INFECTION AND IMMUNITY, Mar. 1990, p. 788–793
0019-9567/90/030788-06$02.00/0

Vol. 58, No. 3

Tumor Necrosis Factor Enhances Neutrophil-Mediated Killing of *Plasmodium falciparum*

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Received 1 September 1989/Accepted 30 November 1989

We developed a radiometric assay by which the antiplasmodial effects of phagocytic cells can be quantitated. This assay was used to examine the effects of recombinant human tumor necrosis factor alpha (TNF-α) on the killing of *Plasmodium falciparum* by human neutrophils. Data presented demonstrated that neutrophils engulf and destroy *P. falciparum*, but substantial killing of parasites required the presence of either heat-labile or heat-stable opsonins. While recombinant TNF-α at concentrations of 5 to 50,000 U/ml showed no direct effects on the parasite, this cytokine augmented the antimalarial activity of neutrophils at doses of 20 to 250 U/10^6 neutrophils. The results suggest that TNF-α is an important component of the immune phagocytic effector mechanisms which are involved in destruction of the malarial parasite.

Tumor necrosis factor alpha (TNF-α) is a polypeptide cytokine whose release can be triggered by a range of stimuli. TNF-α not only functions as a mediator of the deleterious alterations in cellular homeostasis as a result of many invasive infectious agents (7, 34), it is also considered beneficial to the host for defense against infections and malignancy (7, 13–18, 40). It has been shown that several species of malarial parasites and their soluble antigens induce the release of TNF-α from mouse peritoneal macrophages (3, 4). Significant levels of TNF-α have been found in sera from *Plasmodium falciparum*-infected individuals (26, 43) and *Plasmodium berghei*-infected mice (24, 25). Although the pathophysiological effects of TNF-α have been frequently addressed (1, 10, 11, 36), its role in host protection against *Plasmodium* infections has not been clearly defined. While TNF-containing tumor necrosis serum has been shown to be cytotoxic to *Plasmodium yoelii*, *P. berghei* (46), and *P. falciparum* (27), recombinant TNF-α (rTNF-α) did not affect the viability of *P. yoelii* (47) under in vitro conditions. In fact, it has now been shown that lipid peroxidases present in tumor necrosis serum may account for most of the anti-*P. falciparum* activity (41). Contrary to the in vitro observations, repeated injections of rTNF-α prolonged the survival of the mice by markedly reducing the parasitemia (12, 47). One interpretation of these results is that TNF-α may act by activating macrophages and neutrophils. It has now been clearly established that TNF-α increases the antimicrobial activity of neutrophils (16–18). Since neutrophils have been shown to phagocytose and inhibit growth of *P. falciparum* (8, 9, 29, 48), we examined the ability of TNF-α to modulate the antimalarial activity of human neutrophils. The results demonstrate that rTNF-α augments the killing of *P. falciparum* by neutrophils.

MATERIALS AND METHODS

Sera. Immune serum (IS) refers to serum obtained from individuals who were long-term residents in malaria endemic areas in Papua New Guinea or the Solomon islands. These samples contained high titers of antimalarial antibodies (C. M. Rzepczyk, R. Ramasamy, D. A. Ho, P. L. Battistuta, D. Parkinson, T. J. Doran, and M. Honeyman, Eur. J. Immunol., in press) and <10 hemolytic units of complement. Normal serum (NS) was obtained from group AB^+ Australians who had not been to malaria endemic areas. A portion of this was heated at 56°C for 30 min to inactivate complement activity and is referred to as heated normal serum (HNS).

Parasite. Asynchronous culture of *P. falciparum* (FC 27 K^- strain) was maintained in O^+ erythrocytes with malaria medium, which consisted of medium RPMI 1640 (Flow Laboratories) supplemented with 10% heat-inactivated AB^+ serum, at 37°C in 5% CO_2–5% O_2 in air. In experiments, 10^8 erythrocytes per ml with 4 to 5% parasitemia were used.

Treatment of neutrophils with TNF-α. Neutrophils were prepared from the blood of healthy volunteers by the rapid single-step technique (21) involving centrifugation of the blood on Hypaque-Ficoll medium (d = 1.114). After 30 min of centrifugation, the neutrophils were recovered from the second leukocyte band. These were 96 to 99% pure and >99% viable as judged by the trypan blue exclusion method.

rTNF-α produced by Genentech Inc. (South San Francisco, Calif.) was kindly provided by G. R. Adolf from Boehringer Ingelheim, Vienna, Austria. The specific activity of this preparation was 6×10^7 U/mg (>99% pure), and it contained <0.125 U of endotoxin per ml (*Limulus* amoebocyte lysate assay).

