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Cellular aspects of immunoregulation in malaria

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Malaria infection dramatically induces two nonspecific perturbations in immune responsiveness. polyclonal B cell activation and immunosuppression. Polyclonal activation occurs early in infection and results in secretion of antibodies that lack antiplasmodial specificity. Immunosuppression occurs later in infection and is characterized by blunted humoral and cellular immune responses to heterologous (nonplasmodial) as well as plasmodial antigens. Previous studies have suggested that defects in macrophage function may be responsible for immunosuppression in malaria. In what way these cells might be altered in their immunoregulatory role during infection has not been clearly defined. One function of macrophages that is modified in malaria is the ability to secrete in vitro *the monokine lymphocyte-activating factor (LAF). Adherent spleen cells obtained from mice early in* Plasmodium berghei *or* P. yoelii *infection secrete supernormal amounts of LAF. Adherent cells obtained later in infection show subnormal LAF-secreting activity and secrete an immunosuppressive substance. These modulations in macrophage function may be related to the quantity of parasite material ingested by these cells and might help explain the conversion of macrophages from a helper to a suppressor role in malaria.*

Although the mechanisms responsible for protective immunity in malaria remain largely a mystery, knowledge is accumulating regarding the profound generalized perturbations of the immune system that accompany malaria infections. The two most dramatic nonspecific immunological consequences of malaria are the production of a variety of antibodies that lack plasmodial specificity (polyclonal hypergammaglobulinaemia (*1*, *2*) and, paradoxically, also the induction of a state of immunological hyporesponsiveness (see Table 1). These two perturbations occur at different times during the infection. Polyclonal B cell activation is recognized early in the first few days of malaria infection (*3*), while immunosuppression occurs later (*4*). Although the relationship of these antigen nonspecific responses to the development of protective immunity is at present uncertain, these alterations in immune regulation during malaria may nonetheless be important. For example, immunosuppression might account for the sluggishness with which protective immunity develops in human malaria (*5*). The greater severity of intercurrent infections (such as measles, gastroenteritis, and respiratory infections (*6*) in malarious children might also be related to a generalized depression of certain immune responses in these hosts. Finally, the immunosuppression might theoretically represent an obstacle to be overcome in the development of an effective malaria vaccine. An understanding of the basis of immunosuppression in malaria might therefore provide insights into mechanisms whereby the parasite evades the host defences, as well as define those components of the immune system that might have to be artificially modified by a malaria vaccine.

Several studies, carried out largely in rodents infected with malaria, have clearly demonstrated that humoral as well as cellular immune responses may be depressed at certain times during the infection. Clinical studies, however, have revealed such alterations less consistently (Table 1). Of particular interest is the observation that in addition to certain responses to heterologous (nonplasmodial) antigens and mitogens that are depressed during malaria, some specific responses to malaria antigens are also depressed (*4*). Spleen cells of mice infected with lethal and nonlethal *Plasmodium yoelii* proliferated less freely *in vitro* in response to a homologous malaria antigen preparation than did uninfected controls (*4*). In contrast, peripheral blood lymphocytes from children who were previously sensitized (by acute infections) to *P. falciparum*, showed no depression of *in vitro* proliferative responses to falciparum antigen during acute falciparum malaria infection (*10*). One possible explanation for this dichotomy between the response of mouse spleen cells and that of human peripheral blood lymphocytes is that important immunoregulatory events in malaria may occur primarily in the spleen and to a lesser extent at other sites. Support for such a concept of compartmentalization of immunosuppression in malaria comes from

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Table 1. Immunosuppression in malaria. results of representative studies[a]

