Plasmodium falciparum Induces Apoptosis in Human Mononuclear Cells

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The level of spontaneous apoptosis in short-term lymphocyte cultures was evaluated in different human immunodeficiency virus-negative groups of either healthy control individuals or patients with clinical malaria. The mean percentage of spontaneous apoptosis found in patients during a malaria attack was significantly higher than in sex- and age-matched healthy controls. The healthy asymptomatic individuals were individuals with different degrees of exposure to Plasmodium falciparum as reflected by their various mean levels of specific anti-P. falciparum (immunoglobulin G and M) antibodies. The percentages of apoptotic nuclei were found to be significantly higher in lymphocytes from subjects living in an area where malaria is holoendemic than in lymphocytes from subjects less exposed. Concentrations of soluble plasma interleukin-2 receptor were also higher in subjects from areas where malaria is endemic than in other groups, revealing different levels of lymphocyte activation. Of particular relevance to the in vivo situation, a P. falciparum schizont-rich extract induced a systematic and significant elevation of apoptotic nuclei at day 6 in 87.5% (35 of 40) of the subjects tested. In addition studies with different concentrations of extract, [3H]thymidine incorporation was concomitant with a low or limited level of apoptosis. Taken together, our results strongly suggest that acute as well as chronic asymptomatic P. falciparum infections were consistently associated with a marked increase in the level of mononuclear cell apoptosis. This process could be implicated in some of the alterations reported for the proliferative T-cell responses in areas where malaria is endemic.

Malaria is a disease for which a major activation of the immune system has been demonstrated to occur in subjects living in areas of high endemicity (8). This activation, evidenced by a raised level of soluble plasma interleukin-2 receptor (sIL2R), was suggested to play a major role both in the defense mechanism against the parasite and in pathogenesis (24). In some reports, it was demonstrated that high levels of circulating sIL2R were correlated with low in vitro lymphocyte spontaneous proliferation, suggesting that activated cells might have been withdrawn from the circulation (9, 16). Moreover, drastic changes induced by Plasmodium falciparum in several different parameters of immune function, such as a decreased number of circulating T lymphocytes (36, 37) and in vitro depression of the proliferative response of peripheral blood mononuclear cells (PBMC) to malaria antigens (23, 28), were reported.

Apoptosis is a widely studied mechanism of cell death involved in a large range of pathological as well as physiological events. The general characteristics of apoptosis are well established and occur through distinctive morphological and molecular characteristics, including chromatin condensation, fragmentation of DNA into oligonucleosome-size pieces, swelling, and progressive cell degradation (2, 7). Evidence shows that in most circumstances, apoptosis serves a biologically meaningful, homeostatic function mainly in development and growth regulation. Indeed, apoptosis was described in some situations as a protective mechanism against disease by eliminating unwanted (damaged, precancerous, or excessive) cells (2). But recent work suggested that in some cases, apoptosis may be involved in some pathological dysfunctions and diseases (6). One of the hypotheses put forward was that persistent exposure to activation may lead to immune dysfunction and either loss of ability to respond to an antigen (anergy) or induction of an abnormal program of cell death (1, 13, 19).

Following our initial observation of increased levels of apoptosis during and following a malaria attack (30), we have further investigated the possibility that the phenomenon of apoptosis is associated with some of the perturbations described above. In this study, we first confirm and extend our previous report showing that subjects with acute P. falciparum infection have elevated percentages of in vitro lymphocyte apoptosis in comparison with healthy individuals. These high percentages of apoptosis detected in vitro could persist for at least 1.5 months. The levels of spontaneous apoptosis, i.e., apoptosis detected in short-term cultures of PBMC, from human immunodeficiency virus (HIV)-negative, healthy African individuals living in areas with different malaria transmission levels were evaluated and compared with those of PBMC of healthy Caucasian subjects with limited exposure to malaria. In the present study, we demonstrated that apoptosis levels were significantly correlated with exposure to P. falciparum which was associated with high levels of sIL2R. Finally, the effect of P. falciparum antigens (a schizont-enriched extract obtained after adaptation and cultivation of a local parasite isolate) was also evaluated, and we observed that the parasite was responsible for an increase in the level of in vitro apoptosis of PBMC. These observations are discussed in relation to the different effects potentially induced by the parasite upon the host’s immune system.

