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Effector Cells Involved in Nonspecific and Antibody-Dependent Mechanisms Directed against *Plasmodium falciparum* Blood Stages In Vitro

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We have evaluated in in vitro conditions the possible cooperative effect of antimalarial antibodies with several human blood cell types. When used alone, immunoglobulin G from African adults who had reached a state of premunition against malaria was found to have no or very limited direct effect on invasion and multiplication of *P. falciparum* asexual blood stages. In contrast, these antibodies induced a marked specific inhibition of parasite growth in the presence of normal blood monocytes, and the inhibition did not appear to be strain dependent. No similar antibody-dependent cellular inhibitory effect was found using human blood polymorphonuclear leukocytes, lymphocytes, platelets, or adherent spleen cells. However, these cells could all exert in vitro some non-antibody-dependent inhibitory effect when present at high effector/target cell ratios.

In human malaria, relatively few data are available on the effectors of the immune system in subjects who have reached a state of premunition. Nevertheless, the involvement of antibodies has been clearly established in three separate in vivo experiments examining more than 30 subjects: when immune immunoglobulin G (IgG) from protected adults was injected into nonimmune children, *Plasmodium falciparum* parasitemia decreased markedly (10, 12, 19).

Once a cultivation method for *P. falciparum* asexual blood stages was established, it provided a means to modelize under in vitro conditions the mechanisms underlying the observed in vivo effect. Our previous studies in vitro did not indicate that antibodies from protected subjects had any major direct effect on the asexual blood stage cycle. Rather, these studies clearly suggested that IgG cooperates with blood monocytes in an antibody-dependent cellular cytotoxicity (ADCC) mechanism (17).

In the present study, we investigated the possible involvement of other blood and tissue cell types in ADCC by comparing the effects of these cells with those of antibodies alone. Because of the relative fragility of infected erythrocytes (RBC), classical ADCC assays could not be carried out in a reliable way. The cytotoxic effect was in fact measured indirectly by assessing in vitro parasite growth. Therefore, the assay will thereafter be called ADCI (antibody-dependent cellular inhibition).

MATERIALS AND METHODS

Sera. We studied 34 sera which presumably contained protective antibodies since they were obtained from immune African adults. The premunition state of the donors was defined on clinical and epidemiological grounds, namely, continuous exposure to malaria since childhood in the absence of any preventive measures, such as drug prophylaxis, or control measures against mosquitoes.

IgG was prepared from 16 of these sera by ion-exchange chromatography on DEAE-trisacryl (IBF) in Tris hydrochloride (pH 8.2) buffer. The IgG fraction was thereafter dialysed with RPMI, concentrated to the original serum volume, and sterilized on 0.22-μm-pore-size Millex filters. Immunofluorescence was used to check the titer of antimalarial antibodies in the IgG prepared.

Control sera and control IgG were obtained (i) from healthy French blood donors with no history of malaria, and (ii) from *P. falciparum* primary attack cases in French travellers, collected during or shortly after the attack (such individuals have high antibody titers but have no clinical protection against reinfection). Control samples were kept in similar conditions (at −20°C) and for about the same duration as the African sera.

***P. falciparum* growth inhibition assay.** We used two African strains (UPAS and NF 54) and one Thai isolate (FCPS-T23) of *P. falciparum*. The strains were cultured in RPMI medium plus 10% human serum as described (17) and were used within 2 months after thawing of the stabilates. Both NF 54 and FCPS-T23 strains retained their ability to form knob protrusions at the RBC membrane level, as shown by electron microscopic studies (P. Oliaro et al., unpublished data). Only those cultures with a high in vitro rate of proliferation were used in the inhibition assays.

The effects of patient serum, of purified IgG, of cells alone, and of cells and antibodies upon *P. falciparum* growth were assessed in 48-h cultures of *P. falciparum* without medium replacement, performed in 24-well plates containing 0.5 ml of medium per well. Unless otherwise stated, cultures were asynchronous, the initial parasitemia was 0.3 to 0.5%, and the hematocrit was 2%. Patient serum as control serum was added to RPMI medium at a concentration of 10%, and IgG was added at a final concentration corresponding to 10% of its original concentration in the donor serum, with 10% normal culture serum. The number of effector cells in each well is expressed here as a ratio with relation to total RBC and not to infected RBC, since the latter varied during the culture period. We used effector cells at ratios of 1/10 to 1/200 RBC (except for platelets, added at ratios of 1/1 to 1/40 RBC). Each plate included as controls normal human serum (or IgG from normal subjects plus 10% normal serum), patient serum alone (or IgG from patient serum plus 10% normal serum), and effector cells with normal serum (or effector cells with normal IgG plus 10% normal serum).

