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# Cooperation Between Antibodies and Monocytes that Inhibit In Vitro Proliferation of *Plasmodium falciparum*

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The cooperative effect between nonsensitized monocytes from normal individuals and malarial antibody in depressing parasite multiplication was investigated in an in vitro assay. The addition of purified normal monocytes to *Plasmodium falciparum* cultures in the presence of serum from immune individuals markedly inhibited the proliferation of the parasite in vitro: the parasitemia observed was about 22 times lower than that in the presence of immune serum alone. This cooperative effect was found to be effective over a wide range of monocyte/erythrocyte ratios (1:10 to 1:160) and serum concentrations (1/10 to 1/80). Immunoglobulin G extracted on protein A-Sepharose was as effective as total serum in this system. These data suggest that cooperation between nonsensitized cells and immunoglobulins could be an important effector mechanism against *P. falciparum* parasites in vivo.

The infection of humans by malaria parasites elicits an initial immune response, with specifically sensitized T cells and high titers of antibodies, which is generally unable to control infection. However, in individuals repeatedly infected over several years, a state of incomplete resistance, called premunition, appears very gradually. Despite extensive studies with laboratory models of malaria and, to a lesser extent, with humans, the mechanisms mediating protective immunity remain unknown.

The main experiments from which important information has been gained concerning the effectors of the immune response in humans are those of passive transfer. Immunoglobulin G (IgG) extracted from the serum of naturally immunized individuals caused a dramatic fall in the number of asexual parasites when injected into nonimmune recipients (11, 19). Moreover, this protection seemed to be strain independent (20). However, upon addition of serum from immune individuals to *Plasmodium falciparum* cultures, we found (unpublished data), as have others (3, 23, 29), that antibody only partially decreased the multiplication rate and sometimes increased it, but never stopped in vitro proliferation.

Since immune IgG has proved to be more effective in vivo than in vitro, its effectiveness may be related to the involvement of other components of the immune system, particularly nonsensitized cell populations. Indeed, it has been shown recently (4) that addition of immune serum together with large numbers of lymphocytes (lymphocyte/target ratio = 50:1) inhibits markedly the in vitro proliferation of *P. falciparum*. However, in this experiment the cells were isolated from malaria-sensitized individuals, and all mononuclear cell types, including lymphocytes, monocytes, and some polymorphonuclear leucocytes, were used. On the other hand, we have previously shown, using an in vitro method, that human peripheral blood monocytes are involved in the clearance of the free blood stages of *P. falciparum*, the merozoites, after being armed in vivo (17) or in vitro (16) by cytophilic IgG from immune individuals.

The present study was designed to investigate the effect of cooperation between normal monocytes and malarial antibodies on in vitro proliferation of *P. falciparum* in comparison to the effect of antibodies alone.

## MATERIALS AND METHODS

**Monocytes.** The monocytes used in the inhibition assay were obtained from healthy individuals with no past history of malaria. Mononuclear cells isolated from heparinized blood by use of a high-density Ficoll-Hypaque gradient (2) were separated into two populations based on their ability to adhere to serum-coated plastic dishes (18). After three washes of the dishes with cold culture medium, the cell population adhering to the culture dish consisted of 90 to 95% monocytes, and the remaining cells were lymphocytes and neutrophils. Viability, measured by dye exclusion, ranged from 85 to 95%.

**Sera.** Sera containing malarial antibodies were collected from 10 West African adults who were selected because they had been continuously exposed to malar-

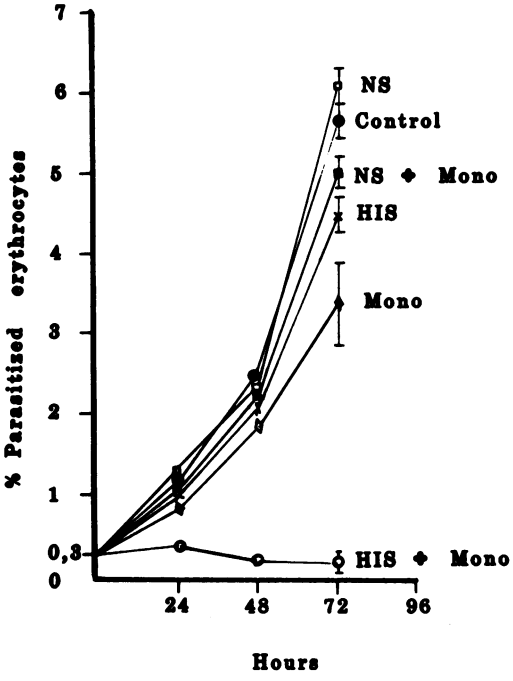


FIG. 1. In vitro proliferation of *P. falciparum* (strain FCM3/FMG) in the presence of various sera or monocytes with sera in 3 days of culture. Control, RPMI 1640 plus 10% human umbilical cord serum; NS, RPMI 1640 plus 10% serum from normal individuals; HIS, RPMI 1640 plus 10% serum from hyperimmune individuals; Mono, monocytes isolated from normal individuals (monocyte/erythrocyte ratio = 1:40). Each point is the mean ± standard deviation of two sera tested in duplicate.