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Subjects and blood samples. A total of 26 patients (8 Caucasians and 18 Africans) with a clinically defined *P. falciparum* attack were studied. Most of these patients were hospitalized in the Hôpital Principal de Dakar. Human peripheral blood samples were also obtained from 95 healthy adult donors (controls) during the rainy season. These donors were aged- and sex-matched groups of subjects differing only by the degree of exposure to *P. falciparum*. Parameters such blood formula were controlled and found comparable among all groups. The first group of African subjects (n = 37, mean age ± standard deviation = 35.6 ± 9.6 years) lived in greater Dakar (Senegal, West Africa), which is an area of low and seasonal malaria transmission (33). Two other groups of African subjects originated from two different villages: Dielmo (n = 39.5 ± 20.7 years) and Ndiop (n = 14.8 ± 3.5 ± 13.6 years). These two villages are situated in a rural area of Senegal, 270 km southwest of Dakar. Entomological and parasitological surveys showed that in Dielmo (an area where malaria is holoendemic) there was a high and perennally parasitic transmission, whereas in Ndiop (an area where malaria is mesoendemic), parasite transmission was clearly seasonal (33). Means of 15.6 and 3.2 infective bites per person per month were recorded in Dielmo and Ndiop, respectively, during our study period (1992 to 1993). In Dakar, the mean number of infective bites ranged from 0.382 to 0.014 per person per year, according to a recent study (32). The fourth group was composed of Caucasian subjects (n = 12, mean age of 41.1 ± 9.5 years) who had spent generally a few months in Dakar and had no history of clinical malaria infection.

Venous blood samples were collected in dry-iced tubes to which 250 U of heparin (Liquemin; Roche) was added per 10 ml of blood. The blood samples of the subjects from Dielmo and Ndiop were transferred by road to the Pasteur Institute in Dakar in less than 5 h, using isotherm boxes in order to maintain the temperature between 15 and 25°C. The subject blood was centrifuged for 15 min at 4°C until use. For some samples during the rainy season. With their informed consent, all subjects were tested for the presence of HIV infection, and none was found positive in the cohort enrolled for this study.

Preparation of the *P. falciparum* extract. The Dielmo isolate used in this study was obtained from an inhabitant of Dielmo in November 1991 and successfully cultivated thereafter as previously described (31). *P. falciparum* extracts were prepared by different methods, depending on later use. For the extract used in cellular culture, schizont enrichment was performed by Plasmodiophore (control) (Roger Bellon, Nemours, France) and yielded preparations containing >95% schizont-infected erythrocytes. Both parasitized and control noninfected erythrocytes were washed three times in RPMI 1640 (Gibco Laboratories, Grand Island, N.Y.). After centrifugation, the final pelleted erythrocytes were washed 4 in volumes of distilled water, aliquoted frozen at −70°C until use. The lysate was used in the different tests at a final dilution of 3:2,000, which was found to induce a peak of cellular [H]thymidine incorporation between day 6 and 7. In some experiments, the effects of different dilutions (from 0.5/2,000 to 6/2,000) of the parasite extract on the percentage of detectable apoptotic nuclei and on the level of [H]thymidine incorporation were evaluated in parallel. An extract of another strain of *P. falciparum* (Palo Alto, FUP/66 Marburg strain) was then tested simultaneously with the Dielmo parasite crude extract.

The extract used for quantitation of antimalarial antibodies was prepared by another method. When the parasitemia was around 10%, sorbitol-synchronized cultures which had reached the schizont stage were concentrated by differential centrifugation, and the enriched mature schizont fraction was immediately isolated, aliquoted, and frozen at −70°C until use. The parasite extract was thawed only once, and the extraction procedure was carried out immediately. A *P. falciparum* suspension was concentrated by centrifugation of schizont-infected erythrocytes with 0.1% saponin in 0.06 M NaCl, ultrasonication in the presence of sodium azide (Sigma) (PBS-BSA-Az). The diluted monoclonal antibodies were added to 5 × 10⁵ cells, which were then incubated for 15 min at 4°C. After two washes in PBS-BSA-Az, the supernatant was removed and the cells were incubated with 20 μg of 7AAD per ml for 20 min at 4°C. The cells were centrifuged once, and the supernatant was removed and replaced by a solution of 20 μg of antigen D (Sigma) per ml in PBS-BSA-Az to which was added 1% paraformaldehyde. After overnight incubation at 4°C, the samples were analyzed on a FACSscan flow cytometer.