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All tests and controls were performed in duplicate wells. At the end of the 48-h culture period, *P. falciparum* growth was assessed microscopically on thin smears from each well, stained with Giemsa, and expressed as the percentage of parasitized RBC counted over 10,000 RBC. Microscopic examination allowed us not only to measure the parasite growth but also to study the morphological appearance of the parasites as well as that of the effector cells. The above protocol was occasionally modified as follows: some cultures were of 72-h duration, some were tested using IgG at 20% of its original concentration in the donor serum, and other cultures were synchronized by two successive treatments of the infected RBC with sorbitol at a 4-h interval. For some cultures performed using polymorphonuclear neutrophils (PMN) as effector cells, the cells were renewed every 24 h with fresh PMN from the same donor. With platelets the cultures were kept on a rotating platform at 10 rpm.

Preparation of effector cells for in vitro tests. (i) Monocytes. Mononuclear cells from healthy donors with no history of malaria were separated from heparinized blood by centrifugation in Ficoll-paque solution (Pharmacia, Uppsala, Sweden) according to the technique of Böyum (2). Monocytes were further separated from lymphocytes by adherence to treated plastic dishes. This step was carried out in each well of the plates used to perform the *P. falciparum* growth inhibition assay, as follows. Fibronectin-treated culture wells were obtained by adding fresh plasma from the cells' donor to each well. After washing with RPMI medium without serum, a 1-ml suspension of 10^6 mononuclear cells in RPMI medium plus 10% normal human serum was added to each well. The plates were incubated for 2 h at 37°C in a 5% CO_2–air mixture. Each well was thoroughly washed with RPMI, particularly in the angles of the dish, to remove nonadherent cells. The method permitted recovery of about 1×10^5 to 2×10^5 adherent cells, among which 90 to 95% were monocytes and the remaining were lymphocytes.

Further purification of monocytes were also attempted by treating adherent cells with several monoclonal antibodies directed to lymphocyte markers (OK T3, T11, BL 14, B1) at a final dilution of 1/10, in the presence of complement, for 30 min at 37°C, followed by four washes in RPMI medium.

(ii) Lymphocytes. Peripheral blood lymphocytes were prepared by serial passages of mononuclear cells on three successive fibronectin-treated culture dishes to separate them from adherent cells. This method allowed us to reach a purity of 80 to 90% depending on the level of blood monocytosis of the donor.

By selecting donors with very low counts of blood monocytes and by in vitro depletion of adherent cells as described above, preparations with 94 to 96% purity could be reached. Further purification of lymphocytes was attempted by treating the above preparation with antibodies directed to the monocyte-macrophage lineage (either OK M1 or a monoclonal antibody kindly supplied by L. Edelman) in the presence of complement. Cells were counted in a hemacytometer and added to *P. falciparum* cultures at lymphocyte/RBC ratios ranging from 1/10 to 1/200.

(iii) PMN. PMN were isolated from mononuclear leukocytes on Ficoll gradients as described above. PMN were then partly separated from the RBC pellet by sedimentation for 30 min at 37°C in a high-molecular-weight medium (10 ml of RBC suspension in Hanks solution with 3 ml of a 5% Dextran T-500 solution [Pharmacia Fine Chemicals]). The supernatant was centrifuged at $200 \times g$ for 10 min at 4°C. To the cell pellet was added an 8.3% ammonium chloride solution. After incubation for 10 min at 4°C, the cells were centrifuged at $200 \times g$, washed twice, and suspended in Hanks solution. The purity of PMN obtained by this method was always higher than 95%. Cells were added to *P. falciparum* cultures at PMN/RBC ratios of 1/10 to 1/200.