Neutrophils were treated with rTNF-α by adding 100 U of rTNF-α in 50 μl of Hanks balanced salt solution to 50 μl of cells (10^6) in microdilution plates at 37°C for 30 min. In some experiments, neutrophils were treated with a range of rTNF-α concentrations.

Growth inhibition assay. Experiments were conducted in 96-well flat-bottom microdilution plates (Linbro; Flow Laboratories, Inc., McLean, Va.), comparing nine different treatment groups. *P. falciparum*-infected erythrocytes were incubated in the presence of IS (group 1), NS (group 2), HNS (group 3), neutrophils plus IS (group 4), neutrophils plus NS (group 5), neutrophils plus HNS (group 6), rTNF-α-treated neutrophils plus IS (group 7), rTNF-α-treated neutrophils plus NS (group 8), and rTNF-α-treated neutrophils plus HNS (group 9). After the pretreatment of neutrophils with rTNF-α or Hanks balanced salt solution, infected erythrocytes (5×10^6 in 50 μl of malaria medium) and 50 μl of serum or malaria medium were added to the wells and mixed, and

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the plates were incubated for a further 2 h at 37°C in 5% CO_2 in air. The wells were pulsed with 1 μCi of [^{3}H]hypoxanthine (Amersham Corp., Arlington Heights, Ill.) and incubated overnight (18 h). Individual well contents were then collected onto glass filter paper with a multiple semiautomated sample harvester (Titertek; Flow Laboratories), and the [^{3}H]hypoxanthine incorporation was measured with a liquid scintillation counter (Beckman model LS 3801). Percent inhibition of growth of the parasite was calculated by the following formula: percent inhibition = (dpm of parasite with serum − dpm of parasite with serum interacted with neutrophils)/dpm of parasite with serum, where dpm = disintegrations per minute. Since neutrophils took up negligible amounts of the label during this period, it was possible to determine accurately the neutrophil-mediated inhibition of [^{3}H]hypoxanthine uptake by the parasites. A small proportion of IS tested showed direct *P. falciparum* inhibitory properties, and these sera were not included in the present study.

Microscopic studies. Neutrophil-parasite interaction was also studied by preparing cytocentrifuge smears of the microdilution plate contents. These were stained with Giemsa (Gurr; BDH, Poole, England) and examined by microscopy at ×1,000 magnification.

Expression of results and statistical analysis. A total of 22 experiments were conducted with neutrophils from different individuals, using 24 different samples of IS and pooled NS from 10 individuals. The results were compared by the two-tailed Student *t* test.

RESULTS

Figure 1 summarizes the results from 22 experiments and also gives the statistical analyses between different treatment groups. The neutrophils showed a small (4%) inhibition of [^{3}H]hypoxanthine uptake by *P. falciparum* in the presence of HNS. However, when serum complement activity was left intact, the degree of inhibition was greater (14%). A more marked inhibition of parasite growth (24%) was seen when IS was used. The effect of IS on parasite growth inhibition was dose dependent at an IS concentration range of 5 to 25% (Fig. 2). This level of inhibition was also seen when the IS was heated at 56°C for 30 min. Interestingly, the capacity of neutrophils to inhibit parasite growth under all these conditions was significantly increased if neutrophils were pretreated for 30 min with rTNF-α (Fig. 1). A dose-dependent enhancement of neutrophil antiparasitic activity was observed with rTNF-α at 1 to 250 U/10^6 neutrophils (Fig. 3). No further enhancement was observed between 250 and 1,000 U.

Similar effects were found when the ammonium sulfate pooled immunoglobulin cut of IS was used. Percent inhibition in three experiments was 39.6 ± 18.9 and 71.0 ± 1.2 in the absence and presence of rTNF-α, respectively. Microscopic examination of cytocentrifuged smears of neutrophil-*P. falciparum* cultures showed that rTNF-α-treated neutrophils actively phagocytosed the parasites in the presence of either complement- or antibody-containing serum (Fig. 4). The neutrophils phagocytosed merozoites, rings, trophozoites, and schizont stages.

In the absence of neutrophils, no inhibition of growth (by radiometric assay) or degeneration (by microscopic examination) of the parasite was observed when *P. falciparum* was incubated with rTNF-α alone at concentrations of 5 to 50,000 U/ml.

