Trisacryl (IBF, Biotechnics, Villeneuve-la-Garenne, France) according to the manufacturer's specifications. The IgG was dialyzed against RPMI 1640, 35 mM Hepes, 23 mM NaHCO<sub>3</sub> medium, and concentrated to the original IgG concentration in the serum and sterilized on 0.22- $\mu$ m pore size Millex filters (Millipore Continental Water Systems, Bedford,

MA). The presence and purity of the IgG collected was ascertained in dot blot assays with mAbs specific for human IgG, IgM, or IgA, and with serial serum dilutions in dot blots using a total extract of *P. falciparum* blood stage proteins as antigen and the original serum as control. IgG from a pool of >1,000 healthy French blood donors prepared by the Cohn method (CRTS, Lille, France) was used as control in all antibody-dependent cellular inhibition (ADCI) assays where the PIAG was tested. Sterile IgG were kept at 4°C until use.

**Parasites.** *P. falciparum* blood stage parasites were isolated from the Thai patients on day 0 before starting IgG treatment. One aliquot from each isolate was cultured in RPMI plus 10% human serum as described (8), and the others were cryopreserved in liquid nitrogen. Among the eight isolates, seven could be adapted to culture conditions and were also cryopreserved at various times after in vitro adaptation. In addition, recrudescence parasites were collected from each patient on day 21–29, cultured, and cryopreserved. Control cultures were performed with the NF54 *P. falciparum* line, which is thought to originate from Africa (9). When required, parasite cultures were synchronized by two Sorbitol treatments at 8-h interval (10) and, after in vitro maturation, were concentrated by flotation on plasmagel (11).

**Invasion and Growth Inhibition Assays.** *P. falciparum* inhibition assays were performed in duplicates in 96-well plates (Nunc, Roskilde, Denmark) at a 2% hematocrit. Unless otherwise indicated, there was no medium replacement during the 18- and 48-h tests. The effect of purified IgG on RBC invasion by *P. falciparum* merozoites was tested in 18-h cultures initiated with mature schizonts at 1–2% parasitemia from synchronized cultures. The direct effect of IgG on *P. falciparum* growth was assessed in 48-h cultures of nonsynchronized parasites at a 0.2–0.5% initial parasitemia. In both assays, test and control IgG were added to the culture medium at 5, 10, and 20% of their original concentration in the donor serum. Parasitemia was estimated in thin smears from each well by microscopic examination of >10,000 erythrocytes.

**ADCI Assay.** Blood mononuclear cells from healthy French donors were separated on Ficoll-Hypaque density gradient (Pharmacia Fine Chemicals, Uppsala, Sweden) according to the technique of Böyum (12). The number of monocytes in the cell suspension obtained was estimated by the nonspecific esterase (NSE) stain (13). The mononuclear cell suspension (in RPMI) was thereafter distributed in a 96-well plates at the rate of  $2 \times 10^5$  monocytes (NSE<sup>+</sup> cells) per well. After a 90-min incubation at 37°C in a 5% CO<sub>2</sub>-air mixture, nonadherent cells were removed from wells by washings with RPMI. This method permitted the recovery of  $\sim 10^5$  adherent cells per well, among which, >90% were monocytes.

In the wells containing the adherent monocytes, *P. falciparum* asynchronous cultures were added at a ratio of 200 RBC per one monocyte. The culture medium (RPMI + 10% human serum) was supplemented with each of the purified IgG to be tested at 10% of their initial concentration in the donor serum (or 5, 10, 20, and 40% for some experiments). Control wells consisted of: (a) culture alone; (b) culture and test IgG without monocytes; (c) culture and control IgG; and (d) culture, control IgG, and monocytes. Unless otherwise stated, the assay duration was 48 h. Starting at 0.2–0.5%, parasitaemia generally reached 1–3%.

The specific growth inhibitory index (SGI), which takes into account the possible inhibition induced by either cells or antibodies (IgG) alone, used as controls in each experiment, was calculated as follows:  $SGI = 100 \times [1 - (\text{percent parasitemia with monocyte and IgG} / \text{percent parasitemia with IgG}) / (\text{percent parasitemia with monocyte and control IgG} / \text{percent parasitemia with control IgG})]$ .

