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were correlated with low in vitro lymphocyte spontaneous proliferation, suggesting that activated cells might have been withdrawn from the circulation (9, 16).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 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 controls 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 additional studies with different concentrations of extract, [3H]thymidine incorporation was concurrent 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.
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Materials and Methods

Subjects and blood samples. A total of 26 patients (8 Caucasians and 18 Africans) who were 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 (control groups) sampled during the dry season. Donors were age- 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 the groups. The first group of African subjects (n = 37, mean age ≤ standard deviation = 35.6 ± 9.6 years) lived in greater Dakar (Senegalese, West Africa), which is an area of low and seasonal malaria transmission (33). In Dielmo and Ndiop, 3.2 infective bites per person per month were recorded in Dielmo and Ndiop (mean age ≤ standard deviation = 35.3 ± 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 living endemic) there was a high and perennial parasite transmission, whereas in Ndiop (an area where malaria is mesoendemic), parasite transmission was clearly seasonal (33). Means of 15.6 and 13.2 infective bites per person per month were recorded in Dielmo and Ndiop, respectively, during our study period (1992 to 1995). In Dakar, the mean number of infective bites ranged from 3.82 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 ± 9.3 years) who had spent generally few months in Dakar and had no history of clinical malaria infection.

Venous blood samples were collected in heparin-coated tubes to which 250 U/L of heparin (Liquifluor Rodhe) 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 isothex boxes in order to maintain the temperature at 0°C and 23°C. The subjects of the other groups were processed directly in the Pasteur Institute facilities. We determined that the levels of apoptosis of lymphocytes from the subjects living in the two rural areas where malaria is living endemic were higher than those of we had also examined from the blood samples, to Dakar. For this control, the blood samples from different volunteers were taken in Dielmo or Ndiop and then transferred to Dakar. The same volunteers came to Dakar on the same day, and they were sampled a second time. When transferred and freshly drawn blood samples were subsequently treated simultaneously, the percentages of apoptosis detected in each paired samples had systematically comparable values.

We also sampled 56 subjects from areas where malaria is endemic (14 subjects from Dielmo and 42 subjects from Ndiop) during the rainy season to evaluate the influence of the rate of parasitemia transmission. 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 Plassmegal (Laboratoire Roger Bellon, Neuilly sur Seine, France) sedentarisation, yields preparations containing >5% schizont-infected erythrocytes. Both parализed and control noninfected erythrocytes were washed three times in RPMI 1640 (Gibco Laboratories, Grand Island, N.Y.). After centrifugation, the final pellet was re-suspended in 4 volumes of distilled water, aliquoted, and frozen at −70°C until use. The lysate was used in the different tests at a final dilution of 32,000, which was found to induce a peak of cellular P3'-5'-nucleotidase incorporation between day 6 and 7. In some experiments, the effects of different dilutions from 0.52,000 to 62,000 of the parasite extract on the percentage of detectable apoptotic nuclei were studied. For the extraction of flat-bottom polystyrene microtiter plates (Nunclon; Nunc, Roskilde, Denmark), and the plates were incubated at 4°C overnight. After the plates were washed five times with phosphate-buffered saline (PBS)-1% Tween, 20 μl of 1% bovine serum albumin (BSA; Sigma, Chemical Company, St. Louis, Mo.) in PBS was added to each well, and the plates were incubated for 1 h at 37°C to block the antigen-free surface of the wells. Thereafter, the diluted (1/200) plasma samples were added to the wells (200 μl per well) and incubated at 37°C for 2 h. Each plasma sample was tested in duplicate either in antigen-coated or control erythrocyte-coated wells for IgM and total IgG. After being washed, the plates were incubated for 1 h at 37°C with biotinylated F(ab')2 fragments to human IgG Fe (α IgG,000) or human IgM Fe (α IgM,000) (Cappel, Organon Teknika Corporation, West Chester, Pa.).