	Human malaria		Rodent malaria	
Parameter	Decreased	Normal	Decreased	Normal
antibody responses	tetanus toxoid (*6, 7*) salmonella "O" (*6*) meningococcal polysaccharide (*8*)	salmonella "H" (*6*)	tetanus toxoid (*12*) sheep erythrocytes (*13*) pneumococcal polysaccharide (*14*)	bacteriophage ØX 174 (*13*)
cutaneous delayed hypersensitivity		PPD, SKSD, candida, DNCB (*6*)	sheep erythrocytes (*15*)	
in vitro lymphocyte transformation	PHA (*9*)	PHA (*6*), malaria antigens (*10*)	PHA, con A, LPS (*16*) malaria antigens (*4*)	
primary *in vitro* antibody response			HRBC (*17*) TNP Ficoll (*4*)	
T lymphocytes	circulating (*11*)		thymic (*18*) lymph node (*18*) splenic (*19*)	
B lymphocytes	circulating (*11*)		thymic (*18*) lymph node (*18*) splenic (*19*)	

[a] Numbers in parentheses indicate references.

the work of Weidanz & Rank (*20*), who noted that malaria induced a depression in the splenic antibody response to sheep erythrocytes (SRBC) but had little effect on antibody formation in draining lymph nodes.

One explanation for immunosuppression in malaria was suggested by Loose et al. (*21*). On the basis of investigations of the *in vivo* induction of plaque-forming spleen cells against SRBC in *P. berghei*-infected rats, they concluded that the immunological defect resided in macrophage function. Specifically, they suggested that in malaria, macrophages might be deficient in their ability to process antigens in the induction of antibody synthesis. Subsequently, Warren & Weidanz (*17*) examined the ability of spleen cells from uninfected and *P. yoelii*-infected mice to mount *in vitro* primary antibody responses to horse erythrocytes (HRBC). When cultured *in vitro* in a modified Mishell–Dutton system in the presence of HRBC, spleen cells of malaria-infected mice produced fewer plaque-forming cells (PFC) to HRBC than did control spleen cells. When adherent cells (predominantly macrophages) were removed from the malarious spleen cell populations and replaced by adherent cells from uninfected mice prior to culture, the *in vitro* primary antibody responses were restored to normal. More recently, Weinbaum et al. (*4*) have reported studies that confirm and extend these observations. They observed that *in vitro* primary antibody (PFC) responses of malarious spleen cells to TNP-Ficoll (a T-independent antigen) were less than those of control spleen cells. Again, replacement of malarious adherent cells by normal adherent cells in these cultures restored responses to normal. In addition in their study, malarious adherent cells could transfer a suppressive effect when added to a population of nonadherent control spleen cells. The results of these studies did not explain in what manner the adherent cells (macrophages) might be immunosuppressive, however.

In the last few years, a body of evidence has accumulated from *in vitro* studies to suggest that among their other functions in promoting T cell–B cell collaboration in immune responses, macrophages can secrete soluble mediators that exert a nonspecific modulating effect on lymphocyte responsiveness (*22*). Best studied among these is lymphocyte-activating factor (LAF), a product of macrophages that can be directly mitogenic to thymocytes and can also augment the proliferative responses of thymocytes to lectin mitogens, such as concanavalin A (con A) and phytohaemagglutinin (PHA) (*23*). Since the biochemical identity of LAF is at present unknown, it is defined operationally by its *in vitro* effects (thymocyte mitogenicity and enhancement of responses to lectin mitogen). Soluble factors elaborated by macrophages with immunosuppressive effects *in vitro* have also been recognized and in some cases their identity has been established (*24*). It was therefore of interest to study whether malaria might have an effect on the ability of macrophages of infected animals to elaborate *in vitro* substances that could modulate lymphocyte responsiveness to the lectin mitogen, con A, and to other mitogenic and antigenic stimuli (*25*).