**Antibody Assays.** Western blot analysis was performed using

mature schizonts separated from *P. falciparum* cultures by flotation on plasmagel, which were then washed in RPMI (11). Parasite extracts from each patient isolate and from NF54 strain were subjected to electrophoresis in a 7.5% polyacrylamide gel containing SDS (14), and proteins were electroblotted onto nitrocellulose filters (BA 85; Schleicher & Schuell, Inc., Dassel, FRG) (15). Nitrocellulose strips were blocked in 5% nonfat milk (Régilait, Saint-Martin-Belle-Roche, France) Tris buffer (50 mM Tris-HCl, pH 8, 0.15 M NaCl) and incubated with human sera diluted 1:100 in the same buffer. After washings, the strips were incubated with <sup>125</sup>I-labeled (16) anti-human IgG or IgM (Biosys, Compiègne, France) diluted 1:2,000, washed, and autoradiographed at -70°C. Western blots were scanned using a scanning densitometre (GS300; Hoefer Scientific Instruments, San Francisco, CA), areas under each peak calculated, and were expressed in arbitrary units compared with a positive control serum run on the same gel.

An indirect fluorescent antibody (IFA) assay was performed on thin smears of infected RBC (2-4% parasitaemia) from each patient isolate, using serial dilutions from 1:200 to 1:102,400 of each serum in PBS (pH 7.4), and FITC-conjugated anti-human IgG (diluted 1/100) (Nordic Immunology, Tilburg, The Netherlands) as a second serum. The ring-infected erythrocyte surface antigen (RESA) IFA was performed as described by Perlmann et al. (17) using glutaraldehyde-fixed ring-infected erythrocytes from patient B. In addition, specific malarial antibodies were also measured using the counter current immunoelectrophoresis (CIEP) method performed on cellulose acetate membranes as formerly described (18).

## Results

The antibody preparation that we tested in vitro had the following properties in vivo. A total of eight Thai patients were treated by the pool of PIAG. Six of them at a total dose of 100 mg/kg over 3 d, and two at a single dose of either 20 mg/kg or 200 mg/kg. In patients treated with 100 mg/kg, the intravenous inoculation of the Ig preparation was very well tolerated. Fever disappeared in a mean of 73 h, that is, as fast or faster than by drug treatment (19). Parasitemia decreased in each case, by an average of 543-fold, and thereafter, remained at low levels for 7-12 d (Fig. 1 a). At 20 mg/kg, clearance of parasitemia and fever occurred fast (68 and 52 h, respectively), but the lowest parasitaemia reached was higher than in patients receiving 100 mg/kg (Fig. 1 b). At 200 mg/kg, parasitemia diminished in two steps. A 160-fold reduction step occurred in 2 h, together with an intense but transient spleen reaction, and was followed by a second reduction step within the next 48 h (Fig. 1 b).

During the follow-up, as the transferred Ig were eliminated, parasitemia reincreased progressively. In three cases, the PIAG was injected a second time (at a rate of 100 mg/kg daily for 2 d) to determine if the first injection may have selected for a parasite population with different antigenic features. In each of the three cases, parasitemia decreased by a mean of 1,310-fold (Fig. 1 c). In contrast, in the control group of 40 patients, who were also recrudescence cases after quinine failure but were not treated with PIAG, parasitemia increased up to 53,000/mm<sup>3</sup> before or shortly after receiving Fansimef. In one control patient, control IgG from healthy French blood donors was given at 100 mg/kg and parasitemia remained fluctuating between 8,000 and 12,000/mm<sup>3</sup> for 5 d until drug treatment was given. Since the 11 treatments performed with African IgG in eight Thai patients had a consistent clinical and parasitological effect, it can be concluded that there is no indication of a major geographic restriction of the antigens target of protective antibodies.