In some instances PMN prepared the next day from the same donor were added to *P. falciparum* cultures on day 1 for 48-h assays or on day 1 and day 2 for 72-h assays.

In one experiment, PMN were obtained from a patient with a chronic granulomatous disease. Another experiment was performed with PMN from a patient with filariasis, resulting in a PMN preparation containing 60% eosinophils and 35% neutrophils.

(iv) Platelets. Purified platelet suspensions were obtained from healthy blood donors during cytapheresis on a blood cell separator (Fenwall CS 3000). The cells, suspended in plasma with adenine-citrate-dextrose (ACD), were washed once in RPMI buffer, suspended in RPMI plus 10% serum, and added to *P. falciparum* cultures at platelet/RBC ratios of 1/1 to 1/40.

(v) Tissue macrophages. Adherent cells of the monocyte-macrophage lineage were prepared from spleen and liver biopsies of patients with no previous history of malaria (whole spleen or liver fragments obtained from D. Rigault, Hôpital Begin, Saint-Mandé, France, and C. Chigot, Hôpital Salpétrière, Paris). Spleen fragments were gently disrupted with forceps in sterile RPMI. After sedimentation of unseparated fragments, the cells present in the supernatant were washed once and spleen macrophages were prepared by adherence to treated plastic culture dishes as described above for monocytes. The resulting cell preparation contained very large and active macrophages of 40 to 70 μm in diameter, smaller macrophages, and monocytes, but few contaminating lymphocytes and epithelial cells. Therefore, the preparation is referred to as "adherent spleen cells."

Liver fragments were perfused with a collagenase solution as described by Guguen-Guillouzo et al. (13), and adherent liver cells were separated from hepatocytes by adherence as described above for monocytes. A majority of the cells recovered were Kupffer cells; the remaining were hepatocytes, lymphocytes, epithelial cells, monocytes, and other unidentified cell types.

Mode of expression of results and statistical analysis. The inhibition of parasite growth obtained by using either antibodies alone or cells alone was expressed as a percentage of the mean parasitemia from duplicate wells compared with mean values from control wells (control serum without antibodies and cells).

In experiments involving both effector cells and antibodies, a different mean of expression of the results was used since each component could have an individual effect. The ADCI was evaluated by calculating the specific growth-inhibitory index (SGI), which took into account the possible growth inhibition induced by either cells or antibodies alone, used as controls in each experiment, as follows: SGI = 1 − [(% parasitemia with cells and antibodies/% parasitemia with antibodies)/(% parasitemia with cells and control serum/% parasitemia with control serum)].

Student's *t* test was used to compare results from several experiments.

RESULTS

Effects of sera and IgG from immune individuals. When RPMI medium was supplemented with 10% serum from either immune or healthy individuals, large variations were recorded in *P. falciparum* in vitro growth as compared with

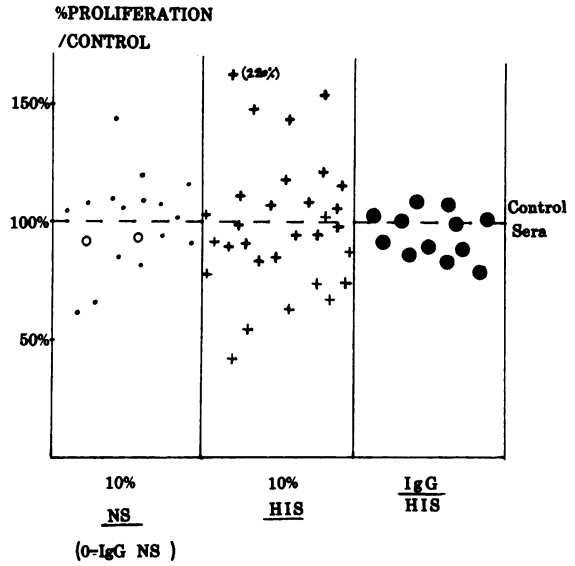

FIG. 1. Direct effect of sera and antibodies. The in vitro growth of *P. falciparum* was measured in the presence of either 10% normal serum from various healthy individuals (NS, ·), 10% serum from African adults (HIS, +), or the IgG fraction from these sera (IgG/HIS, ●). In this figure, the degree of parasite reproductive increase in the presence of tested serum or IgG is expressed as a percentage of that in control wells (containing our standard AB serum culture) in each experiment.

results obtained with the batch of human type AB serum used to maintain the *P. falciparum* lines in vitro (see Fig. 1, in which results are expressed as a percentage of controls).