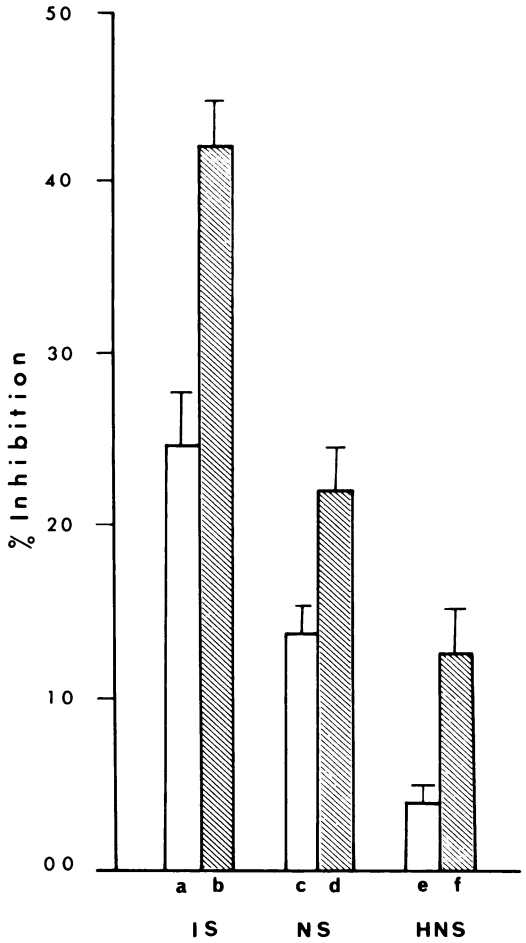

FIG. 1. Inhibition of [^{3}H]hypoxanthine uptake by *P. falciparum* after interaction with neutrophils (open bars) and rTNF-α-activated neutrophils (hatched bars) in the presence of IS, NS, and HNS. Results are presented as the mean ± standard error of the mean (bars) from 22 experiments. The two-tailed Student *t* test showed the following: a versus b, $P < 0.005$; c versus d, $P < 0.05$; e versus f, $P < 0.025$; a versus c, $P < 0.05$; b versus d, $P < 0.001$; a versus e, $P < 0.001$; b versus f, $P < 0.001$.

DISCUSSION

The results presented in this report indicate that the greatest damage to *P. falciparum* by neutrophils occurred in the presence of both antimalarial antibodies and TNF-α. It is also evident that the augmentation of *P. falciparum* damage by TNF-α was not due to a direct effect of the cytokine. Serum containing TNF activity has been shown to be cytotoxic for various *Plasmodium* species (27, 46). Our findings suggest that factors other than TNF-α in this serum are likely to be responsible for these effects, a conclusion consistent with more recent findings (12, 41). The observation that TNF-α augments the neutrophil-mediated parasite damage in the presence of HNS is in agreement with the previous findings that TNF-α stimulated the neutrophil response against nonopsonized zymosan (33). Since TNF-α is known to alter neutrophil surface receptors (5, 23, 39), it is possible that the interaction between neutrophil receptors and lectins on infected erythrocytes is optimized because of an increased affinity of the receptors. TNF-α has also been shown to enhance the expression of complement receptor type 3 (23, 39). It is therefore conceivable that the increased