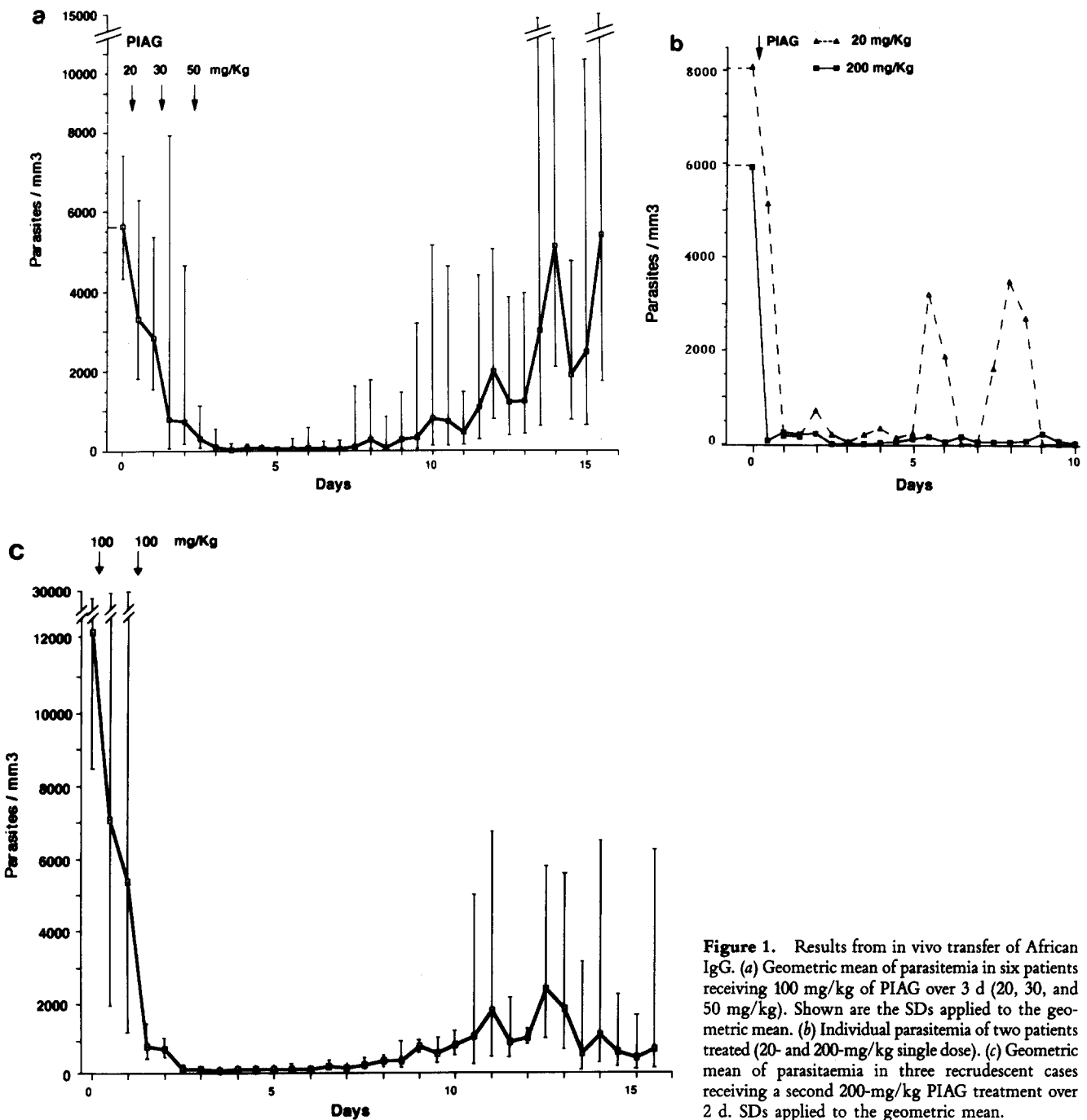
The content in antibodies to *P. falciparum* antigens in the pool of donors and in the receivers' sera was analyzed by IFA and Western blots. Table 1 shows that the receivers had on day 0, that is, before IgG transfer, whole antigen IFA titers at least as high as those of the pooled donors, and that more than half of them had in fact higher titers. This result is consistent with previous studies demonstrating an absence of correlation between the results from the IFA assay and the state of clinical immunity (20). IFA-RESA titers were, however, higher in the African pool than in the receivers, an expected finding since it has been shown that on average titers increase with exposure to the disease (21).

Similarly, when Western blots were performed with *P. falciparum* blood stage antigens, they revealed the existence of significant amounts of antibodies to most of the parasite polypeptides in each of the receiver's serum (Fig. 2). Scanning of these Western blots was used to measure the area under the curve and, after integration, to evaluate the total amounts of antibodies to parasite polypeptides. As for IFA, results reveal, for example, that three patients (Fig. 2, lanes C, D, and E) had more than twice as much antimalarial antibodies as the PIAG. From this result, it can be concluded that the difference between donors and receivers was more of a qualitative rather than of a quantitative order. The clear clinical and parasitological improvement obtained by the injection of the PIAG suggests that this pool and the receivers' Ig differ by