ia and presumably had reached a state of premunition. These subjects had high levels of fluorescent antibodies to malarial antigens and had not recently taken antimalarial drugs. Control sera were obtained from healthy French subjects who had never been in areas endemic for malaria. The effect of immune or normal serum, and of their cooperation with normal monocytes, on the proliferation of *P. falciparum* was assessed in asynchronous cultures of the FCR3/FMG strain maintained in vitro by use of a modification (12) of the method of Trager and Jensen (27).

**Inhibitory test.** The inhibitory tests were performed in flat-bottom 12-well tissue culture plates (Linbro). Each well was filled with 0.7 ml of RPMI 1640 medium supplemented with one of the following: (i) 10% heat-inactivated human umbilical cord serum as a parasite growth control, (ii) 10% of each of the normal sera to be tested (iii) 10% of each of the immune adult sera, or (iv) IgG extracted on protein A-Sepharose CL-4B at 10% of the original IgG concentration in the serum plus 10% heat-inactivated human umbilical cord serum. *P. falciparum*-infected erythrocytes (RBC) were added to obtain an 8% cell suspension with a 0.3% initial parasitemia. In the wells in which the cooperation between antibodies and monocytes was investigated, purified monocytes were added to obtain a monocyte/RBC ratio of 1:40, unless otherwise stated. All tests were performed in duplicate for each serum or serum-monocyte combination tested. The cultures were incubated at 37°C, and the medium was changed every 24 h (using medium supplemented with the same serum used to initiate the culture). The parasite concentration was counted per 20,000 RBC on Giemsa-stained smears made after 0, 24, 48, and 72 h of culture. Another group of tests was performed by the same method except that the culture period used was limited to 24 instead of 72 h.

## RESULTS

In the first experiment (Fig. 1) we compared the effect of purified monocytes from normal individuals, normal serum, serum from immune individuals and combinations of monocytes with serum on inhibition of parasite proliferation in vitro. In 3-day cultures of *P. falciparum*, the parasites proliferated in vitro in the presence of all except one of the combinations tested. The proliferation rates were only slightly lower with immune serum than with normal serum or normal monocytes, and the parasitemia decreased only in the presence of both normal monocytes and immune serum. After 72 h of culture, the parasitemia with this combination was 22 times lower than that with all other components tested.

The second group of experiments was set up to evaluate the effectiveness of various concentrations of immune serum (1/10 to 1/80) and various monocyte/erythrocyte ratios (1:10 to 1:160) (Fig. 2) on parasite proliferation. In 24-h asynchronous cultures of *P. falciparum* and for all concentrations of serum and cells tested, the associative effect of monocytes and immune serum notably inhibited the proliferation of the parasite. The effect was maximum for monocyte ratios of 1:10 to 1:40 (Fig. 2); however, inhibition remained important when fewer cells were used. No striking dose response was noted for the serum concentrations tested. Compared with control proliferations in the presence of monocytes and normal serum, inhibition rates reached 95% in the presence of monocytes and immune serum at 1/10 and 1/20 dilutions, 85% at the 1/40 dilution, and 80% at the 1/80 dilution.

The inhibitory effect of monocytes with immune serum can be attributed to a cooperation between cells and the IgG fraction of the immune serum (Fig. 3). Monocytes with purified IgG were as or more effective in reducing parasite proliferation than were monocytes with the same complete serum. On the other hand, purified IgG without cells was practically not effective. In this case, inhibition was even lower than with the complete serum.