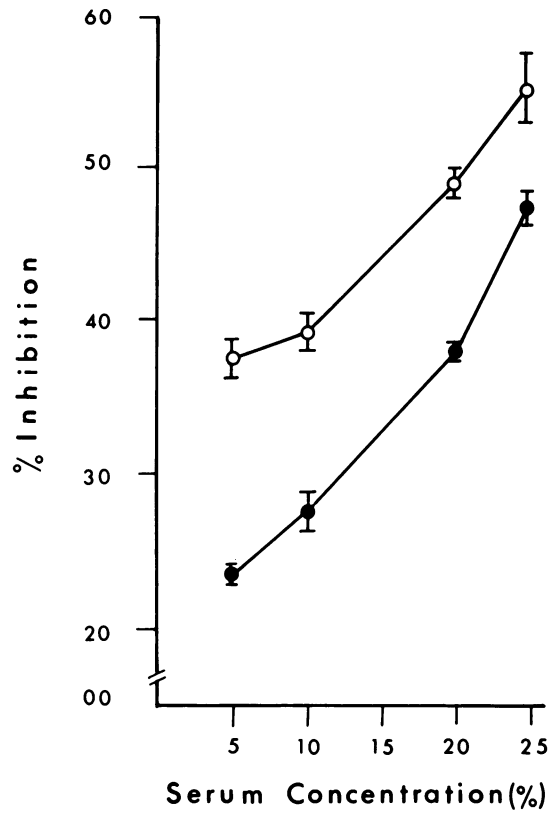

FIG. 2. Effect of IS concentration on the inhibition of [^{3}H]hypoxanthine uptake by *P. falciparum* (5 × 10^6 infected erythrocytes per well) in the presence of either neutrophils (●) or neutrophils (1 × 10^6) pretreated with 100 U of TNF-α (37°C, 30 min) (○). The results are presented as the mean ± standard error of the mean (bars).

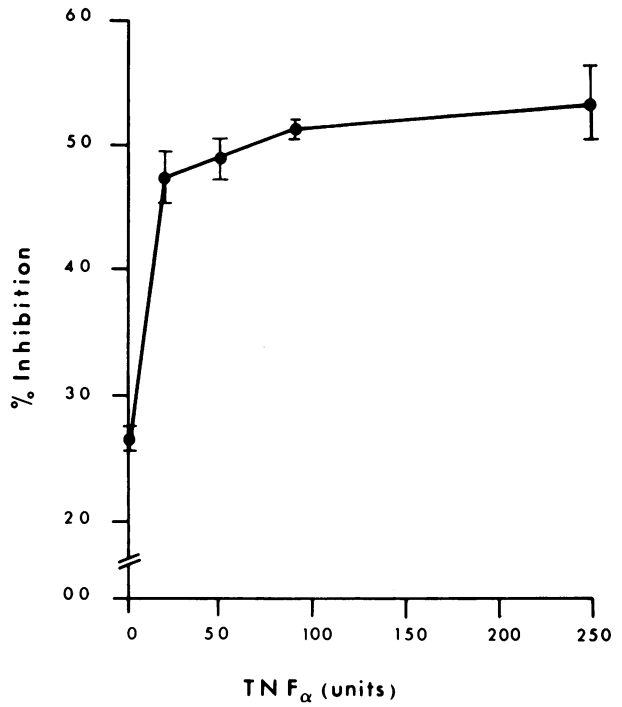

FIG. 3. Effects of different concentrations of rTNF-α (1 to 250 U/10^6 neutrophils) on the anti-*P. falciparum* activity of neutrophils in the presence of IS, expressed as percent inhibition of [^{3}H] hypoxanthine uptake by the parasite. Results are expressed as the mean ± standard error of the mean (bars). Percent inhibition by neutrophils in the absence of TNF-α was 26.0 ± 0.8.

damage in the presence of complement results from functional changes in this receptor. It is also tempting to speculate that the effects of TNF-α in the presence of antibody are related to its increased expression of neutrophil Fc receptors (39). Among a number of possible roles of antibody in immunity to malaria is that of opsonization and promotion of phagocytosis (28, 32). Extensive examination of cytocentrifuged smears of neutrophil-*P. falciparum* cultures showed that rTNF-α-treated neutrophils always actively phagocytosed the parasites in the presence of either complement- or antibody-containing serum.

The mechanisms by which TNF-α alters neutrophils for antimicrobial activity have been partly identified. The capacity of TNF-α to alter the Fc, complement, and other adherence receptors (5, 39, 44) is likely to be important in its effects on neutrophil damage to malarial parasites. In addition, however, TNF-α is known to prime neutrophils for increased oxygen radical production (6, 18, 20, 22, 33, 45) and increased release of lysosomal components in response to a variety of stimuli, including opsonized fungi and amoebae (18, 20, 37). The capacity of antibody and complement to promote *P. falciparum*-induced oxygen radical production has been established (42). However, the role of oxygen intermediates in the killing of *P. falciparum* appears controversial. While the importance of oxygen-derived reactive species has been emphasized (30, 38), neutrophils defective in oxidative metabolism still inhibited the growth of *P. falciparum* (31). More importantly, hydrogen peroxide in the presence of myeloperoxidase forms a potent anti-*P. falciparum* system (35). The ability of TNF-α to augment the release of granule constituents and reactive oxygen intermediates from neutrophils is likely to contribute to the enhanced killing of *P. falciparum* by neutrophils observed in our study, particularly in view of previous findings from this laboratory which showed that lymphokine-treated neutrophils from normal subjects but not those from a myeloperoxidase-deficient patient were activated for amoeba killing (19).

