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Letter to the Editor

What Is the Cause of Lymphopenia in Malaria?

Two recent reports conclude that Fas-induced apoptosis plays an important role in the lymphopenia of *Plasmodium falciparum* malaria in humans (5) and in *Plasmodium coatneyi*-infected macaques (6). In both studies, the authors base their conclusion on concomitant findings of increased levels of soluble Fas ligand in serum and lymphopenia. An important element in their arguments is the finding of spontaneous ex vivo T-cell apoptosis in *P. falciparum* patients from Senegal (1, 7). However, to our knowledge no other reports exist supporting the hypothesis that high levels of apoptotic cells in the peripheral blood are a general feature of *P. falciparum* malaria. We have never been able to detect significant increases in the proportion of peripheral T cells showing evidence of apoptosis in several studies of many malaria patients in Ghana, except once in a moribund cerebral malaria patient (our unpublished data). Indeed, Matsumoto et al. could detect apoptotic peripheral blood mononuclear cells (not necessarily T cells) in moribund animals only (6), in line with our findings but at variance with the Senegalese data where apoptotic cells were readily detected even in asymptomatic parasite carriers (7).

Lymphopenia is a well-established feature of *P. falciparum* malaria but is replaced by lymphocytosis in a matter of a few days after initiation of drug therapy, before gradually normalizing over the next couple of weeks (4). How this can occur if the lymphopenia is the result of widespread apoptosis is difficult to imagine. Rather, we have proposed that the initial lymphopenia reflects disease-induced reallocation of T cells to sites of inflammation (2), followed by reemergence of such cells upon cure (4). Although apoptosis appears not to be involved in these changes, it can be speculated that the eventual return to predisease homeostasis is mediated by apoptosis of excess "battle-worn" T cells (Fig. 1). Whether such postmalaria apoptosis occurs remains uncertain, let alone whether it is detectable in the peripheral circulation and is mediated through Fas. Although the papers by Kern et al. (5) and Matsumoto et al. (6) provide evidence of both lymphopenia and increases in soluble Fas ligand, both fail to demonstrate any evidence that one is the consequence of the other. Until such evidence becomes available, we feel that reallocation/reemergence remains the most plausible explanation of the observed lymphopenia in malaria.

This hypothesis is supported by a number of independent studies, most recently by an investigation of peripheral and splenic cellular changes in *P. chabaudi*-infected mice (3).

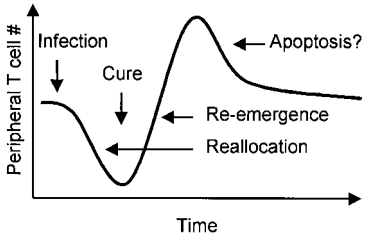

FIG. 1. Schematic diagram of perturbations in numbers of peripheral T cells following *Plasmodium* infection.

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Authors' Replies

(i) Kern and Wellinghausen. The marked decrease in the number of circulating lymphocytes, particularly of the T-cell population, in acute human malaria has stimulated research to explain this finding. On the basis of previous studies in Ghana, Hviid et al. (3) propose a model to explain the perturbations in numbers of peripheral T-cells following *Plasmodium* infection. According to their hypothesis apoptotic events occur soon after drug treatment and cure. Lymphopenia during parasitemia before treatment is explained by reallocation of lymphocytes to sites of inflammation. Formerly depressed lymphocyte counts in nonimmune individuals increase after specific antiparasitic treatment by a factor of 2 to 3 within 7 days (P. Kern, K. Braker, and C. Hemmer, unpublished observation), which supports the hypothesis of reemergence and redistribution of lymphocytes.

In our recent concise report (4), we investigated the kinetics of the apoptotic marker soluble Fas ligand (sFasL, CD95 ligand) during acute falciparum malaria of nonimmune adults. These data should add further knowledge on the pathogenesis of the observed T-cell lymphopenia in acute malaria. FasL is extremely elevated at the time of diagnosis and decreases during antiparasitic treatment, suggesting an association with T-cell lymphopenia. As clearly indicated, we have not analyzed T cells for expression of Fas receptor (CD95 receptor) or investigated the turn over and the cellular expression of Fas. Our findings were quite unexpected and led us to speculate about alternative explanations for the T-cell lymphopenia. We pro-