Sera from healthy individuals sometimes induced an increase of growth, sometimes a decrease, as compared with the control (our standard serum batch), and this effect was sometimes strain dependent (i.e., one serum could inhibit the growth of a given strain but increased the growth of another strain, and the result was reproducible) (data not shown). The same variation in the effect of total serum was observed when sera from African adults were used. Inhibition was only occasionally more pronounced than with sera from healthy individuals (Fig. 1).

When purified IgG from those immune sera that induced a marked inhibition of *P. falciparum* growth at 10% concentration was added at similar concentrations (2 to 3 g/liter) to the standard medium (RPMI plus 10% AB serum), very limited or no inhibitory effect was recorded (Fig. 1). The results obtained with immune IgG therefore suggest that most of the growth inhibition effect recorded using total serum is related to the non-IgG fraction in that serum.

Inhibitory effects of cells. All the cell types we studied were found able, in the presence of normal serum, to inhibit to some extent the in vitro multiplication of *P. falciparum* (Fig. 2). The effect was clearly dependent on the number of cells added to cultures. A marked inhibitory effect was observed when effector cells were present at concentrations greater than 1/40 RBC (or, for platelets, >1/5), which can be considered unrealistically high as compared with the in vivo situation. The degree of inhibition depended also on the duration of the experiment; it was greater in 72-h than in 48-h cultures and was variable from donor to donor, particularly for monocytes and PMN. Cells from patients who had experienced in the previous week a viral infection (i.e., acute respiratory tract infection) had a greater inhibitory effect than did uninfected controls (i.e., total inhibition occurred at the monocyte/RBC ratio of 1/80). Cells with altered respiratory burst functions, such as PMN from a patient with a chronic granulomatous disease, were found to be as inhibitory as those from normal donors, thus confirming previous results (16).

Microscopic examination of 48-h cultures indicated that a percentage of effector cells had been destroyed. The percentage of lysis was greater for platelets and PMN than for monocytes and lymphocytes. Among the remaining healthy cells, phagocytes showed signs of activation, and some of

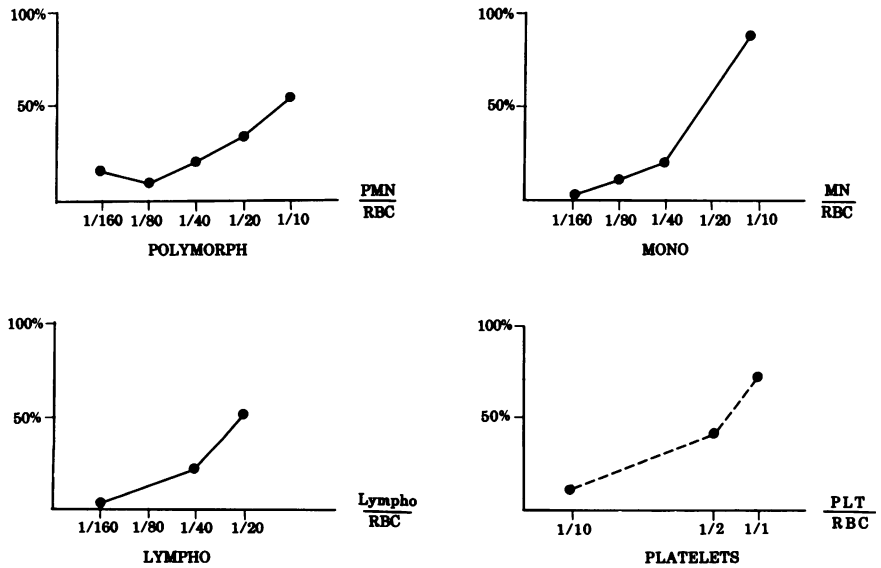

FIG. 2. Inhibition effect of various blood cells. Results are expressed as the percentage of inhibition of *P. falciparum* in vitro growth performed in the presence of 10% normal serum compared with control cultures without cells (pooled data from 5 to 19 experiments depending on the cell type used). Effector cell concentrations are expressed as a ratio to RBC. POLYMORPH, PMN; MONO, MN, monocytes; LYMPHO, lymphocytes; PLT, platelets.