**Table 1.** Anti-*P. falciparum* Antibody Levels in PIAG and Thai Patient Sera before Transfer

Sera tested	PIAG	A	B	C	D	E	F	G	H
IFA	1,600	1,600	1,600	3,200	51,200	12,800	1,600	3,200	6,400
IFA RESA	250	<5	<5	20	<5	10	<5	<5	80
CIEP	1+	1+	3+	1+	1+	1+	1+	1+	1+

Shown are reciprocal titers obtained using either acetone-fixed smears of schizonts of strain Thai B (IFA) or glutaraldehyde (1%)-treated and air-dried ring-infected RBC (IFA RESA). Results of CIEP are expressed in the number of precipitating bands. Sera are from the patients A to H, taken on day 0, that is, before PIAG injection.



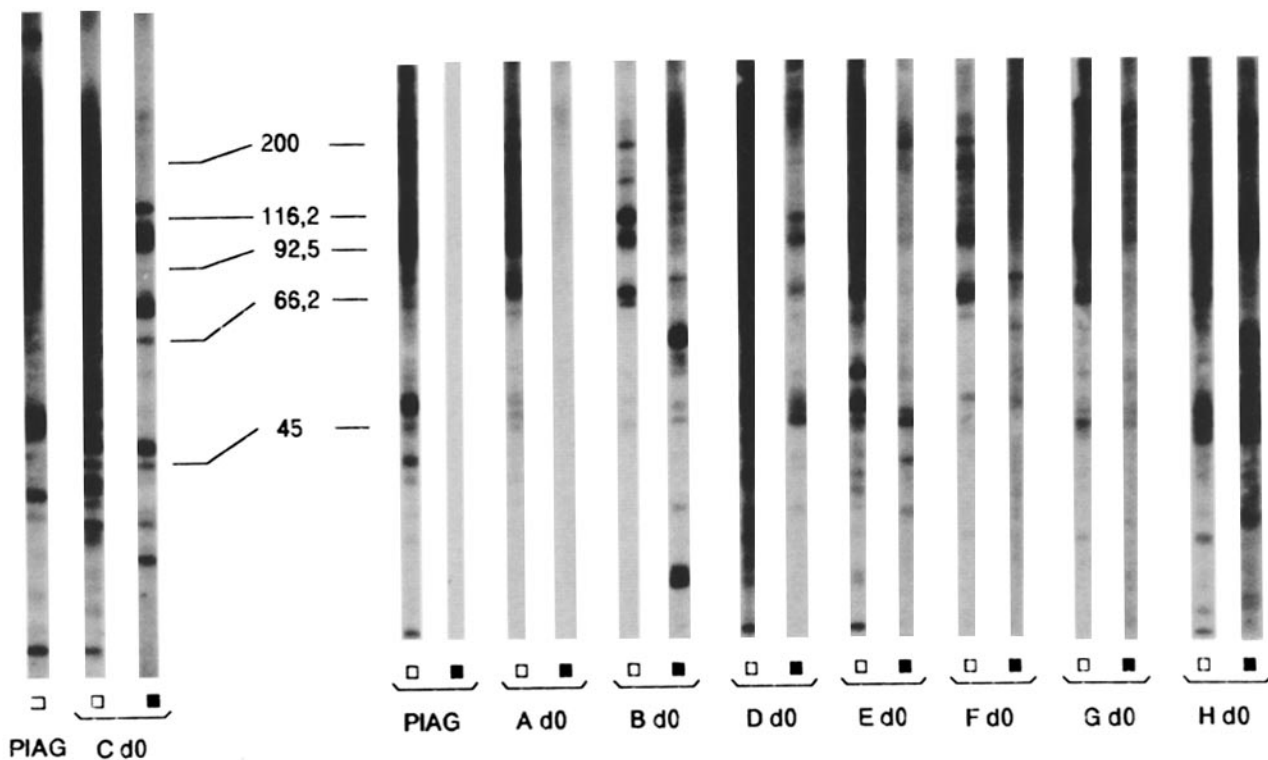
**Figure 1.** Results from in vivo transfer of African IgG. (a) Geometric mean of parasitemia in six patients receiving 100 mg/kg of PIAG over 3 d (20, 30, and 50 mg/kg). Shown are the SDs applied to the geometric mean. (b) Individual parasitemia of two patients treated (20- and 200-mg/kg single dose). (c) Geometric mean of parasitaemia in three recrudescence cases receiving a second 200-mg/kg PIAG treatment over 2 d. SDs applied to the geometric mean.

a limited number of critical antibody specificities. This is particularly true when one considers that in the blood of patients receiving a total dose of 200 and 20 mg/kg, the PIAG was in fact diluted at least 10 times and 100 times, respectively, while the receivers' Ig were obviously undiluted.