RESULTS OF EXPERIMENTS

Adherent spleen cells with the characteristics of macrophages were obtained from uninfected control mice and from mice on different days of infection with *P. berghei* or *P. yoelii*. Cells were incubated in serum-free medium for 48 hours and culture supernatants were then tested for their ability to augment the con A-stimulated incorporation of tritiated thymidine by C3H/HeJ thymocytes. This assay tests for the presence of LAF. It was observed that macrophages from mice early in their malaria infections (days 1–3) elaborated supernormal LAF activity into the culture medium. Macrophages from mice later in infection (days 4 and 5) elaborated subnormal amounts of LAF. Adherent cells from mice that recovered from *P. yoelii* elaborated supernormal LAF. These observations indicated that macrophage function was indeed being altered in a biphasic manner during murine malaria infection. By virtue of increased LAF secretion, macrophages appear to have a potential antigen nonspecific helper effect early in malaria. In this connexion, it was also possible to determine that adherent cell supernatants which contained supernormal LAF activity could also polyclonally stimulate normal spleen cells *in vitro* to produce antibody (as assessed in a reverse plaque assay) (Rosenberg & Wyler, unpublished observations, 1978). Later in infection (days 4 and 5) macrophages presumably lose this helper capability, since they secrete subnormal quantities of LAF *in vitro*. Because of evidence suggesting an active suppressor role of macrophages in the later part of malaria infection (*4*), it seemed of interest to test whether adherent cells from infected mice could elaborate a suppressor factor *in vitro*. To test this, culture supernatants from adherent spleen cells were added to cultures of normal spleen cells (unfractionated population). The magnitude of tritiated thymidine incorporation by these spleen cells in response to various stimuli (PHA, con A, lipopolysaccharide (LPS), malaria antigen) was determined in spleen cells cultured in the presence or absence of adherent cell supernatant. Culture supernatants of adherent cells obtained from uninfected mice and from mice on days 1–3 of infection had no significant effect on stimulated spleen cell proliferation. In contrast, adherent cell supernatants from mice on days 4 and 5 of the infection suppressed responses to PHA, con A, and malaria antigen, but not to LPS. The suppressive material in supernatants was non-dialysable, heat-stable (boiling for 10 min), and resistant to neuraminidase treatment. Suppressive activity was, however, abolished by treating supernatants with ribonuclease, pronase, or trypsin. The suppressive factor was not interferon, because suppressive supernatants contained no detectable interferon activity. Since the elaboration of the suppressive factor was unaffected by treating adherent cells with indomethacin, it was possible to conclude that it was not a prostaglandin, but its precise biochemical nature remains obscure.

The next consideration was how macrophages might be converted to a suppressor role during malaria. One possibility was suggested by the following study. Adherent spleen cells from uninfected BALB/c mice were allowed to ingest increasing concentrations of *P. berghei*-infected erythrocytes from syngeneic mice. Culture supernatants from these phagocytic macrophages were collected and assayed for LAF activity. From these studies it was possible to determine that as macrophages ingested increasing concentrations of parasite material, they secreted supernormal concentrations of LAF. However, when very large amounts of parasite material were ingested, these cells secreted subnormal amounts of LAF. Since parasitized erythrocytes themselves elaborated no substances with effects on thymocyte proliferation, it seemed likely that the alterations in LAF activity in macrophage culture supernatants represented alterations of macrophage function related to the amount of parasite material ingested.

DISCUSSION

From these *in vitro* studies it seems reasonable to propose the following hypothesis to help explain the role of macrophages in modulating antigen-nonspecific responses in malaria. Macrophages accumulate rapidly in the spleen of malaria-infected animals under the influence of products of activated lymphocytes (*26*). Phagocytosis of parasite material ensues, resulting first in the macrophage acquiring a nonspecific helper role by virtue of its secreting supernormal LAF and, perhaps, also a polyclonal B cell stimulatory substance. As an increasing number of macrophages are recruited to ingest parasite material, these cells are then converted from their helper role to a suppressor role. They secrete a suppressor factor and may also be abnormal in other immunoregulatory functions. The compartmentalization of these events to the spleen might result from the particular opportunity for macrophage–parasite interaction in this location.