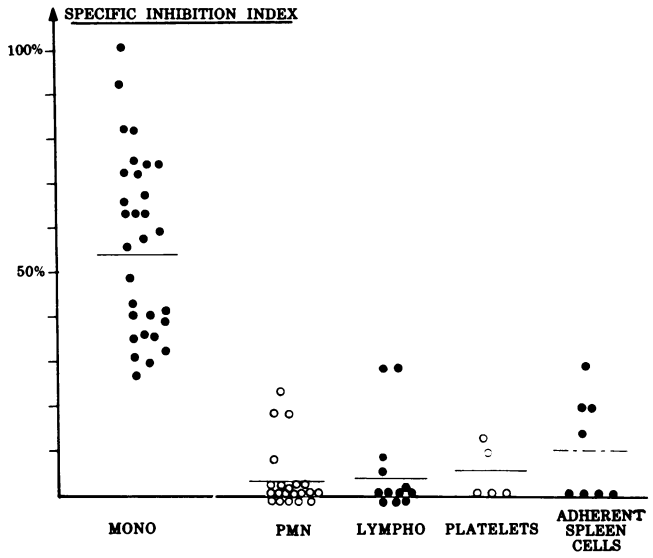

FIG. 3. Results from the ADCI assay performed with various cells. SGI was calculated as described in Materials and Methods in 48-h cultures using cells from normal donors and RPMI plus 10% serum, supplemented with IgG from immune subjects at 10% of the donor plasma concentration. With lymphocytes (LYMPHO) are shown only the results from preparations containing less than 12% monocytes (MONO).

them contained parasite debris (i.e., pigment). At high effector cell/RBC ratios, the few intraerythrocytic parasites which remained at the end of the culture had an altered morphology, many being condensed ring stages devoid of vacuole, resembling the "crisis forms" described by Taliaferro and Cannon (23, 24).

On the basis of the above results, all further experiments aimed at assessing the cooperative effect between cells and antibodies were carried out with effector cell/RBC ratios of 1/80 except for platelets, which were used at a ratio of 1/10.

ADCI effect on *P. falciparum* growth. (i) Monocytes. In medium containing 10% IgG from immune subjects, monocytes from normal individuals were found in each case to be able to induce a specific inhibitory effect greater than that induced by either monocytes or IgG alone. In Fig. 3 are summarized the results from 34 ADCI assays performed with IgG from 16 immune individuals and cells from 12 normal donors in 12 separate experiments (and IgG from normal donors in control wells).

The SGI index ranged from 26 to 100% (Fig. 3). The SGI was influenced by several factors. First, it was generally greater when the starting parasitemia and the multiplication rate of the parasite were high. For example, in one 72-h experiment the starting parasitemia was 1.3% and the final parasitemia was 16% with IgG alone, 20% with monocytes alone, and 3.7% with monocytes and IgG. The resulting SGI was 72%. In contrast, cultures starting at a parasitemia of 0.2 to 0.3% and ending at parasitemia of 1 to 2% resulted in an SGI generally close to or below 50%. The SGI was low when a given monocyte preparation had a nonspecific inhibitory effect, since the resulting specific increase in inhibition due to IgG-cell cooperation was reduced.

In contrast, the SGI did not vary much depending on the parasite line used in experiments performed with cells from the same donor. The *P. falciparum* culture lines used were found to be equally susceptible to the antibody-dependent cytotoxicity of monocytes. This apparent absence of strain specificity in the ADCI effect is in agreement with former experiments (17). Monocyte samples that were 100% pure could not be obtained even by use of lymphospecific monoclonal antibody mixtures. However, for a given IgG preparation the cooperative effect was similar when monocyte preparations containing either 20 or 3% lymphocytes were used.

Most of the parasites remaining at the end of the ADCI experiment were at the ring or trophozoite stage with altered morphological features. Trophozoites had a condensed appearance, with a retracted cytoplasm and no vacuole. These characteristics were not morphologically different from those observed in the absence of antibody, only they occurred at lower cell concentrations. In contrast, Giemsa staining did not visualize any morphological alteration of the few remaining schizonts. At the end of the experiment, about 25 to 35% of monocytes contained pigment and other parasite debris.