In vitro studies were undertaken to investigate the biological effect of the clinically effective IgG upon the parasites from seven of the eight patients, and upon an unrelated African isolate. In the merozoite invasion assay, addition of 1, 2.5, and 5 mg/ml of PIAG (that is, ~5, 10, and 20% of the

original IgG concentration in the donor serum) had no or only a very moderate influence on the rate of parasite penetration in RBC (Table 2).

In a 48-h growth assay, which explored both invasion and intra-erythrocytic maturation, more surprising results were recorded. As compared with control medium, with and without control IgG, the presence of PIAG in the culture resulted in an improved growth of the African isolate, and of some of the Thai patients' parasites, while the remaining were unaffected (Table 2 and Figs. 3 and 4). These results

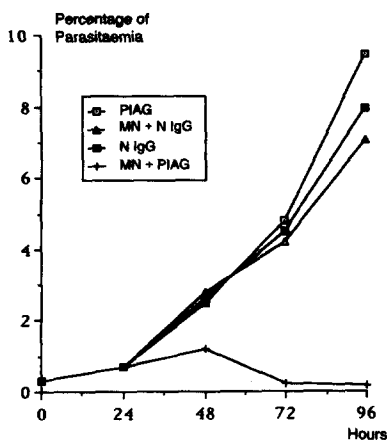


**Figure 2.** Comparative analysis of the antibody content in the African pool (PIAG) and in each of the receivers before Ig transfer (sera A-H on day 0) by Western blots performed on extract of parasites from patient C, revealed with  $^{125}\text{I}$ -labeled anti-human IgG ( $\square$ ) or IgM ( $\blacksquare$ ).

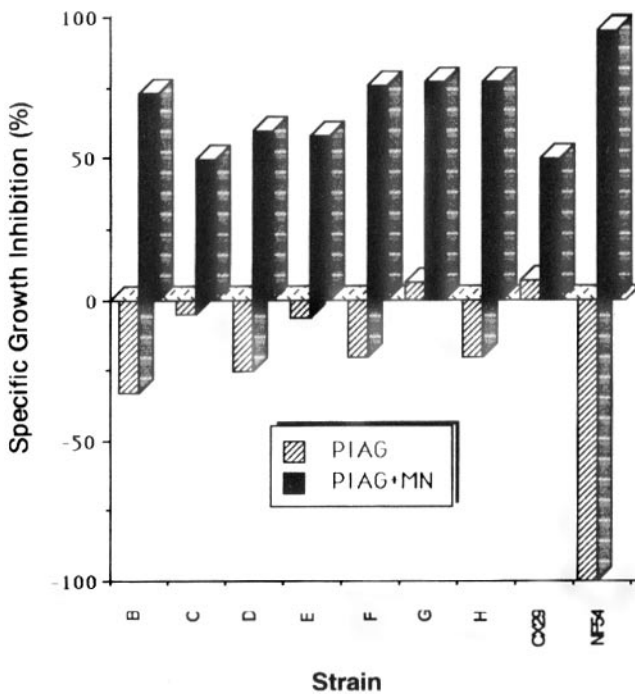
were consistent in >12 separate experiments performed with NF54 isolate, although the intensity of increase varied, and in two to five experiments performed with each of the patients strains at 2.5-mg/ml concentration of PIAG. The same absence of significant inhibition of parasite growth or invasion was observed in experiments in which we used IgG prepa-

rations from five other African immune adults, as well as with IgG preparations from six European primary attack cases (not shown). Finally, IgG extracted from the initial samples (day 0) of four of the Thai patients were also ineffective in the same invasion and growth inhibition assays (not shown).