In several different experiments, the 7AAD staining technique was used simultaneously with the PI staining technique, and the results obtained with the two techniques were highly comparable (Spearman rank correlation coefficient r = 0.893, P = 0.029). Therefore, these two labeling techniques could be independently used to determine and confirmed that B cells as well as the different T-cell subpopulations (CD4+ and CD8+) were potentially affected by the process of apoptosis.
RESULTS

Unusually high levels of spontaneous apoptosis are detected in short-term cultures of lymphocytes from individuals with clinical malaria. Table 1 shows characteristics of the three groups of subjects studied (Caucasians, Africans living in an area with low malaria transmission, and Africans from areas where malaria is holoendemic and mesoendemic, sampled during the dry season. In each group, we analyzed the mean levels of parasite-specific antibodies and the mean levels of parasite-specific antibodies in healthy subjects and in individuals with clinical malaria, Ndiop (mesoendemic) and Dielmo (holoendemic), respectively. The lowest levels were found in subjects from a low-malaria-transmission area (Dakar). The highest mean level of parasite-specific antibodies was found in Africans from areas with parasite-specific antibodies. Second, the mean percent of specific antibodies was significantly lower (P = 0.001) in lymphocytes obtained from Caucasian subjects (mean 6.5%) than in those obtained from the Africans with parasite-specific antibodies. The usual biological parameters were comparable between these different groups of subjects, suggesting that the subjects differed only in the degree of exposure to Plasmodium falciparum.

<table>
<thead>
<tr>
<th>Group of subjects</th>
<th>Clinical status</th>
<th>% Apoptosis at day 3</th>
<th>Specific anti-P. falciparum antibodies optical density [range]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy (Ndiop)</td>
<td>With clinical malaria (Dielmo and Ndiop)</td>
<td>3.8 [7.0–24.0]</td>
<td>0.715 [0.077–1.320]</td>
</tr>
<tr>
<td>Healthy (Ndiop)</td>
<td>Healthy (Dielmo)</td>
<td>5.8 [15.6–35.5]</td>
<td>0.045 [0.013–0.181]</td>
</tr>
<tr>
<td>Healthy (Dielmo)</td>
<td>With clinical malaria (Dielmo and Ndiop)</td>
<td>22.5 [70.0–48.0]</td>
<td>0.106 [0.067–0.336]</td>
</tr>
<tr>
<td>Healthy (Dielmo)</td>
<td>Healthy (Dielmo)</td>
<td>9.1 [14.7–43.0]</td>
<td>0.251 [0.021–1.289]</td>
</tr>
<tr>
<td>Healthy (Ndiop)</td>
<td>With clinical malaria (Dielmo and Ndiop)</td>
<td>29.7 [5.0–9.0]</td>
<td>0.522 [0.030–2.000]</td>
</tr>
<tr>
<td>Healthy (Ndiop)</td>
<td>Healthy (Dielmo)</td>
<td>21.8 [2.9–8.2]</td>
<td>0.575 [0.054–2.016]</td>
</tr>
<tr>
<td>Healthy (Dielmo)</td>
<td>With clinical malaria (Dielmo and Ndiop)</td>
<td>22.1 [5.5–10.0]</td>
<td>0.0001 [0.0002–1.0000]</td>
</tr>
<tr>
<td>Healthy (Dielmo)</td>
<td>Healthy (Dielmo)</td>
<td>6.5 [3.8–5.6]</td>
<td>0.0001 [0.0002–1.0000]</td>
</tr>
<tr>
<td>Healthy (Ndiop)</td>
<td>With clinical malaria (Dielmo and Ndiop)</td>
<td>10.0 [30.0–48.0]</td>
<td>0.0001 [0.0002–1.0000]</td>
</tr>
<tr>
<td>Healthy (Ndiop)</td>
<td>Healthy (Dielmo)</td>
<td>8.2 [2.9–8.2]</td>
<td>0.0001 [0.0002–1.0000]</td>
</tr>
<tr>
<td>Healthy (Dielmo)</td>
<td>With clinical malaria (Dielmo and Ndiop)</td>
<td>5.5 [3.8–5.6]</td>
<td>0.0001 [0.0002–1.0000]</td>
</tr>
<tr>
<td>Healthy (Dielmo)</td>
<td>Healthy (Dielmo)</td>
<td>3.8 [2.9–8.2]</td>
<td>0.0001 [0.0002–1.0000]</td>
</tr>
<tr>
<td>Healthy (Ndiop)</td>
<td>With clinical malaria (Dielmo and Ndiop)</td>
<td>5.6 [12.5–33.2]</td>
<td>0.0001 [0.0002–1.0000]</td>
</tr>
<tr>
<td>Healthy (Ndiop)</td>
<td>Healthy (Dielmo)</td>
<td>2.9 [0.9–8.2]</td>
<td>0.0001 [0.0002–1.0000]</td>
</tr>
<tr>
<td>Healthy (Dielmo)</td>
<td>With clinical malaria (Dielmo and Ndiop)</td>
<td>3.8 [2.9–8.2]</td>
<td>0.0001 [0.0002–1.0000]</td>
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<td>Healthy (Dielmo)</td>
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</tr>
<tr>
<td>Healthy (Ndiop)</td>
<td>With clinical malaria (Dielmo and Ndiop)</td>
<td>5.5 [3.8–5.6]</td>
<td>0.0001 [0.0002–1.0000]</td>
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<td>Healthy (Ndiop)</td>
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<td>Healthy (Dielmo)</td>
<td>With clinical malaria (Dielmo and Ndiop)</td>
<td>5.6 [12.5–33.2]</td>
<td>0.0001 [0.0002–1.0000]</td>
</tr>
</tbody>
</table>
degree of exposure to *P. falciparum* of individuals living in areas differing in endemicity. As a consequence, when considering the relationships between specific antibody levels and the percentage of apoptotic nuclei detected, we found a strong correlation ($r = 0.56$, $P < 0.001$ for IgG; $r = 0.46$, $P < 0.001$ for IgM).