Finally, no similar ADCI effect was recorded when the assay was performed using IgG extracted from sera of five *P. falciparum* primary attack cases instead of immune IgG. The SGI varied between −28 and 9% (data not shown).

(ii) PMN. Out of 20 experiments, none showed a significant inhibitory effect resulting from the cooperation of PMN with IgG (summarized in Fig. 3). In most instances the parasitemia reached after 48 and 72 h of culture was identical to that obtained using PMN and control serum or control IgG. Some degree of inhibition was recorded in only 3 of 20 assays, but the SGI was always lower than 30%, and the results were not confirmed by additional assays performed with the same antibodies and the same *P. falciparum* line.

Although the majority of PMN kept normal morphological features up to the end of the culture, their in vitro life span is known to be shorter than that of monocytes. Since the absence of ADCI mediated by PMN could possibly be attributed to the premature death of the cells in vitro, we attempted to circumvent this difficulty by adding, after 24 h of culture, PMN freshly prepared from the same donor. No specific inhibition was recorded in eight assays performed that way.

In one case, PMN were prepared from one donor on 3 consecutive days and added to culture on days 0, 1, and 2. The resulting parasitemia was influenced by the concentration of cells—the nonspecific inhibitory effect reached 40%—but no additional inhibition due to the specific PMN-IgG cooperation was recorded.

Thus, in the conditions of our assay, PMN, in contrast to monocytes, do not seem to cooperate with IgG.

Finally, similar results were obtained with a 60% rich eosinophil preparation, indicating that such cells may not be able to mediate ADCI.

(iii) Lymphocytes. Preliminary experiments using total mononuclear cells resulting from Ficoll separation revealed that this preparation, which contains monocytes and lymphocytes, induces a specific inhibition of *P. falciparum* growth in the presence of antibodies (data not shown).

Using partially purified lymphocyte preparations depleted of adherent cells, a moderate and inconsistent specific inhibition was recorded. At that stage it was observed that the resulting SGI was related to the percentage of monocytes contaminating the lymphocyte suspension; i.e., a preparation containing 85% lymphocytes and 15% monocytes resulted in 12% inhibition, whereas a preparation of 95% lymphocytes and 5% monocytes induced no detectable inhibition.

Attempts to further purify the lymphocyte subset did not reach 100% purity. Lymphocytes containing only a few contaminating cells could be obtained reproducibly by selecting donors with low percentages of monocytes (i.e., less than 60 monocytes per μl) and further depleting the adherent cells.

In Fig. 3 are summarized results obtained using lymphocytes contaminated by less than 12% monocytes (3 to 12%). All SGIs recorded were lower than those obtained with monocytes, and examination of slides did not show any modification of parasite morphology. In contrast, higher SGIs were obtained by using lymphocyte preparations containing up to 40% monocytes; with mononuclear cell preparations containing 5, 10, 20, and 40% monocytes, the resulting SGIs were 8, 19, 24, and 55%, respectively. These results show that the ADCI apparently exerted by some lymphocyte preparations is a function of the percentage of monocytes they contain. These data indicate that human peripheral blood lymphocytes are not able to exert ADCI against *P. falciparum* in vitro.

(iv) Platelets. Using platelets from five different donors at a ratio of 1 to 10 RBC in culture with immune IgG, no significant inhibition of *P. falciparum* growth was found which could be related to a cooperation between platelets and antibodies (Fig. 3). Results were the same when fresh platelets were added at 24 h of culture. Compared with control cultures (platelets and normal IgG), no additional alteration of parasites could be detected.

(v) Adherent spleen cells. The adherent subset from spleen cels of three different donors was used with IgG from three immune African adults. These cells induced no or only moderate antibody-dependent growth inhibition effects (Fig. 3). The mean SGI recorded using these cells was about 5 times lower than that obtained with peripheral blood monocytes. The cell preparations we used contained a majority of large tissue macrophages but also numerous lymphocytes, monocytes, and other cell types poorly defined by morphological criteria.