In view of the former evidence that IgG may cooperate efficiently with blood monocytes (5, 6), further investigations were made using the ADCI assay. In this assay, monocytes from healthy blood donors were present at a ratio of 1:200 RBC, and PIAG was used at a 10% concentration (2.5 mg/ml), which is about its initial in vivo concentration when administered at a rate of 100 mg/kg. In contrast with IgG alone, a consistent antibody-dependent monocyte inhibitory effect was recorded in a 48-h assay, with each of the seven Thai patients' isolate and the African strain (Figs. 3 and 4). The SGI, which takes into account the possible nonspecific effect of monocytes or IgG alone, reaches 95% for NF54 and varies between 50 and 80% for the Thai isolates in a 48-h assay. An example of an ADCI experiment showing the growth rate in control and test cultures, from which the SGI is calculated, is given in Fig. 3. This figure also demonstrates that when cultures are kept >48 h, with daily medium changes, the intensity of the ADCI effect increases with time. Results were similar when using in vitro PIAG samples obtained before and after the partial pepsine treatment required to purify IgG for intravenous use (not shown). IgG extracted from five individual African adults on DEAE-trisacryl were also effective (not shown). Finally, the SGI was not found



**Figure 3.** Antibody-dependent cellular inhibition assay (ADCI). 96-h cultures were performed using *P. falciparum* isolate from patient C with daily medium change, either in the presence ( $\Delta$ , +) or absence ( $\square$ ,  $\blacksquare$ ) of monocytes from healthy donors, using control IgG ( $\Delta$ ,  $\blacksquare$ ) or the African IgG used in the in vivo transfer experiment ( $\square$ , +) at a 2.5-mg/ml concentration in RPMI + 10% human serum medium.



**Figure 4.** Summary of the 48-h ADCI assays performed with nine *P. falciparum* isolates and the African IgG (PIAG). Results are expressed as the specific growth inhibition in percent, which takes into account the possible inhibitory or facilitation effect of either cells or antibodies alone. Results obtained by ADCI appear as black bars (monocytes + antibodies). Controls in the presence of antibodies alone appear as hatched bars (the negative SGI represents an increase of *P. falciparum* growth as compared with control IgG). (B–H) Thai strains isolated from patients B to H before PIAG transfer. (CX29) The strain isolated from the Thai patient C on day 29 after transfer upon recrudescence of parasitemia. (NF54) An African strain used as control in each experiment.

to be influenced by the presence or absence of complement in the serum used for culture (not shown).

The IgG of four patients taken before they were passively protected were ineffective in the same ADCI assay. Results (Fig. 5) ranged from very slight inhibition (SGI = 5%) to increase of growth (SGI = -22%). In contrast, IgG extracted from day 4 samples of the same patients, that is, 4 d after receiving 10% of PIAG (100 mg/kg), showed a clear ADCI effect. In this case, in which the protective antibody was in fact diluted at least 1:20 in the receiver serum, that is ~1:200 in the culture well, the SGI reaches 34 and 46% (Fig. 5). Thus, results obtained in the ADCI assay using IgG taken either before or after PIAG in vivo transfer appear to correlate closely with clinical and parasitological observations.

We did not find a close relationship between the intensity of inhibition in the ADCI assay and the antibody concentration (above results and other in vitro assays performed with 1, 5, and 10% IgG; not shown), but such is also the case for results obtained in vivo using either 20, 100, or 200 mg/kg, which were not directly dose dependent (though recrudescences occurred earlier at 20 mg/kg than at 100 and 200 mg/kg).

**Table 2.** Evaluation of the Direct Effect of PIAG on Parasite Invasion and Growth

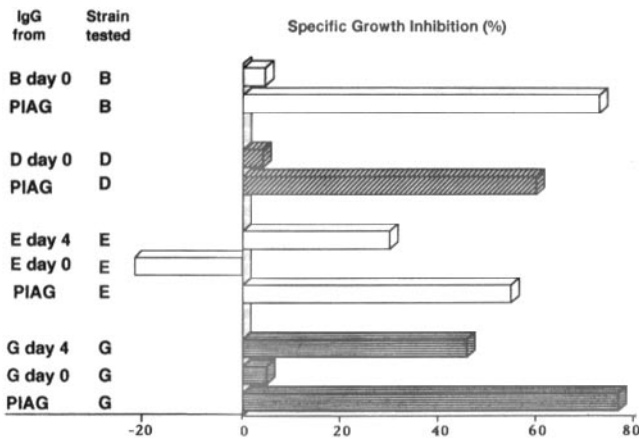
Strain	IgG concentration mg/ml	Invasion assay (percent final parasitemia)		Growth assay (percent final parasitemia)	
		Control IgG	PIAG	Control IgG	PIAG
NF54	2.5	1.8	1.8	2.2	4.4
	5	1.8	1.7	2.2	3.3
B	2.5	1.1	1	3	4
	5	1.1	1	ND	ND
C	2.5	5.7	7.9	8.3	9.6
	5	5.7	7.7	8.3	9.4
D	2.5	2.9	2.5	3.2	3.2
	5	2.9	2.4	ND	ND
E	2.5	1.2	1.2	2.6	2.8
	5	1.2	1.3	ND	ND
F	2.5	ND	ND	2.4	2.8
G	2.5	ND	ND	1.9	1.8
H	2.5	ND	ND	2.9	3.5
Cx29	2.5	9.6	11.5	1.6	1.5
	5	9.8	9.5	ND	ND