Cell preparation and cultures. PBMC were isolated from heparinized whole blood by Histopaque-1077 density gradient (Sang Touch, Dielmo, St. Louis, Mo.) and washed three times in PBS. Cultures were prepared in flat-bottom 24-well Nunclon plates at a concentration of 106 cells per ml in culture medium containing RPMI 1640 supplemented with 10% (vol/vol) heat-inactivated pooled human AB serum (CNTS, France), 1 mM glutamine, 35 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 1% sodium pyruvate, 2 g of sodium bicarbonate per liter, and 10 ng of gentamycine per liter. The plates were then incubated at 37°C in a water-saturated atmosphere containing 5% CO2. The percentages of apoptotic nuclei were determined 72 h after the initiation of the culture, a delay chosen after preliminary experiments. When the effect of parasitic antigens was tested, 10 μl of the mixture-enriched parasitic extract was added to the cell suspensions, and the apoptosis levels were evaluated both at day 3 and day 6. Systematic control cultures consisted of nonparasitized erythrocytes treated exactly in the same way as the parasitized erythrocytes. To compare the effect of the P. falciparum antigen with a control stimulus, we studied in parallel the effect on apoptosis levels of purified protein derivative (PPD), used at a final concentration of 2.5 μg/ml. When lymphocyte proliferative responses were studied, culture medium RPMI 1640 containing 96-well round-bottom Nunclon plates at a concentration of 106 cells per ml in a total volume of 200 μl [3H]thymidine (1 μCi per well) was added after 6 days of culture, 16 h before harvesting, and incorporation of radioactivity was measured by liquid scintillation counting.

Quantification of apoptosis. (i) PI staining technique. Apoptosis was quantified by staining nuclei with propidium iodide (PI; Sigma) and analyzing fluorescence with a FACScan (Becton Dickinson, Immunocytometry Systems, San Jose, Calif.) as originally described by Nicoletti et al. (22) on thymocytes and as adapted by Gougon et al. (11) on human cells. Briefly, following either 72 or 6 days of incubation, the cells were collected after centrifugation at 200 g for 10 min. The pellet was gently resuspended in 1.5 ml of a hypotonic fluorochrome solution of PI: 50 μg of PI per ml was diluted in 0.1% sodium citrate with 0.1% Triton X-100 (Sigma). The suspension was incubated overnight at 4°C and analyzed with a FACScan flow cytometer to determine the PI fluorescence of individual nuclei. Cytometric analysis of the red fluorescence channel allows to distinguish the different types of nuclei expressed as the percentage of gated nuclei in each region. Apoptotic nuclei appeared as a broad hypodiploid DNA peak that was easily discriminated from the narrow peak of nuclei with normal (diploid) DNA content and from nuclei originating from activated cells with hyperdiploid DNA content.

(ii) 7AAD staining technique. In some experiments, another technique of quantification of apoptotic cells was used. This technique consisted of staining the cells with 7-amino-acidocyanin D (7AAD; Sigma) for discrimination between live and early apoptotic cells. The advantage of the latter method is that it is the spectral characteristics of 7AAD and confirm the combination of this DNA dye with fluorescein isothiocyanate and phycoerythrin cell surface staining. This technique was performed as described by Schmidt et al. (25). In short, each of the fluorescein isothiocyanate- or phycoerythrin-stained monoclonal antibodies (Becton Dickinson) was diluted at 1/100 to a final concentration of 3 μg per ml. A 1% 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 washing in PBS-BSA-Az, the supernatant was removed and replaced by a 20 μg of 7-AAD 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 acridine 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 FACScan 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 ρ = 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.

Evaluation of IL2R. IL2R was evaluated by ELISA, using an Immunotech IL2R kit (Immunotech S.A., Luminy, Marseille, France) according to the manufacturer's instructions.

Statistical analysis. Nonparametric Kruskal-Wallis and Mann-Whitney tests were used to compare the continuous variables between the different groups. Correlation analysis was performed using the Spearman rank correlation test. The following results were collected and calculated to perform the analysis: results obtained for each individual at days 3 and day 6, the Wilcoxon nonparametric paired test was used. P value of <0.05 was considered significant.
RESULTS

Unusually high levels of spontaneous apoptosis are detected in short-term cultures of lymphocytes from individuals with clinical malaria and from healthy sensitized asymptomatic subjects. Table 1 shows characteristics of the three groups of subjects studied (Caucasians, Africans living in an area with low malaria transmission [Dakar], and Africans from areas where malaria is holoendemic [Dielmo] and mesoendemic [Ndiop]) sampled during the dry season. In each group, we analyzed the percentages of apoptosis and the levels of specific anti-P. falciparum antibodies in healthy subjects and in individuals with clinical malaria.

When comparing healthy subjects with those with clinical malaria, we found that the mean percentages of spontaneous apoptosis determined at day 3 (i.e., the percentage of apoptotic nuclei found after 3 days of lymphocyte culture without any exogenous stimulus) were higher in short-term lymphocyte cultures from individuals with malaria than in those of the corresponding matched healthy HIV-negative controls (P < 0.001). It is notable that the level of apoptosis was evaluated in some cases ex vivo (i.e., just after mononuclear cell isolation), and high levels of apoptosis (up to 16% of apoptotic nuclei) were already detectable in patients with malaria. Such a marked increase of spontaneous apoptosis was long lasting and was detectable for up to 6 weeks.