The findings of this study emphasize the importance of both TNF-α and antibody in immunity to *P. falciparum*. The importance of cell-mediated immunity and also natural killer cells has previously been emphasized in immunity to malaria (1, 2; Rzepczyk et al., in press). Stimulation of T cells by malarial antigens could lead to release of gamma interferon, which activates macrophages and natural killer cells for release of TNF-α.

The possible role of TNF-α in the pathophysiology of malaria has been emphasized (1, 7, 10, 11, 36). A series of experiments support the hypothesis that excessive amounts of TNF-α produce many of the abnormalities observed after the injection of an endotoxin and during clinical endotoxemic shock. However, TNF-α has been shown to produce a wide range of biological effects in humans, and some of these effects, for example, pyrexia, may be beneficial by causing retardation of parasite development (25). Our results suggest that TNF-α plays an important role in immunity to malaria by activating neutrophils for increased killing of the parasite. This may also explain the discrepancy between the lack of direct antiplasmodium activity of TNF-α in vitro and its ability to reduce parasitemia in vivo (12, 47).

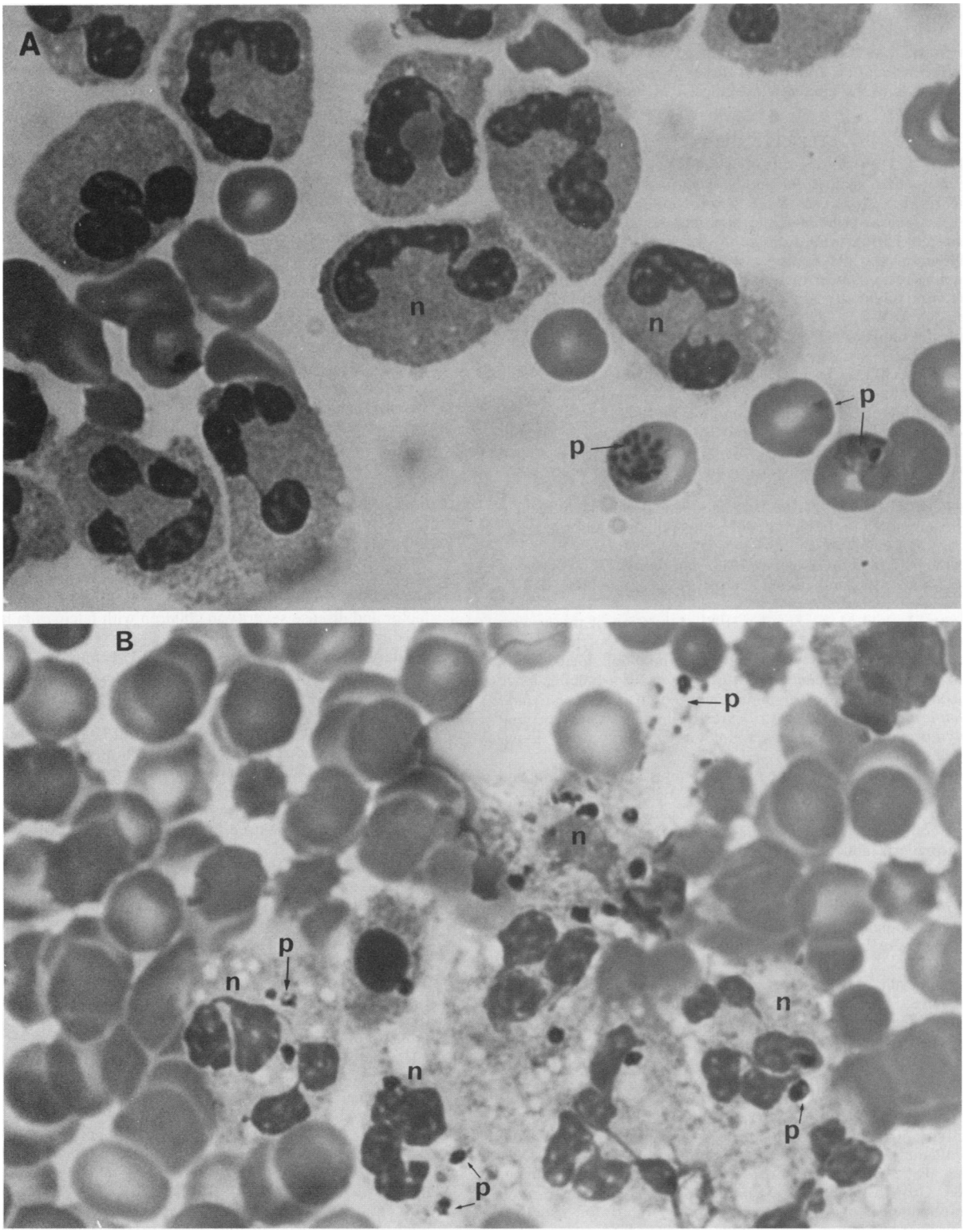

FIG. 4. Photomicrographs of stained smears of cultures showing no evidence of phagocytosis of parasites (p) by neutrophils (n) in the presence of HNS (A) or TNF-α-pretreated neutrophils having phagocytosed various stages of *P. falciparum* in the presence of IS (B).

ACKNOWLEDGMENTS

We thank Timothy Jaeger for skillful technical assistance and Karen Forsyth of The Walter and Eliza Hall Institute for Medical Research, Melbourne, for providing *P. falciparum* to initiate cultures.

This work was supported by grants from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases and the Children's Medical Research Foundation of South Australia.