The influence of each component tested on parasite growth varies greatly from one individ-

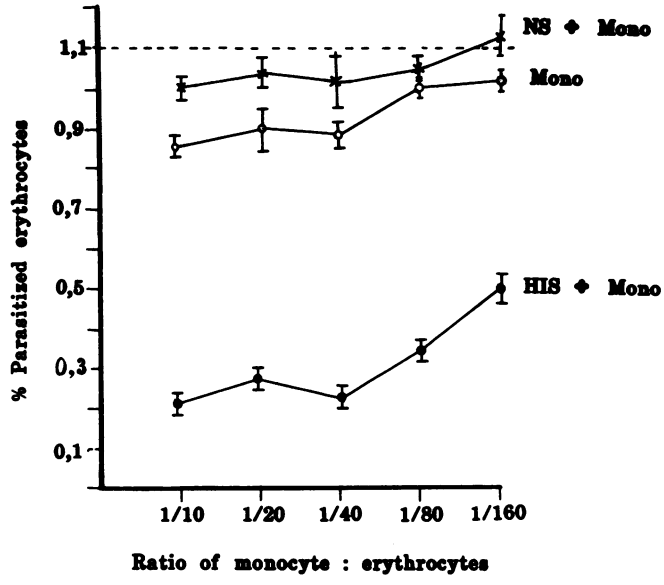


FIG. 2. In vitro proliferation of *P. falciparum* in the presence of various quantities of normal monocytes in RPMI 1640 medium supplemented with either 10% normal serum (NS) or 10% serum from hyperimmune individuals (HIS). The duration of culture was 24 h, and the starting parasitema was 0.3%. Each point is the mean ± standard deviation of two sera tested in duplicate.

ual serum to the other, as can easily be demonstrated when using a larger group of normal or immune sera (Fig. 4). However, the monocyte-IgG system clearly remains the most effective. The presence of individual immune sera resulted in inhibition of proliferation in six cases and an increase of proliferation in four other cases. The simultaneous presence of normal monocytes and immune serum resulted, during the 24-h period of observation, in total inhibition of growth in four cases and 50 to 90% inhibition in six other cases.

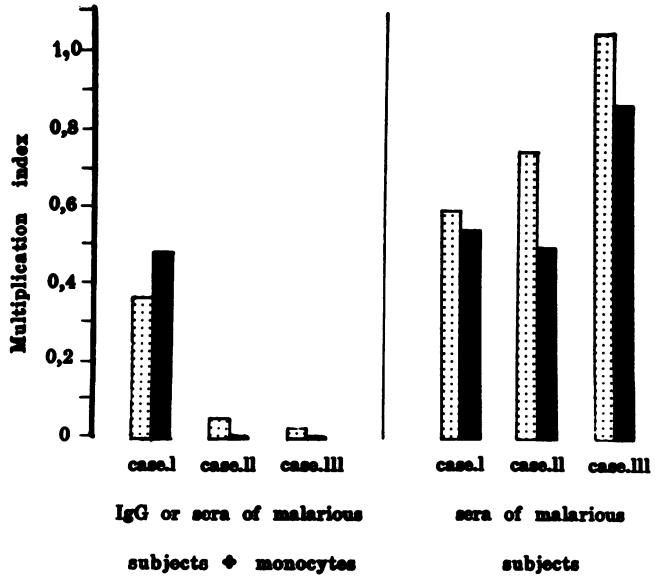


FIG. 3. In vitro multiplication index of *P. falciparum* cultures performed in the presence of 10% serum from hyperimmune subjects ■ or IgG extracted from these sera ▨, alone or combined with normal monocytes (monocyte/erythrocyte ratio = 1:40). The duration of culture was 24 h, and the starting parasitemia was 0.3%.

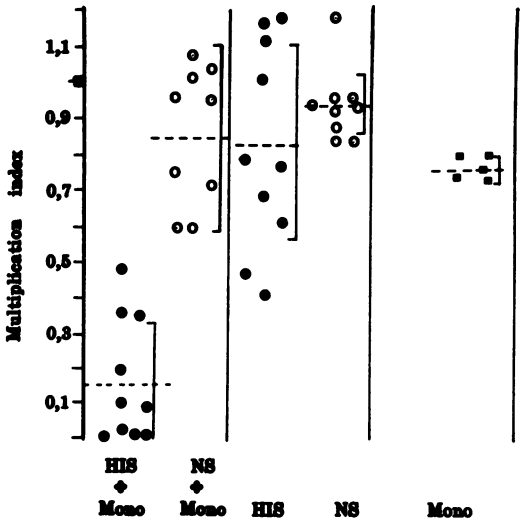


FIG. 4. In vitro multiplication index of *P. falciparum* cultures performed in the presence of various lots of normal monocytes, normal sera, or immune sera. ■, monocytes (Mono) isolated from normal individuals (monocyte/erythrocyte ratio = 1:40); ○, serum from normal individuals (10% in RPMI 1640 medium) or the same serum in the presence of normal monocytes (monocyte/erythrocyte ratio = 1:40); ●, serum from hyperimmune subjects (10% in RPMI 1640 medium) or the same serum in the presence of normal monocytes. The duration of culture was 24 h, and the starting parasitemia was 0.3%. The test was performed in duplicate.