In addition to alterations in macrophage function, the direct mitogenic effect of malarial parasite-derived material on lymphocytes might be important in immunoregulation in malaria. In a previous study, aqueous extracts of *P. falciparum*-infected erythrocytes nonspecifically stimulated peripheral blood lymphocytes from control adults and cord blood (*27*). Greenwood (*28*) postulated that this mitogenic effect might represent nonspecific B cell stimulation, since

this would help explain the polyclonal hypergammaglobulinaemia in malaria. This hypothesis seems unlikely in view of a recent study (*29*) in which it was possible to determine that *P. falciparum*-infected erythrocytes contained a substance that nonspecifically stimulated human T but not B cells. By preparing purified populations of peripheral human T and B cells, we observed that only the former subpopulation proliferated *in vitro* in response to *P. falciparum* extract. Even mixtures of 10% T cells and 90% B cells failed to proliferate significantly in response to the extract. It now remains to be determined whether helper or suppressor T cells are being preferentially activated by the parasite substance. Clearly, this direct activation might have important immunoregulatory consequences *in vivo*. Direct nonspecific stimulation of helper T cells in concert with supernormal LAF production by macrophages could thus be instrumental in polyclonal B cell activation. Conversely, stimulation of suppressor T cells in concert with a macrophage-derived suppressor substance might be an important determinant of immunosuppression in malaria. Much, however, remains to be learned about how the activation or suppression of immunocompetent cells is brought about in malaria.

How might knowledge of immunoregulation be important in our overall understanding of malaria and solving the challenge of immunoprophylaxis? Several infectious diseases in addition to malaria are associated with immunosuppression (*30*). In some cases these other infections are chronic, suggesting a possible causal relation between immunosuppression and the failure to develop adequate host-defence mechanisms. Therefore, it is not unreasonable to consider that the slow development of protective immunity in malaria or the persistence of parasites in some infections (most notably, *P. malariae*) might be related to depression of certain immune responses required for the induction or expression of parasiticidal processes. Clearly, these defence mechanisms must be more precisely defined before the relationship between immunoregulation and host defence can be established.

The decreased ability of humans and rodents infected with malaria to respond to antigens administered peripherally (subcutaneously or intramuscularly) (see Table 1) raises the concern that the same blunted responses might occur to malaria antigens. Thus, children with chronic malaria might not be immunizable with a malaria vaccine. The observations that malaria antigen-specific responses by spleen cells are depressed in infected mice (*4*) indicates that the immunosuppression is not restricted to heterologous (nonmalarial) antigens. On the other hand, if immunosuppression is compartmentalized to the spleen why are responses to *peripherally* administered antigens also blunted? While this question has not been addressed specifically in malaria, there is evidence from other systems that immunoregulatory cells might migrate between the spleen and peripheral lymphoid sites (*31*). Thus, in malaria, the apparent compartmentalization of suppression in the spleen (*20*) does not exclude the opportunity for development of immunosuppression at peripheral sites as well.

Understanding immunoregulation in malaria might have practical importance. The choice of adjuvants to be used in malaria vaccines might be dictated by their relative ability to prevent or overcome the immunosuppressive effects of infection. Screening adjuvants by employing *in vitro* assays, such as those described above, might be of value. In addition, fractionation of parasite material that, by nonspecific (as well as by specific) effects, can stimulate predominantly helper rather than suppressor T cell populations may be important in vaccine development. In summary, then, the analysis of immunoregulatory pathways in malaria may prove to be directly pertinent to issues of immunoprophylaxis.

RÉSUMÉ

IMMUNORÉGULATION CELLULAIRE DANS LE PALUDISME

L'infection paludéenne provoque deux types dramatiques de perturbation non spécifiques dans les réponses immunitaires: l'activation polyclonale des cellules B et l'immunosuppression. L'activation polyclonale se produit précocement et entraîne la sécrétion d'anticorps qui ne possèdent pas de spécificité antiplasmodique. L'immunosuppression, qui peut survenir à un stade plus avancé de l'infection, est caractérisée par l'affaiblissement des réponses immunitaires humorale et cellulaire aussi bien à l'égard des antigènes antiplasmodiques qu'à l'égard d'autres antigènes. Des études antérieures ont suggéré que l'immunosuppression dans l'infection paludéenne peut provenir de déficiences dans la fonction des macrophages, mais on n'a pas encore élucidé le mécanisme à l'origine de l'altération progressive du rôle immunorégulateur qu'ils jouent au cours de l'infection. L'une des fonctions des macrophages qui est affectée par le paludisme est la capacité de ceux-ci de sécréter *in vitro* le facteur monokin d'activation des lymphocytes (LAF). On a constaté que des quantités supernormales de LAF étaient secrétées par des cellules splénıques adhérentes, prélevées sur des souris au début d'une infection à *Plasmodium berghei* ou à *P. yoelii*. Les cellules adhérentes prélevées à un stade

ultérieur de l'infection avaient une activité subnormale en ce qui concerne la sécrétion de LAF et sécrétaient une substance immunosuppressive. Ces modulations dans la fonction des macrophages peuvent être en rapport avec la quantité de matériau parasitaire ingérée par les cellules en cause.