When considering the asymptomatic subjects of the different groups, we found very different mean levels of specific anti-P. falciparum antibodies, which reflected the differences in malaria transmission. The highest mean levels of parasite-specific antibodies were found in subjects from areas where malaria is holoendemic (Dielmo) and mesoendemic (Ndiop), while lower mean levels were observed in subjects from a low-malaria-transmission area (Dakar). The lowest levels were found in Caucasians. Concurrently, we analyzed the mean levels of spontaneous apoptosis in the different groups of asymptomatic subjects. The following observations confirmed that the parasite infection was very likely potentially associated with elevated levels of apoptosis. First, following 3 days of in vitro culture, the mean percentage of spontaneous apoptosis was lower (P \leq 0.001) in lymphocytes obtained from Caucasian subjects (mean ± standard deviation = 8.2% ± 2.9%) with low-level or no detectable specific antibodies than in lymphocytes originating from any of the sensitized African subjects with parasite-specific antibodies. Second, the mean percentages of apoptosis of lymphocytes obtained from African subjects living in the two villages located in areas with high malaria endemicity, Ndiop (mesoendemic) and Dielmo (holoendemic), were the most elevated (21.4% ± 5.5%). Third, there was no detectable difference between the mean percentage of apoptosis found in Dielmo (22.1% ± 5.6%) and that found in Ndiop (21.8% ± 5.8%) in the lymphocytes from individuals with comparable levels of specific antibodies. Fourth, these mean levels of apoptosis were, in contrast, significantly higher (P \leq 0.0001) than the mean percentage of apoptosis found in lymphocyte cultures obtained from age- and sex-matched healthy African subjects living in greater Dakar (13.7% ± 3.8%), an area of comparatively low and seasonal transmission where malaria is hypoendemic, as reflected by a low mean level of malaria-specific antibodies. The usual biological parameters such as hemoglobin phenotype or blood formula were largely comparable between these different groups of subjects, suggesting that the subjects differed only in the degree of exposure to P. falciparum.

Therefore, there existed an apparent potential relationship between the level of detectable spontaneous apoptosis and the...
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).

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 of subjects from 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 \((95.9 \pm 47.7 \text{ pM, } n = 23)\) and Ndiop \((82.1 \pm 39 \text{ pM, } n = 14)\) than in samples of individuals with reduced exposure to *P. falciparum* living in greater Dakar, either Africans \((59.2 \pm 43.1 \text{ pM, } n = 31)\) or Caucasians \((53.03 \pm 48.3 \text{ pM, } n = 12)\).

### 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*</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 ± 4.4 ((n = 14))</td>
<td>2.26 ± 2.2 ((n = 14))</td>
<td>102.6 ± 86.7 ((n = 14))</td>
</tr>
<tr>
<td></td>
<td>Dry</td>
<td>1.25</td>
<td>22.1 ± 5.6 ((n = 32))</td>
<td>2.38 ± 1.9 ((n = 32))</td>
<td>95.9 ± 47.7 ((n = 23))</td>
</tr>
<tr>
<td>Ndiop</td>
<td>Rainy</td>
<td>2.6</td>
<td>18.7 ± 5.07 ((n = 42))</td>
<td>2.09 ± 1.02 ((n = 42))</td>
<td>70.8 ± 30.1 ((n = 13))</td>
</tr>
<tr>
<td></td>
<td>Dry</td>
<td>0</td>
<td>21.8 ± 5.8 ((n = 14))</td>
<td>1.06 ± 0.46 ((n = 14))</td>
<td>82.1 ± 39 ((n = 14))</td>
</tr>
</tbody>
</table>

* 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 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.

**FIG. 4.** Effects of different concentrations of two different parasite extracts on the percentages of apoptotic nuclei and the [3H]thymidine uptake by lymphocytes. Extract 1 was prepared from the Dielmo parasite isolate, and extract 2 was prepared from a Palo Alto (FUP/06 Marburg) strain. The subject A is from Dakar, while the subjects B and C are from Dielmo. The lymphocytes of subject A showed [3H]thymidine uptake in the presence of the two extracts, the lymphocytes of the subject B were reactive only with extract 2, and none of the extracts induced [3H]thymidine incorporation the lymphocytes of subject C.
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 energy (23, 28). One can hypothesize that permanent exposure of 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 particular group of sensitized and/or reactive cells eliminated 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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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 

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\text{[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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