## DISCUSSION

The above-described results demonstrate that moderate variations in the proliferation rate of *P. falciparum* in vitro are induced when serum or cell populations from normal individuals are added to the medium. These variations are also observed when serum from subjects immune to malaria is used. A distinct reduction of parasite replication was only obtained when the antibodies were allowed to cooperate with nonsensitized peripheral monocytes.

The development of cultivation methods for *P. falciparum* has opened new areas of research by allowing the in vitro study of selected components of the immune system when added to parasite cultures. However, the reproducibility of the method remains unsatisfactory, and proliferation rates of the parasite are influenced by a number of nonspecific factors, primarily human serum (15).

When we tested the in vitro effect of serum containing malarial antibodies, we observed that its activity alone was moderate and inconsistent. Only 2 of the 10 sera tested were clearly inhibitory, and on the average the difference between inhibition by normal and immune sera was not statistically significant ($P > 0.6$). There have been a number of studies showing that in vitro proliferation of *P. falciparum* is reduced by the addition of small quantities of serum from *P. falciparum*-immunized Aotus monkeys (5–7, 23). However, this reduction was also observed with serum from normal Aotus monkeys at concentrations higher than 5%. With pooled (22) or individual (3, 23, 29) serum from immune human subjects, inhibition rates, when present, were variable; the majority were in the range of 0 to 50%. For two sera only, rates of over 50% were reported (3). All of the sera tested were from individuals supposedly protected, and it has been proposed that in vitro inhibition by antibodies may vary depending on the strain of parasite used (29). In view of the results presented in this paper, it is also possible that variations in the inhibitory effect of serum are related to the occasional presence of leukocytes contaminating the fresh RBC added for subcultures.

We observed lower inhibition rates with purified IgG alone than with serum, which suggests the possible involvement of nonantibody serum inhibitors such as tumor necrosis factor, endotoxins (10, 26, 28), interferons (13, 14, 24), or simply other immunoglobulin classes. To our knowledge, high levels of in vitro inhibition have been obtained only by using monoclonal antibodies resulting from the artificial immunization of mice (21) and not with naturally occuring antibodies.

The synergistic effect of blood monocytes with immune IgG is clearly demonstrated in the present study. Each of these components had variable and limited effects when tested separately in culture, and their effect when used together was greater than the sum of their separate effects. Moreover, the antibody-dependent activity of monocytes was effective over a wide range of monocytes/target ratios.

The monocytes used in our experiments were isolated from donors nonsensitized to malaria. However, adherence to plastic surfaces and in vitro maintenance lead progressively to activation and dedifferentiation of these cells. In the first group of experiments, over 72 h, many of the monocytes had transformed into macrophages. Therefore, tests of shorter duration were subsequently performed. In any case, both macrophages derived from monocytes and monocytes cultivated not longer than 24 h were ineffective unless serum or IgG was added, even in small quantities. With limitations due to in vitro conditions, these results indicate that cooperation of antibodies with nonsensitized cells is probably a more effective antimalarial mechanism than that of antibodies alone. It is conceivable that in vivo other phagocytic cells such as splenic macrophages, polymorphonuclear leucocytes, and endothelial cells could act

similarly. Mature schizonts are adherent to endothelial cells (25), ensuring a close contact between phagocytes and merozoites when they are released from schizonts.

The mechanisms underlying the antimalarial activity of monocytes and IgG remain speculative. Monocytes can exert an antimicrobial effect through a variety of substances and mechanisms. Their ability to ingest and destroy *P. falciparum* merozoites in vitro has been demonstrated (17) and is dependent on cytophilic IgG (16). A number of free parasites captured in this way will not reinvade RBC, but additional mechanisms should be acting simultaneously. The contact of malarial antigen, i.e., the merozoite surface antigen, with antibodies attached via their Fc region to monocytes and macrophages may trigger the release by these cells of very active soluble mediators capable of killing the intraerythrocytic parasites. This hypothesis is supported by several recent experiments showing the antimalarial activity of monokines such as tumor necrosis factor on both *Plasmodium vinckei* in vivo (9, 10) and *P. falciparum* in vitro (Haidaris et al., unpublished data cited by Allison and Eugui [1]) and of free oxygen radical generators in vivo and in vitro (1, 8).

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