On the basis of the low or absent inhibition recorded with that cell mixture, the data suggest that spleen macrophages do not cooperate with antibody and that the inconsistent inhibitory effect occasionally observed is likely related to the presence of low numbers of blood monocytes in the preparation used.

Only one preparation of adherent liver cells could be tested. This preparation, containing about 60% Kuppfer cells, induced SGIs of 0, 20, and 30% with IgG from three immune donors (data not shown). The low purity of the Kuppfer cells used prevented us from reaching any clear conclusion about their involvement in the ADCI effect.

DISCUSSION

In three separate in vivo experiments, purified immune IgG was proved to confer protection when passively transferred in humans acutely infected with *P. falciparum* (10, 12, 19).

The present data confirm our previous findings on the low direct effect of antibodies in vitro, namely, that IgG from immune subjects, prepared either by protein A (17) or DEAE fractionation (this study), does not have any direct effect on parasite growth detectable in vitro when used at 10% or even 20% of its in vivo concentration. These results are not in agreement with experiments performed by several other research groups using whole serum or IgG from monkeys or humans (3, 8, 21, 25). Our data suggest that most of the inhibitory effect that was sometimes observed with total serum is related to the non-IgG fraction in those sera. A similar nonimmunoglobulin toxic factor called CFF has been identified previously in sera from East African malarial subjects (14). In contrast, immune IgG from Indonesian subjects was found to have a direct effect in vitro, but so far no antibody with such an effect has been detected in sera from protected African adults (this study; J. B. Jensen, personal communication).

The striking difference between the in vivo and in vitro effects of antibodies led us previously to search for a cooperation between antibodies and effector cells. In a preliminary study the involvement of blood monocytes could be demonstrated (17). In other parasitic diseases, particularly in schistosomiasis, several cells such as eosinophils, macrophages, and platelets were found to be effectors of major defense mechanisms when triggered by specific antibody and target parasites, inducing the release of mediators cytotoxic to parasites (7).

In the present study, we attempted to evaluate the involvement of several circulating cell types. In view of the fragility of the parasitized erythrocyte, which in our opinion precluded the classical ADCC assays, we chosed to evaluate the antibody-cell cooperation by measuring its net effect on the in vitro multiplication rate. This ADCI assay has the additional advantage of assessing both effector cells and parasite morphology.

Of the many blood cell types known to exert ADCC against other pathogens, our in vitro study leads to the conclusion that only monocytes mediate an ADCI effect on *P. falciparum* blood stages. Results obtained with blood monocytes clearly confirm and extend our previous observations (17). Results demonstrate a consistently greater effect of monocyte-IgG cooperation compared with that of other cells or of each component alone. The intensity of the monocyte effect was similar for several different healthy blood donors and did not apparently vary much depending on the strain. These results, which extend to additional strains the strain independency formerly observed, are important to stress in view of the large number of variant antigens identified in malaria parasites (26). Together with former results obtained by ADCI and phagocytosis assays (17, 18; P. Bayard and P. Druilhe, unpublished results), our observations suggest that the target antigen(s) involved in ADCI is not strain specific, or else, if variant antigens are involved, sera from African adults must contain each of the corresponding antibody specificities in sufficient amount.

In the present study we made no attempt to investigate the mode of action of monocytes. The possibility that medium exhaustion could be responsible for the observed effect on parasites was considered, but in our opinion can be excluded for the following reasons. A concentration of 10^5 mononuclear cells per 0.5 ml of medium is not known to be capable of exhausting RPMI medium in 48 h. The same ADCI effect has been recorded in our previous studies (17) in which medium was replaced daily. No similar effect was recorded with the other effector cells we studied, used at the same concentration, nor was it observed with monocytes when using IgG from primary attack cases. The latter cells and sera (with high immunofluorescent-antibody titers and likely high content in immune complexes) are important controls of the monocyte-IgG specific effect.

Our results do not confirm previous reports suggesting that lymphocytes could be involved in ADCI against *P. falciparum* blood stages (4, 5). They clearly show that preparations of whole mononuclear cells cooperate with

antibodies but that monocytes and not lymphocytes are the effector subset in such preparations. On the contrary, the presence of purified lymphocytes induced in many of our experiments a slight increase in parasitemia compared with controls (not shown).