pose that FasL-mediated apoptosis of lymphocytes might play a role in the lymphopenia of acute malaria, although a direct association can, of course, not be shown. Our suggestions are supported by animal experiments provided by Matsumoto et al., who observed similar changes in their well-elaborated animal model (5). In contrast to Balde et al. (1) and Matsumoto et al. (5), who showed evidence of apoptosis in the whole population of mononuclear cells only, we found statistically significant association of the sFasL level in serum with T-cell count but not with B-cell or monocyte count. Since T cells play a predominant role in Fas/FasL-mediated mechanisms, a putative association is further stressed. However, further details about possible mechanisms of interaction and their relevance are clearly needed. As discussed in our report (4), the soluble "death" ligand FasL not only acts on the cell-bound "death" receptor Fas but also can inhibit the interaction of cell-bound FasL with Fas receptor and, thus, may even have adverse functions.

The reallocation hypothesis does not contradict the fact that signalling through FasL/Fas in early infection leads to active apoptotis and thus to a decrease of lymphocytes. It might be speculated that the elimination of T lymphocytes to apoptosis is instrumental in dampening the immune response, which is clinically reflected by profound immunosuppression, i.e., oral thrush and labial herpes, etc. We believe that reallocation and apoptosis occur in parallel. To achieve lymphocyte homoeostasis during human malaria a balance between proapoptotic and antiapoptotic mechanisms may be assumed, comparable to the situation in other infectious diseases, such as human immunodeficiency virus infection (2).

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(ii) Matsumoto et al. Peripheral T lymphopenia is a well-established feature of patients with falciparum malaria. Although its cause remains unclear, reallocation of activated T cells has been considered a likely explanation of it (3, 7, 8). On the other hand, recent studies have shown that apoptosis in the periphery increases in malaria (1, 11), as reported for other infectious diseases (2, 4, 6, 12). Following these reports, a series of studies have demonstrated that malaria-induced T-cell apoptosis might be caused by a Fas-mediated event (5, 9), including our recent study using two species of macaques with different susceptibilities to *Plasmodium coatneyi* (10). Fas-induced apoptosis thus appears to be involved in T lymphopenia during malaria infection.

As pointed out by Hviid and Kemp, there is thus far no direct evidence that T lymphopenia is the consequence of malaria-induced Fas expression on T cells and the increase in the soluble Fas ligand (sFasL) level in serum in our macaque model. However, we still consider it likely that Fas-mediated T-cell apoptosis is involved in T lymphopenia during malaria infection. As shown in our recent study (10), T lymphopenia together with the rapid and remarkable increase of sFasL, DNA fragmentation in peripheral blood mononuclear cells, and decrease in Fas-positive T-cell level could be demonstrated only in susceptible macaques (Japanese macaques) and not in resistant macaques (cynomolgus macaques). The hypothesis of Fas-mediated apoptosis is the most adequate explanation of T lymphopenia in *P. coatneyi*-infected Japanese macaques.

It seems likely that the cause of T lymphopenia in malaria is not singular but multiple and that the major causes differ from each other, since malaria infection induces a wide variety of host reactions in each host-parasite system. This view is consistent with the fact that studies on malaria-associated T lymphopenia have shown variable results, leading to different interpretations of this phenomenon. We speculate further that the major cause of T lymphopenia changes during the course of infection even in the same host. In the early phase of disease, T lymphopenia is mainly due to reallocation of activated T cells, but with progress of the disease, Fas-mediated apoptosis plays an increasingly important role in T lymphopenia. This hypothesis agrees with the observations that the degree of T lymphopenia correlates with disease severity (7) and that apoptotic cells are, as mentioned by Hviid and Kemp, difficult to detect except in moribund patients.

In order to demonstrate this hypothesis, we have to clarify the trigger of T-cell activation as well as the source of sFasL. Malaria attack stimulates the immune system in the early phase of disease, resulting in the activation of T cells. This activation augments Fas expression and FasL production in activated T cells and/or other FasL-producing cells. We have already demonstrated that serum samples from *P. coatneyi*-infected monkeys including Japanese and cynomolgus macaques contain a factor(s) which activates only Japanese macaque peripheral lymphocytes (unpublished data).

T lymphopenia appears to play a crucial role in the pathogenesis of malaria but is not yet well characterized. Further studies on the mechanism of T lymphopenia will increase understanding of malarial immunopathology.

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