LITERATURE CITED

1. **Allison, A. C.** 1989. The role of cell-mediated immune responses in protection against *Plasmodia* and in the pathogenesis of malaria, p. 501–513. *In* W. H. Wernsdorfer and I. McGregor (ed.), Malaria, principles and practice of malariology, 1st ed. Churchill Livingstone, Edinburgh.
2. **Allison, A. C., and E. Eugui.** 1983. The role of cell-mediated immune responses in resistance to malaria, with special reference to oxidant stress. Annu. Rev. Immunol. **1**:361–392.
3. **Bate, C. A. W., J. Taverne, and J. H. L. Playfair.** 1988. Malarial parasites induce TNF production by macrophages. Immunology **64**:227–231.
4. **Bate, C. A. W., J. Taverne, and J. H. L. Playfair.** 1989. Soluble malarial antigens are toxic and induce the production of tumour necrosis factor *in vivo*. Immunology **66**:600–605.
5. **Berger, M., E. M. Wetzier, and R. S. Wallis.** 1988. Tumour necrosis factor is the major monocyte product that increases complement receptor expression on mature human neutrophils. Blood **71**:151–158.
6. **Berkow, R. L., and M. R. Dodson.** 1988. Biochemical mechanisms involved in the priming of neutrophils by tumor necrosis factor. J. Leukocyte Biol. **44**:345–352.
7. **Beutler, B., and A. Cerami.** 1988. The common mediator of shock cachexia, and tumor necrosis. Adv. Immunol. **42**:213–231.
8. **Brown, J., and M. E. Smalley.** 1981. Inhibition of the *in vitro* growth of *Plasmodium falciparum* by human polymorphonuclear neutrophil leucocytes. Clin. Exp. Immunol. **46**:106–109.
9. **Celada, A., A. Cruchaud, and L. H. Perrin.** 1984. Independence of complement on *in vitro* immune phagocytosis of *Plasmodium falciparum* parasitized erythrocytes by human monocytes and polymorphonuclear leukocytes. Int. Arch. Allergy Appl. Immunol. **73**:363–366.
10. **Clark, I. A.** 1987. Cell-mediated immunity in protection and pathology of malaria. Parasitol. Today **3**:300–305.
11. **Clark, I. A., and G. Chaudhri.** 1988. Tumour necrosis factor may contribute to the anaemia of malaria by causing dyserythropoiesis and erythrophagocytosis. Br. J. Haematol. **70**: 99–103.
12. **Clark, I. A., N. H. Hunt, G. A. Butcher, and W. B. Cowden.** 1987. Inhibition of murine malaria *Plasmodium chabaudi in vivo* by recombinant interferon-γ or tumor necrosis factor, and its enhancement by butylated hydroxyanisole. J. Immunol. **139**: 3493–3496.
13. **Damonneville, M., J. Wietzerbin, V. Pancre, M. Joseph, A. Delanoye, A. Capron, and C. Auriault.** 1988. Recombinant tumour necrosis factor mediates platelet cytotoxicity to *Schistosoma mansoni* larvae. J. Immunol. **140**:3962–3965.
14. **Denis, M., and K. Chadee.** 1989. Cytokine activation of murine macrophages for in vitro killing of *Entamoeba histolytica* trophozoites. Infect. Immun. **57**:1750–1756.
15. **de Titto, E. H., J. R. Catterall, and J. S. Remington.** 1986. Activity of recombinant tumor necrosis factor on *Toxoplasma gondii* and *Trypanosoma cruzi*. J. Immunol. **137**:1342–1345.
16. **Djeu, J. Y., D. K. Blanchard, H. H. Halkias, and H. Friedman.** 1986. Growth inhibition of *Candida albicans* by human polymorphonuclear neutrophils: activation by interferon-γ and tumour necrosis factor. J. Immunol. **137**:2980–2984.
17. **Ferrante, A.** 1989. Augmentation of the neutrophil response to *Naegleria fowleri* by tumor necrosis factor alpha. Infect. Immun. **57**:3110–3115.
18. **Ferrante, A.** 1989. Tumor necrosis factor alpha potentiates neutrophil antimicrobial activity: increased fungicidal activity against *Torulopsis glabrata* and *Candida albicans* and associated increases in oxygen radical production and lysosomal enzyme release. Infect. Immun. **57**:2115–2122.
19. **Ferrante, A., N. L. Hill, T. J. Abell, and H. Pruul.** 1987. Role of myeloperoxidase in the killing of *Naegleria fowleri* by lymphokine-altered human neutrophils. Infect. Immun. **55**:1047–1050.