Results obtained using other phagocytes are surprising. Both PMN and adherent spleen cells apparently did not act as monocytes did in our assay. In previous studies using assays other than ADCI, monocytes and PMN behaved similarly. First, the same rate of antibody-mediated merozoite phagocytosis was obtained by using monocytes and PMN in epidemiological studies (Bayard and Druilhe, unpublished data). Second, in a chemiluminescence assay both monocytes and PMN were found to be able to release reactive oxygen intermediates when triggered by merozoites and specific antibodies (F. Lunel, B. Descamps-Latscha, and P. Druilhe, submitted for publication). Furthermore, the chemiluminescence index recorded with PMN was on average 3 times higher than that recorded with monocytes.

It may be argued that the PMN could not be maintained metabolically active over the longer delay required to measure ADCI than to measure chemiluminescence. However, no ADCI effect was observed even when PMN were renewed daily. In addition, in vitro activation of the PMN was suggested by the higher rate of phagocytosis of parasites or parasite debris observed in assays performed with immune IgG than in those with control IgG, and by morphological signs of activation (e.g., enlarged cytoplasm-containing vacuoles).

This discrepancy between the results obtained with PMN and monocytes in chemiluminescence and ADCI assays does not support the hypothesis that reactive oxygen intermediates are the active mediator involved in monocyte-mediated ADCI.

The adherent spleen cell population we used contained several distinct cell types, including many lymphocytes, some blood monocytes, and a majority of large macrophages. Less firm conclusions can be drawn from the small number of experiments performed with these cells. Despite signs of specific activation (e.g., phagocytosis), as for PMN, the adherent spleen cells had a much less obvious ADCI effect than did monocytes.

Platelets have been shown to exert an ADCC effect against schistosomes in vitro and to release reactive oxygen intermediates when specifically triggered (7). This function was found to be retained even in platelets kept for up to 6 days at 4°C in plasma (A. Joseph, personal communication), and several other functions (e.g., on coagulation) are known to be maintained for up to 10 days. We used freshly prepared platelets and attempted to replace them daily. Even in these conditions, no ADCI effect was detected. Thus, unless some unknown function of these cells can disappear faster in in vitro conditions, we conclude that there is no in vitro indication that platelets may have a role to play in the protective mechanisms evidenced by passive transfer of antibodies.

Apart from their involvement in ADCI, we found that monocytes alone can also inhibit the asexual blood phase when present at high concentrations. On the basis of similar antibody-independent inhibitory effects induced by neutrophils or macrophages, several authors have concluded that PMN and monocytes have an important role to play in the defense mechanisms against malaria (6, 15, 16, 20). Our study confirms this in vitro effect but also shows that it can be exerted by a large variety of cells including monocytes, PMN, spleen and liver macrophages, and platelets and lymphocytes. The in vivo relevance of this non-antigen-specific in vitro effect seems to us more questionable than does that of the antibody-dependent effect. For example, it may simply be an in vitro artifact due to the lysis of some cells and the release of proteases or toxic factors. Alternatively, it may be the in vitro evidence of the nonspecific first line of defense which is known to control parasitemia before a specific immune response is raised: during the acute attack in naive individuals, the parasitemia remains in most instances at levels that are relatively low compared to what would result from the theoretical rate of multiplication (×8 to ×16 each 48 h). The gradual acquisition of an effective immune protection thereafter allows an improved control of the parasitic load: in adults from endemic areas parasitemia is maintained at very low levels (11).

The type of alterations induced in intraerythrocytic parasites by cells alone and by antibody-cell cooperation appeared identical when observed by optical microscopy (this study) and by electron microscopy (P. Olliaro et al., submitted for publication). In view of in vitro results and of in vivo observations it can thus be proposed that in nonimmune subjects the direct stimulation of monocytes and of several other cell types represents a first non-antigen-specific barrier which controls to some extent the parasite growth (9) and that in immune subjects the antibody-monocyte cooperation simply increases the effect mediated by monocytes and results in the superior control of parasite load observed in such individuals.

Our study provides additional indications that antibodies in immune individuals would have no or only limited direct effect compared to their involvement in cell-mediated mechanisms.

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