Suppression of Lymphocyte Transformation by Plasma from Owl Monkeys Acutely Infected with *Plasmodium falciparum*

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Plasma collected from owl monkeys during the acute phase of *Plasmodium falciparum* infection was shown to adversely affect several in vitro responses which are considered to be correlates of cell-mediated immune functions of normal monkeys. In the presence of acute-phase plasma, response of normal monkey peripheral blood lymphocytes to stimulation with phytohemagglutinin, concanavalin A, and pokeweed mitogen was severely reduced, as was the ability of peripheral blood lymphocytes to respond to allogenic and xenogenic histocompatible antigens. The transformation response of peripheral blood lymphocytes from normal humans to phytohemagglutinin and concanavalin A was also suppressed. Since acute-phase plasma was not cytotoxic for peripheral blood lymphocytes, decreased responsiveness did not result from cell destruction. Acute-phase plasma appears to block initial steps in lymphocyte transformation.

Malaria is one of the most severe health problems of people living in tropical areas of the world today (6). The disease produces not only high mortality and morbidity during the acute stages of infection, but humans frequently develop signs of decreased immunocompetency after recovery from *Plasmodium falciparum* infections (4, 7). People residing in hyperendemic areas of Africa are reported to have depressed antibody responses to various antigens (8), a lowered incidence of allergies and autoimmune diseases (3, 7), and decreased lymphocyte responsiveness to mitogen stimulation with phytohemagglutinin (PHA) (9). The factor(s) responsible for immune suppression associated with *P. falciparum* malaria is unknown.

Since experimentation on humans with naturally acquired *P. falciparum* malaria is limited, an animal model using the owl monkey, *Aotus trivirgatus*, has been developed. These animals, which are highly susceptible to *P. falciparum*, usually die 2 to 3 weeks after experimental infection.

In an earlier study we monitored changes in the responsiveness of *Aotus* monkey lymphocytes to mitogen stimulation with PHA, concanavalin A (ConA), and pokeweed mitogen (PWM) during the course of *P. falciparum* infection (10a). *Aotus* monkeys with low-grade parasitemias (<10%) showed no significant alterations in cell-mediated immune responses as judged by

mitogen stimulation; however, a significantly depressed response of peripheral blood lymphocytes (PBL) to PHA and ConA stimulation was observed in animals with parasitemias between 25 and 50%. Greater than 50% parasitemias resulted in further suppression, including depression of PWM responses. In the study just mentioned, PBL were cultured in medium supplemented with autologous plasma. Since serum inhibitors of T-cell responsiveness have been reported in several infectious diseases (1, 5), it seemed possible that plasma from acutely infected animals might have a suppressive effect on lymphocyte transformation. Data from several experiments supporting this hypothesis are reported herein.

MATERIALS AND METHODS

Experimental infection. *A. trivirgatus* subsp. *griseimembra* originating from Colombia were maintained at the University of Hawaii Primate Facility for at least 6 months before experimental infection. Each of the six animals was inoculated intravenously with either 0.3×10^6 fresh or 0.5×10^6 cryopreserved erythrocytic stages of an African strain of *P. falciparum* (Uganda-Palo Alto—FUP). When the animals showed signs of terminal illness due to malaria infection, usually 6 to 14 days after challenge (10a), they were tranquilized and humanely killed by exsanguination. The average parasitemia of these animals was 63% (range 48 to 90%) with an average erythrocyte count of $1.2 \times 10^6/\text{mm}^3$. The plasma from each of the six animals was examined for immunosuppressive activity.

In vitro cell-mediated immunity tests. (i) Mitogen stimulation of PBL. PBL from normal *Aotus*

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monkeys were isolated on ficoll-sodium metrizoate (Nygaard and Co.) and incubated under optimal culture conditions. In brief, each culture contained 1.7×10^6 PBL in 0.15 ml of RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum, 15% pooled normal *Aotus* plasma (NAP) or test plasma, 10 mM HEPES buffer (*N*-2-hydroxyethyl piperazine-*N*-2-ethanesulfonic acid), 20 mM L-glutamine, 100 U of penicillin, and 100 μ g of streptomycin per ml. The following concentrations of mitogens in 0.05 ml of RPMI medium were added to each culture; 0.03 μ l of PHA (Difco Laboratories), 0.083 μ g of ConA (Calbiochem), and 1.7 μ l of PWM (Grand Island Biological Co.). After 2 days of incubation, 1.3 μ Ci of tritiated thymidine (specific activity, 40 to 60 Ci/mmol, New England Nuclear) was added, and the cultures were harvested with a semiautomatic cell harvester 18 to 20 h later. Counts per minute were determined in a Packard-Tricarb scintillation counter.