REFERENCES

1. COHEN, S. & BUTCHER G. A. *Military medicine,* **134**: 1191-1197 (1969).
2. SHAPER, A. G. ET AL *Lancet,* **1**: 1342-1347 (1968).
3. ROSENBERG, Y. J. *Nature (London),* **274**: 170-172 (1978).
4. WEINBAUM, F. I. ET AL *Journal of immunology,* **121**: 629-636 (1978).
5. MCGREGOR, I. A. ET AL. *British medical journal,* **2**: 686-692 (1956)
6. GREENWOOD, B. M. ET AL. *Lancet,* **1**: 169-172 (1972).
7. MCGREGOR, I. A. & BARR. M. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **56**: 364-367 (1962).
8. WILLIAMSON, W. A. & GREENWOOD, B. M. *Lancet,* **2**: 1328-1329 (1978).
9. MOORE, D. L. ET AL. *Clinical and experimental immunology,* **17**: 647-656 (1974).
10. WYLER, D. J. & BROWN, J. *Clinical and experimental immunology,* **29**: 401-407 (1977).
11. WYLER, D. J. *Clinical and experimental immunology,* **23**: 471-476 (1976).
12. VOLLER, A. ET AL *Zeitschrift für Tropenmedizin und Parasitologie,* **23**: 152-155 (1972).
13. BARKER, L. R. *Journal of infectious diseases,* **123**: 99-101 (1971).
14. MCBRIDE, J. S. ET AL *Immunology,* **32**: 635-644 (1977).
15. CRANDALL, R. B. *Journal of parasitology,* **62**: 321-323 (1976).
16. SPIRA, D. ET AL *Clinical and experimental immunology,* **24**: 139-145 (1976).
17. WARREN, H. S. & WEIDANZ, W. P. *European journal of immunology,* **6**: 816-819 (1976).
18. KRETTLI. A. U. & NUSSENZWEIG, R. *Cellular immunology,* **13**: 440-446 (1974).
19. GRAVELY, S. M. ET AL *Infection and immunity,* **14**: 178-183 (1976).
20. WEIDANZ, W. P. & RANK, R. G. *Infection and immunity,* **11**: 211-221 (1975).
21. LOOSE, L. D. ET AL *Proceedings of the Helminthological Society of Washington,* **39**: 484-491 (1972).
22. NELSON, D. S. Nonspecific immunoregulation by macrophages and their products. In: Nelson, D. S., ed. *Immunobiology of the macrophage,* New York, Academic Press, 1976, pp. 235-257.
23. GERY, I. ET AL. *Journal of experimental medicine,* **136**: 128-142 (1972).
24. GOODWIN, J. S. ET AL. *New England journal of medicine,* **297**: 963-968 (1977).
25. WYLER, D. J. ET AL. *Infection and immunity,* **24**: 151-159 (1979)
26. WYLER, D. J. & GALLIN, J. I. *Journal of immunology,* **118**: 478-484 (1977).
27. WYLER, D. J & OPPENHEIM. J. J. *Journal of immunology,* **113**: 449-454 (1974).
28. GREENWOOD, B. M. *Lancet,* **1**: 435-436 (1974).
29. WYLER, D. J. ET AL. *Infection and immunity,* **24**: 106-110 (1979).
30. SCHWAB, J. H. *Bacteriological reviews,* **39**: 121-143 (1975).
31. ROMBALL, C. G. & WEIGLE, W. O. *Cellular immunology,* **34**: 376-384 (1977).