NF54 is a strain thought to be of African origin. B–H are the parasites isolated from Thai patients before PIAG transfer. Cx29 is the recrudescence strain in Thai C patient collected 29 d after the PIAG transfer. PIAG or control IgG were added, at various concentration, to culture medium (RPMI plus 10% human serum). The invasion assay was an 18-h culture of highly synchronized mature schizont-infected erythrocytes at 0.5–2% of parasitemia. The growth assay was a 48-h culture of asynchronous parasites, starting at 0.2–0.5% of parasitemia.

Recrudescence parasites from patient C, taken on day 29 after IgG administration (strain CX29), were found susceptible in vitro to the antibody-dependent monocyte inhibitory effect, as they were in vivo to the same IgG preparation (Fig. 4).

## Discussion

The present study was designed to establish a precise correlation between in vivo events and in vitro results. Because of former drug therapy failure, the patients we studied had harboured parasites for several weeks and therefore developed a significant immune response to parasite polypeptides, as shown by IFA and Western blot assays. The immune response of the patients to parasite antigens had no or limited clinical



**Figure 5.** Results of 48-h ADCI assays with four *P. falciparum* Thai isolates and various IgG preparations. Cultures were performed in presence of monocytes, in RPMI + 10% human serum supplemented with: PIAG (2.5 mg/ml) or homologous IgG isolated from the patient's serum on day 0 before transfer or on day 4 after PIAG transfer. All IgG were used at final concentrations in RPMI corresponding to 10% of their initial concentration in the donor's serum.

consequences in vivo. We also used in vitro an antibody preparation that, when allowed to interact with the same parasites in vivo, had clear detectable parasitological and clinical effects. For the first time, this set-up allowed us to precisely match parasites from each patient, in in vitro conditions, either with the antibody preparations that were clinically effective, or with those non- or insufficiently effective upon the same parasites. This set-up differs from that of former studies where antibodies were from subjects only presumably protected, based on epidemiological and clinical observations, and parasite isolates or strains were only presumably similar to the isolates having infected the antibody donors, but in which no evidence was available that the antibodies studied could be effective in vivo on the parasites tested in vitro.

In the context of the source of parasites and antibodies we studied, and in the in vitro conditions we used, the results strongly support the conclusion that protective antibodies have very limited direct effects upon parasite growth and invasion, but rather, act in cooperation with blood monocytes. Reduction of parasitemia with African adult IgG was consistent in the ADCI assay with each of the patients' isolate, as it was in vivo, while negative or inconsistent results were obtained with the patients' initial (nonprotective) antibodies in the ADCI assay, as well as when using the protective antibodies without effector cells.

In vitro, a direct inhibition exerted by antibodies has been frequently reported when using mAbs and also with sera from immunized animals (22, 23). Studies in which whole human sera have been used are difficult to interpret, as the involvement of non-IgG factors can be suspected (6, 24). Relatively few studies made with IgG from immune subjects are available. In these studies, the prevalence of individuals with blocking IgG was usually low among the subjects studied (25). When present, it was found to be strain restricted (26),

while premunition is apparently an immunity effective against any isolate. Affinity-purified anti-Pf155/RESA antibodies, and some of the sera with high titers of the same antibody, were found effective in blocking merozoite invasion in vitro, though this activity was not consistent in each of the clinically immune subjects (21, 27). Using a highly purified IgG preparation that proved clinically effective, we did not find a significant inhibition of invasion and no inhibition of growth. To the contrary, the PIAG frequently induced (six of eight cases) an increase of growth that reached 100% in one instance, when using NF54 parasites. The reasons for this paradoxical improved growth obtained when adding, in vitro, IgG of a high degree of purity are not clear. This observation is, however, reminiscent of similar phenomena reported with other stages of malaria parasites. An increase in oocysts numbers was repeatedly obtained when mosquitoes were fed gametocytes in the presence of sera containing antigamete antibodies (28), as was an increase in sporozoite numbers recorded in mosquitoes fed gametocytes and ant sporozoite antibodies (29). An increased growth of the asexual blood stages by polyclonal antibodies has also been occasionally reported (25), and more recently, a mAb that enhances merozoite invasion has been described (30). That some particular antibodies may favor parasite survival, rather than block it, is thus already documented. Perhaps more unexpected is the observation that the same antibody preparation that induced parasite clearance in vivo was also responsible for an improved growth in vitro in the absence of effector cells.