Human PBL isolated from five normal volunteers were stimulated with 0.03 μ l of PHA, 8.3 μ g of ConA, and 1.7 μ l of PWM and incubated in duplicate as described above.

Values are expressed as the log of counts per minute for 10^6 lymphocytes $\pm$ standard error of the mean.

(ii) MLR. The *in vitro* one-way mixed lymphocyte reaction (MLR) was employed to determine if acute-phase plasma (APP) could alter lymphocyte recognition and response to histocompatible antigens (HLA). The procedure described by O'Leary et al. (10) was essentially followed. Stimulator cells were prepared by incubating 2×10^6 *Aotus* PBL with 25 μ g of mitomycin C per ml for 30 min at 37°C. After washing three times with medium, the cells were resuspended and equal numbers of stimulator and responder PBL were mixed together and cultured for 5 days with tritiated thymidine added during the last 18 to 20 h of incubation. The results are expressed as average stimulation ratios, which were determined by dividing the counts per minute in stimulated cultures by counts per minute in unstimulated cultures.

Cultivation of transformed cells. APP was tested for the potential of blocking cell division of human lymphocytes which had already differentiated and were thus transformed. Thus, 2×10^6 Raji cells (transformed human B cells) per ml were cultured in the presence of 15% pooled NAP or APP for 4 to 6 days in minimal essential medium (Grand Island Biological Co.) supplemented with 10% heat-inactivated fetal calf serum. Fractions were periodically removed, and the total number of Raji cells per milliliter and percent viability were determined.

Cell viability. Total cell counts were determined using a standard cell-counting chamber, and analysis of viability was carried out with the trypan blue exclusion test.

Statistical evaluation. Logarithmic means of counts per minute or stimulation ratios were compared, and *P* values were determined by using a standard *t*-test analysis.

RESULTS

Mitogen-stimulated NAP PBL. In this experiment, 15% pooled NAP or APP was added

to mitogen-stimulated cultures of PBL from six normal *Aotus* monkeys. Transformation of normal lymphocytes was significantly suppressed in PHA- ($P < 0.05$), ConA- ($P < 0.01$), and PWM- ($P < 0.05$) stimulated cultures in the presence of APP (Fig. 1).

Since the decreased responsiveness observed above could have resulted from a cytotoxic effect of APP, the viability of normal *Aotus* PBL cultured for 4 to 6 days in the presence of pooled NAP and APP was determined (Fig. 2). As can be seen, the total number of viable cells per milliliter was the same under the two culture conditions. Additional studies revealed that APP was not toxic for human PBL.

Mitogen-stimulated normal human PBL. Since APP suppressed transformation of *Aotus*

FIG. 1. Effect of APP on mitogen-stimulated PBL from normal owl monkeys. Values are expressed as the log of counts per minute for 10^6 lymphocytes (cpm/ 10^6 Lym) $\pm$ standard error of the mean (SEM).

FIG. 2. Effect of APP on the viability of normal *Aotus* monkey and human PBL. PBL from three normal *Aotus* monkeys and three normal humans were cultured in medium supplemented with 15% pooled NAP or APP. The total number of cells per milliliter and the percent viability were determined. The average number of viable cells per milliliter $\pm$ standard deviation are presented.

monkey PBL, it was felt that APP might be able to affect the responsiveness of PBL from other species. To investigate this possibility, PBL from five normal humans were cultured in the presence of 15% pooled human plasma (HP), NAP, and APP. The amount of tritiated thymidine incorporated by human PBL was always lower when the cultures were supplemented with NAP compared with HP ($P < 0.05$); however, no significant difference in the stimulation ratios as observed under the two culture conditions (stimulation ratio for PHA with HP 31 ± 5 , with NAP 32 ± 5 ; ConA HP— 30 ± 5 , NAP— 25 ± 6 ; PWM HP— 25 ± 6 , NAP— 15 ± 4) (Fig. 3). Transformation of human PBL in response to PHA and ConA stimulation was drastically suppressed ($P < 0.001$) in the presence of APP compared to NAP as determined either by counts per minute or stimulation ratio. Although transformation in PWM cultures was lowered by the presence of APP, the decrease was not highly significant.