20. **Ferrante, A., M. Nandoskar, A. Waltz, D. H. B. Goh, and I. C. Kowanko.** 1988. Effects of tumour necrosis factor alpha and interleukin-1 alpha and beta on human neutrophil migration, respiratory burst and degranulation. Int. Arch. Allergy Appl. Immunol. **86**:82–91.
21. **Ferrante, A., and Y. H. Thong.** 1982. Separation of mononuclear leucocytes from human blood by the one-step Hypaque-ficoll method is dependent on blood column height. J. Immunol. Methods **48**:81–85.
22. **Figari, I. S., N. A. Mori, and M. A. Palladino, Jr.** 1987. Regulation of neutrophil migration and superoxide production by recombinant tumor necrosis factor-α and -β: comparison to recombinant interferon-γ and interleukin-1α. Blood **70**:979–984.
23. **Gamble, J. R., J. M. Harlan, S. J. Klebanoff, and M. A. Vadas.** 1985. Stimulation of adherence of neutrophils to umbilical vein endothelium by human recombinant tumor necrosis factor. Proc. Natl. Acad. Sci. USA **82**:8667–8671.
24. **Grau, G. E., L. F. Fajardo, P.-F. Piguet, B. Allet, P.-H. Lambert, and P. Vassalli.** 1987. Tumor necrosis factor (cachectin) is an essential mediator in murine cerebral malaria. Science **237**:1210–1212.
25. **Grau, G. E., V. Kindler, P.-F. Piguet, P.-H. Lambert, and P. Vassalli.** 1988. Prevention of experimental cerebral malaria by anticytokine antibodies: interleukin 3 and granulocyte macrophage colony-stimulating factor are intermediates in increased tumor necrosis factor production and macrophage accumulation. J. Exp. Med. **188**:1499–1504.
26. **Grau, G. E., T. E. Taylor, M. E. Molyneux, J. J. Wirima, P. Vassalli, M. Hommel, and P.-H. Lambert.** 1989. Tumor necrosis factor and disease severity in children with falciparum malaria. N. Engl. J. Med. **320**:1586–1591.
27. **Haidaris, C. G., D. Haynes, M. S. Meltzer, and A. C. Allison.** 1983. Serum containing tumor necrosis factor is cytotoxic for the human malaria parasite *Plasmodium falciparum*. Infect. Immun. **42**:385–393.
28. **Hunter, K. W., Jr., J. A. Winkelstein, and T. W. Simpson.** 1979. Serum opsonic activity in rodent malaria: functional and immunochemical characteristics *in vitro*. J. Immunol. **123**:2582–2587.
29. **Kharazmi, A., and S. Jepsen.** 1984. Enhanced inhibition of *in vitro* multiplication of *Plasmodium falciparum* by stimulated human polymorphonuclear leucocytes. Clin. Exp. Immunol. **57**:287–292.
30. **Kharazmi, A., S. Jepsen, and B. J. Andersen.** 1987. Generation of reactive oxygen radicals by human phagocytic cells activated by *Plasmodium falciparum*. Scand. J. Immunol. **25**:335–341.
31. **Kharazmi, A., S. Jepsen, and N. H. Valerius.** 1984. Polymorphonuclear leucocytes defective in oxidative metabolism inhibit *in vitro* growth of *Plasmodium falciparum*. Scand. J. Immunol. **20**:93–96.
32. **Khusmith, S., and P. Druilhe.** 1983. Antibody-dependent ingestion of *P. falciparum* merozoites by human blood monocytes. Parasite Immunol. **5**:357–368.
33. **Klebanoff, S. J., M. A. Vadas, J. N. Harlan, L. H. Sparks, J. R Gamble, J. M. Agosti, and A. M. Waltersdorph.** 1986. Stimulation of neutrophils by tumor necrosis factor. J. Immunol. **136**:4220–4225.
34. **Le, J., and J. Vilvek.** 1987. Biology of disease—tumour necrosis factor and interleukin 1: cytokines with multiple overlapping biological activities. Lab. Invest. **56**:234–245.
35. **Malhotra, K., D. Salmon, L. B. Jacques, and J. L. Vilde.** 1988. Susceptibility of *Plasmodium falciparum* to a peroxidase-mediated oxygen-dependent microbial system. Infect. Immun. **56**: 3305–3309.
36. **Miller, K. L., P. H. Silverman, G. Kullgren, and L. J. Mahlmann.** 1989. Tumor necrosis factor alpha and the anemia associated with murine malaria. Infect. Immun. **57**:1542–1546.
37. **Nathan, C. F.** 1987. Neutrophil activation on biological sur-