As much as the in vitro conditions can be relevant to in vivo situations, these results indicate that the clearance of parasites observed in vivo upon PIAG transfer is most likely not due to a direct effect of antibodies on the parasite asexual cycle, and points toward other modes of action. Anti-Pf155/RESA antibodies were at a higher titer in the donors that were in the receivers, but first, considering the dose of Ig administered, their final concentration in the receivers was very low (in two cases, lower than that of the receivers), and second, they were not found effective in blocking invasion upon the patients strains, at least in our in vitro conditions. Preliminary in vitro studies did not show reversal of parasite adherence to melanoma cells, and found only inconsistent inhibition of adherence (Canques et al., manuscript in preparation). Complement did not appear to play a role in a former study (5), as in this study. Thus, in view of the data collected in vitro, the involvement of effector cells able to cooperate with antibody seemed a likely hypothesis. Evidence in favor of a monocyte-dependent antibody-mediated protection mechanism was first obtained in 1983 (5). This was confirmed in further unpublished and published studies (6), which were also aimed at evaluating the cell types possibly involved. Only blood monocytes were found effective in ADCI, and other cells, such as lymphocytes, platelets, polymorphonuclears, and adherent spleen cells, were not. This antibody-dependent monocyte-mediated inhibitory or cytotoxic effect was confirmed by others (31). In the present study, results from the ADCI assay yielded clear-cut results. There was a direct correlation between the inhibitory effect of a given antibody preparation in this assay and its in vivo potential. Initial antibodies

from patients were consistently ineffective in the ADCI assay, while the African antibodies transferred, and the patients antibodies collected after transfer, were effective. Both initial and recrudescing parasites were susceptible in vitro in ADCI as was the case in vivo with the same Ig preparation. Further in vivo confirmation of the essential role of monocytes appears difficult to achieve, as it would require the in vivo depletion of this cell subset, which, obviously, cannot be envisaged in the natural model.

Based on the correlation found, in the former (5) and in the present study, between the status of acquired protection and the ability of antibodies to cooperate with blood monocytes, we conclude that the ADCI assay can be proposed as one means to select for antigen targets of antibody-mediated mechanisms. Malaria parasite polymorphism is an increasingly evident feature emerging from the characterization of several *P. falciparum* antigens (32). In the seven isolates we studied, this polymorphism was found to concern at least 30% of the polypeptides revealed by the African IgG pool in Western blots performed with extracts from each patient's

isolate (results not shown). It is encouraging for the future of an asexual blood stage vaccine that a single IgG preparation had consistent clinical effects on geographically remote and antigenically diverse parasites. It is also important to stress that no strain restriction was found for the ADCI mechanism in this, as in former studies, where IgG from single immune individuals and other strains of parasites were used (5, 6).

Our results would enable us to conciliate the very old concept of an essentially cellular nature of resistance to malaria, which was based on histo-pathological observations of phagocytes (33, 34), with the relatively more recent evidence (2) for the critical role of antibodies. The relatively rare qualitative differences found in Westerns between antibody specificities present in nonprotected and protected subjects are indicative that only a limited number of critical antibody specificities are responsible for both the in vivo and the in vitro effects. Further investigations on this clinically defined material may give some clues on the basis of acquired protection.

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We thank all participants in the in vivo study: L. Martinache, T. Burnouf, D. Huart (CRTS, Lille), D. Ouattara, M. Saraka, M. Therizol-Ferly (Abidjian), P. Chantanavanich, S. Khusmith, S. Pasuralertsakul, S. Tharavanij (Bangkok), C. Foucault, and J.-J. Fournel (CTS Pitié-Salpêtrière, Paris). We also thank P. H. David for reviewing the manuscript.

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Received for publication 26 April 1990 and in revised form 21 August 1990.

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