The concentrations of sIL2R were studied in the different groups. An apparent relationship existed between the level of sIL2R and the percentages of apoptotic nuclei in in vitro mononuclear cell cultures ($r = 0.54$, $P = 0.001$) (Fig. 1).

**Influence of the rate of parasite transmission on the mean level of apoptosis and cellular activation in Dielmo and Ndiop.**

As shown in Table 1, the percentages of apoptotic nuclei were comparable during the dry season in Dielmo and Ndiop. This was also the case during the rainy season even if the percentages of detectable apoptotic nuclei were lower (Table 2). During both seasons, the mean percentage of apoptotic cells detected in vitro in lymphocyte cultures of individuals from these areas of endemicity remained significantly higher than that of individuals living in greater Dakar. Therefore, the consistently higher levels of apoptosis detected in lymphocytes from the subjects living in Ndiop and Dielmo were not accidental or transient. This observation of long-lasting, elevated percentage of apoptosis was confirmed when successive blood sampling of different individuals was performed throughout the year (data not shown). Our data did not show any correlation between the percentage of apoptotic nuclei and either the presence or absence of circulating parasites following blood smear analysis at the time of sampling (data not shown).

As shown in Table 2, during the rainy season, which is the season of maximal *P. falciparum* transmission both in Ndiop and Dielmo, no significant difference in the percentage of activated cell nuclei was observed between the two villages. In contrast, during the dry season, which corresponds to an almost complete absence of parasite transmission in Ndiop, whereas transmission is perennial in Dielmo, a significant difference in the percentage of activated cell nuclei was noted between the two villages. The percentage of activated cell nuclei detected was lower in lymphocytes from subjects living in Ndiop than that observed in the rainy season ($P < 0.001$), while in Dielmo, the percentages of activated cell nuclei detected were comparable during the two seasons.

The mean levels of sIL2R were evaluated on different occasions in Dielmo and Ndiop. Irrespective of the transmission season, the mean levels of sIL2R were comparable in the samples collected in the two villages (Table 2). During the dry season, the sIL2R levels were also evaluated in the other groups. They were significantly higher in samples of residents of Dielmo ($95.9 \pm 47.7$ pM, $n = 23$) and Ndiop ($82.1 \pm 39$ pM, $n = 14$) than in samples of individuals with reduced exposure to *P. falciparum* living in greater Dakar, either Africans ($59.2 \pm 43.1$ pM, $n = 31$) or Caucasians ($53.03 \pm 48.3$ pM, $n = 12$).

**Effects of the *P. falciparum* extract on apoptosis levels.** Figure 2A shows that the *P. falciparum* extract induced a slight but significant initial decrease of apoptosis at day 3 in 90% (36 of 40) of the mononuclear cell cultures. This was, in sharp contrast, followed by a subsequent rise in the apoptosis levels.