faces. Massive secretion of hydrogen peroxide in response to products of macrophages and lymphocytes. J. Clin. Invest. **80**:1550–1560.

38. **Ockenhouse, C. F., S. Schulman, and H. L. Shear.** 1984. Induction of crisis forms in the human malaria parasite *Plasmodium falciparum* by interferon-activated, monocyte derived macrophages. J. Immunol. **133**:1601–1608.
39. **Perussia, B., M. Kobayashi, M. E. Ross, I. Anegon, and G. Trinchieri.** 1987. Immune interferon enhances functional properties of human granulocytes: role of Fc receptors and effect of lymphotoxin, tumour necrosis factor, and granulocyte-macrophage colony stimulating factor. J. Immunol. **138**:765–774.
40. **Playfair, J. H. L., and J. Taverne.** 1987. Antiparasitic effects of tumour necrosis factor *in vivo* and *in vitro*. CIBA Found. Symp. **131**:192–205.
41. **Rockett, K. A., G. A. T. Targett, and J. H. L. Playfair.** 1988. Killing of blood-stage *Plasmodium falciparum* by lipid peroxides from tumor necrosis serum. Infect. Immun. **56**:3180–3183.
42. **Salmon, D., J. L. Vilde, B. Andrieu, R. Simonovic, and J. Lebras.** 1986. Role of immune serum and complement in stimulation of the metabolic burst of human neutrophils by *Plasmodium falciparum*. Infect. Immun. **51**:801–806.
43. **Scuderi, P., K. S. Lam, K. J. Ryan, E. Petersen, K. E. Sterling, P. R. Finley, C. G. Ray, D. J. Slymen, and S. E. Salmon.** 1986. Raised serum levels of tumour necrosis factor in parasitic infections. Lancet **ii**:1364–1365.
44. **Seow, W. K., Y. H. Thong, and A. Ferrante.** 1987. Macrophage-neutrophil interactions: contrasting effects of the monokine interleukin-1 and tumour necrosis factor (cachectin) on human neutrophil adherence. Immunology **62**:357–361.
45. **Shalaby, M. R., B. B. Aggarwal, E. Rindeknecht, L. P. Svedersky, B. S. Finkle, and M. A. Palladino, Jr.** 1985. Activation of human polymorphonuclear neutrophil functions by interferon-γ and tumour necrosis factors. J. Immunol. **135**:2069-2073.
46. **Taverne, J., H. M. Dockrell, and J. H. L. Playfair.** 1981. Endotoxin-induced serum factor kills malarial parasites in vitro. Infect. Immun. **33**:83–89.
47. **Taverne, J., J. Tavernier, W. Fiers, and J. H. L. Playfair.** 1987. Recombinant tumour necrosis factor inhibits malaria parasites *in vivo* but not *in vitro*. Clin. Exp. Immunol. **67**:1–4.
48. **Trubowitz, S., and B. Masek.** 1968. *Plasmodium falciparum*: phagocytosis by polymorphonuclear leukocytes. Science **162**: 273–274.