MLR. Since laboratory-maintained *Aotus* monkeys are imported from various areas throughout Central and South America, they are truly an outbred species with wide HLA differences. Various combinations of allogenic stimulator PBL were cultured with responder PBL from 12 *Aotus* monkeys in culture medium supplemented with 15% pooled APP or NAP. Lymphocyte responses with stimulation ratios greater than 3.0 were considered to represent significant differences in HLA. The MLR was significantly lowered by the presence of APP ($P < 0.01$) (Fig. 4). The response of *Aotus* PBL to

xenogenic stimulator cells (human PBL) was also significantly affected by APP ($P < 0.01$).

Transformed cells. The in vitro growth curves for cultures of Raji cells cultured in the presence of 15% pooled NAP or APP were the same (Fig. 5). Thus APP did not alter the growth of transformed cells.

DISCUSSION

Plasma from *Aotus* monkeys acutely infected with malaria adversely affected various in vitro lymphocyte responses which are considered to

FIG. 4. Effect of APP on the MLR. Results are average stimulation ratios (SR), which were determined by dividing the counts per minute in stimulated by counts per minute in unstimulated cultures.

FIG. 3. Effect of APP on mitogen-stimulated human PBL. Values are expressed as the log of counts per minute for 10^5 lymphocytes (cpm/ 10^5 Lym) $\pm$ standard error of the mean (SEM).

FIG. 5. Growth of Raji cells in the presence of NAP and APP.

be correlates of cell-mediated immune functions of normal monkeys. APP severely reduced mitogen responsiveness to PHA, ConA, and PWM, as well as altered the ability of PBL to respond to allogenic and xenogenic HLA antigens. Since APP was not cytotoxic for PBL even in the presence of complement, decreased responses were not due to cell destruction.

Aotus monkeys acutely infected with *P. falciparum* malaria have been shown to develop decreased responses to mitogen stimulation during the course of infection (10a). When normal *Aotus* PBL were cultured in plasma from these acutely infected animals, normal PBL developed the same degree of suppressed mitogen responsiveness. The transformation response of normal human lymphocytes to mitogen stimulation was also severely altered by the presence of APP.

The specificity of mitogen stimulation for *Aotus* T- and B-lymphocytes has not been ascertained; however, the specificity is probably similar to that for human PBL in that PHA and ConA are primarily T-cell mitogens (2). Results reported herein suggest that a factor in plasma of acutely infected *Aotus* monkeys preferentially suppresses transformation of T-cells. This is substantiated by the results of the MLR, since this reaction is carried out by T-helper cells (2).

APP blocked lymphocyte transformation in response to several stimuli. However, lymphocytes which had completed certain critical steps in differentiation apparently were not affected by APP. This was demonstrated by failure of APP to affect cell division of Raji cells. Thus APP is not acting as a generalized mitotic inhibitor.

The results reported here are similar to those reported for mice and rats with experimental schistosomiasis. For example, Capron et al. (1) demonstrated that a low-molecular-weight compound, released by adult schistosomes, was capable of depressing the response of mouse spleen cells to ConA and lipopolysaccharide stimulation and to allogenic mouse cells. This product was found to be present in plasma of mice after week 2 of *Schistosoma mansoni* infection. The response of spleen cells to schistosome antigens was also suppressed. These results support the hypothesis that adult schistosomes release a product which is both specifically and nonspecifically immunosuppressive.

The mechanism of action of plasma from monkeys with acute malaria on lymphocyte trans-

formation is unknown. Numerous soluble malarial antigens are present in the sera of humans and *Aotus* monkeys during the acute phase of *P. falciparum* malaria (7, 11). Possibly, these soluble malaria antigens or antigen-antibody complexes, or both, play a role in causing altered immune responses in malaria. Soluble antigens could affect lymphocyte transformation either by competing with mitogens or antigens for receptor sites on macrophages or lymphocytes, blocking initial steps in lymphocyte transformation, stimulating suppressor T-cells, inducing soluble mediators, or inhibiting various critical stages of cell-cell interactions. Further studies in this area are warranted.

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