### TABLE 2. Percentages of apoptotic and activated cell nuclei and levels of sIL2R during different *P. falciparum* transmission seasons

<table>
<thead>
<tr>
<th>Region</th>
<th>Season</th>
<th>Mean no. of infected bites/mo&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Mean % apoptotic nuclei ± SD</th>
<th>Mean % nuclei from activated cells ± SD</th>
<th>Mean concn of sIL2R ± SD (pM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dielmo</td>
<td>Rainy</td>
<td>1.7</td>
<td>$17.5 \pm 4.4$ ($n = 14$)</td>
<td>$2.26 \pm 2.2$ ($n = 14$)</td>
<td>$102.6 \pm 86.7$ ($n = 14$)</td>
</tr>
<tr>
<td></td>
<td>Dry</td>
<td>1.25</td>
<td>$22.1 \pm 5.6^b$ ($n = 32$)</td>
<td>$2.38 \pm 1.9$ ($n = 32$)</td>
<td>$95.9 \pm 47.7$ ($n = 23$)</td>
</tr>
<tr>
<td>Ndiop</td>
<td>Rainy</td>
<td>2.6</td>
<td>$18.7 \pm 5.0^b$ ($n = 42$)</td>
<td>$2.09 \pm 1.02$ ($n = 42$)</td>
<td>$70.8 \pm 30.1$ ($n = 13$)</td>
</tr>
<tr>
<td></td>
<td>Dry</td>
<td>0</td>
<td>$21.8 \pm 5.8$ ($n = 14$)</td>
<td>$1.06 \pm 0.4^b$ ($n = 14$)</td>
<td>$82.1 \pm 39$ ($n = 14$)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean value of the month during which the samples were collected and those of the month before (10a).

<sup>b</sup>A significant difference in values ($P < 0.02$) was found between the two seasons.
observed at day 6 in 87.5% of the lymphocytes tested (35 of 40 cultures) (Fig. 2B), at a time when the parasite-induced [3H]thymidine uptake was maximal. The percentages of apoptosis observed at day 3 as well as at day 6 in cultures in which P. falciparum extract was present were markedly different from those observed in lymphocytes cultures without any exogenous stimulus ($P = 0.001$, Wilcoxon paired test). The effect of a classical stimulus, PPD, was simultaneously studied. At day 3, PPD induced a significant decrease ($P < 0.001$) in the percentages of detectable apoptotic nuclei, and this effect was largely comparable to that found in the presence of parasite antigens (Fig. 3A). In contrast, at day 6, there was no detectable difference between the level of apoptosis observed when the lymphocytes were cultivated alone or when they were cultivated in the presence of PPD (Fig. 3B). Finally, when added to the lymphocyte cultures, control nonparasitized erythrocytes (prepared in exactly the same conditions as the parasitized ones) induced no detectable difference in the percentage of apoptosis compared with that found for the lymphocytes alone. As noninfected erythrocytes were the true controls in our study, this observation led us to assume that the parasite itself was very likely responsible for the induction of apoptosis observed at day 6.

In three independent experiments, extracts of two different P. falciparum strains were added at various concentrations, and simultaneously with the evaluation of the levels of apoptosis, [3H]thymidine incorporation was tested. Figure 4a shows that the lymphocytes of subject A, from Dakar, had low levels of apoptosis and incorporated [3H]thymidine in a dose-dependent manner with each of the two parasite extracts. The lymphocytes of subject B, from Dielmo, showed a very high level of spontaneous apoptosis (Fig. 4b). A decrease of apoptosis levels with different concentrations of the Dielmo parasite extract (extract 1) was associated with an increase in [3H]thymidine incorporation. The lymphocytes of subject C, also from Dielmo, presented always high levels of apoptosis, and no [3H]thymidine incorporation was observed (Fig. 4c). Therefore, in these experiments, occurrence of [3H]thymidine incorporation was concomitant with a low or limited level of apoptosis.

**DISCUSSION**

These results confirmed and extended our initial observation of elevated levels of apoptosis during and following malaria
attacks. Apoptosis was elevated not only in patients suffering from acute malaria infections but also in asymptomatic, healthy. HIV-negative individuals living in areas where malaria is endemic. In areas characterized by a high and permanent rate of parasite transmission, malaria represents a chronic infection wherein the parasite is continuously present following permanent reinfection. Malaria infection was observed to lead to a chronic state of activation (9, 24) but also to a state of anergy (23, 28). One can hypothesize that permanent exposure to the immune system to P. falciparum antigens might induce a deletion of reactive T cells as described for superantigens (17, 18). Our results tended to support such a possibility, as they clearly illustrated that a relationship could exist between P. falciparum exposure and the level of spontaneous apoptosis. Therefore, acute as well as chronic P. falciparum asymptomatic infection was very likely responsible for the induction and/or the amplification of the phenomenon of apoptosis.

The finding that the difference of transmission during the dry season in the two villages studied was not associated with a difference in the levels of detectable apoptotic nuclei was in agreement with our previous observation that malaria infection could have a long-lasting effect (up to several months) on in vitro lymphocyte viability (30). As a consequence, no direct correlation could be found between the level of spontaneous apoptosis detected in vitro and the presence or absence of parasites in the blood of subjects, at least when assessed by blood smears. Moreover, it must be pointed out that negative thick smears did not allow us to completely rule out the possible presence of the parasite at a very low level. In a previous intensive and longitudinal study with daily thick-smear analysis, we showed that during a 4-month period, up to 98% of individuals in Dielmo harbored parasites at least once (33). In addition, when PCR analysis was carried out, it was shown that a number of subjects with parasite-negative blood smears were in fact positive by the PCR technique (14). Therefore, most of the inhabitants of Dielmo and Ndiop probably experienced a permanent asymptomatic parasite infection even if it was not systematically detectable on a single blood film. As a consequence, the potential in vivo incidence of parasite antigens on lymphocytes was probably long lasting if not permanent.

In contrast to what was observed for apoptotic nuclei, significant differences in the percentages of activated cell nuclei were found between the two villages during the dry season. In Ndiop, the clear-cut reduction of malaria transmission observed during this season was associated with a significant decrease of detectable activated cell nuclei compared with what was found during the rainy season. This was not the case in Dielmo, where limited but permanent transmission occurred even during the dry season (33). Therefore, this observation strongly suggested the direct impact of the parasite on mononuclear cell activation, and this hypothesis was strengthened by the positive correlation found between the percentage of apoptotic nuclei and the concentration of sIL2R, a marker of apoptosis (9, 16, 24).

In vitro, the addition of parasite extract to cell cultures consistently affected the level of apoptosis of mononuclear cells. This indicated a direct potential impact of parasite antigens on the in vitro apoptosis because it was not observed either with the control antigen (nonparasitized erythrocytes) or with a ubiquitous control antigen such as PPD. Interestingly, upon exposure to various concentrations of parasite extract, [3H]thymidine incorporation was found when the apoptosis levels were low or decreasing, suggesting that mononuclear cells could either respond to antigenic stimulation by proliferation or be driven to apoptosis.

Taken together, our in vitro observations strongly suggested that parasite-derived antigens could be responsible for the unusually elevated level of apoptosis found in areas where malaria is endemic. It was previously suggested that P. falciparum products could have mitogenic (12) or superantigenic (3) activity, and indeed such activity leads to PBMC apoptosis. There is also the possibility that malaria-associated oxidative stress accounts for the induction of unusual levels of apoptosis (5).

Of notice, the apoptosis percentages detected in lymphocytes from subjects living in areas of endemicity reached the levels of apoptosis found in asymptomatic African HIV-infected subjects living in Senegal (29). A potential consequence of our observation is that the level of in vitro apoptosis, which is considered by some authors as a marker associated with the progressive evolution of HIV infection, should be evaluated with particular caution in areas of endemicity where basic apoptosis levels can already be consistently increased.

This study strongly suggested that, in parallel to viral and bacterial infections (20, 34, 38) and as recently reported for experimental Chagas’ disease (10), a parasite infection such as malaria is very likely to induce a significant long-lasting increase in spontaneous apoptosis levels. This phenomenon could be essential in maintaining a normal balance in the number and the density of renewing cell populations and in the regulation of the polyclonal activation observed in malaria. Apoptosis could also participate in the resolution of the parasite-induced acute inflammation as evoked in other diseases (15, 26). This finding raised the question of the various consequences of such a mechanism both in vivo and in vitro and, in particular, the validity of in vitro proliferative assays measured by [3H]thymidine uptake and widely used in areas of endemicity to detect specific responses to P. falciparum antigens. We previously showed that different lymphocyte subpopulations were involved (30). But among these cells, it remains to determine if the mononuclear cells undergoing apoptosis belong to a particular group of sensitized and/or reactive cells eliminated by the parasite, thus enabling the pathogen to establish itself in the host. If this is the case, then parasite-induced apoptosis could participate in reducing the immune response directed toward critical antigens by increasing the fragility of potential cellular effectors. Complementary studies are being carried out to determine if defined antigens such as parasite-derived synthetic peptides can induce apoptosis in lymphocytes cultured in vitro. This analysis could have considerable impact on the final choice of molecules to include in a vaccine strategy.

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