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Effects of Cytokines, Complement, and Antibody on the Neutrophil Respiratory Burst and Phagocytic Response to *Plasmodium falciparum* Merozoites

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The interaction between *Plasmodium falciparum* merozoites and human neutrophils, as well as the role of cytokines, complement, and antimalarial antibody on this interaction, was examined in vitro by measuring luminol-dependent chemiluminescence and phagocytosis. Merozoites, in the presence of heat-inactivated (56°C/30 min) normal serum, had very little effect on the neutrophil chemiluminescence. This response was significantly enhanced by the addition of normal serum (containing normal complement activity). In the presence of serum or plasma containing anti-*P. falciparum* antibodies (IS) with no detectable complement activity, the merozoites induced a marked response characterized by an increase in initial peak rate of chemiluminescence and a sustained increased rate of chemiluminescence. However, this response was not further increased if IS containing complement activity was used. Pretreatment of neutrophils with either tumor necrosis factor alpha, lymphotoxin, or gamma interferon significantly increased the neutrophil response to IS-treated merozoites, reflected in an increased initial peak rate and sustained increased rate of chemiluminescence. The effects of cytokine treatment of neutrophils and IS opsonization of merozoites were synergistic. In association with the changes in the chemiluminescence responses, IS was shown to promote phagocytosis of merozoites by neutrophils, and this event was further increased by treating neutrophils with the cytokines. The results emphasize the importance of antibody and cytokines in neutrophil-mediated damage of *P. falciparum* merozoites.

For most of its life cycle in humans, *Plasmodium falciparum* is located intracellularly. Merozoites, which represent a brief extracellular stage of the parasite, are liberated following intrahepatic and erythrocytic schizogony. Depending on the *Plasmodium* species, 100 to 30,000 merozoites are known to be liberated from the initial hepatoschizogony (61), and each erythrocytic schizont releases 6 to 32 merozoites which reinvade erythrocytes to initiate a new asexual cycle. Attention has been directed towards a possible development of a vaccine against the merozoite stage of the parasite, and purified antimerozoite antibodies and sera from immune individuals have been shown to inhibit merozoite reinvasion of erythrocytes in vitro and in animal models (8, 15, 44, 49, 51, 54, 59, 66). However, recent evidence suggests that protection in humans is not mediated by the inhibition of merozoite invasion by antibodies (3). In fact, these investigators and others have shown that antimalarial antibodies promote phagocytosis of *P. falciparum* merozoites (3, 13, 36, 62).

Merozoites are exposed to neutrophils infiltrating the liver during hepatocyte schizogony (34) or to neutrophils in the circulation during rupture of erythrocytes infected with mature schizonts. Phagocytosis of merozoites by neutrophils in vivo (63, 64) and in vitro (41, 42) has been reported. Since the existence of exoerythrocytic merozoites is very brief, an efficient immune mechanism is required to prevent their reinvasion. Priming of neutrophils with cytokines and opsonization with antimalarial antibody and complement may enhance the efficiency of neutrophil-mediated killing of merozoites. High levels of circulating tumor necrosis factor alpha (TNF-α) and gamma interferon (IFN-γ) have been detected in *P. falciparum*-infected individuals (5, 26–28, 53), and we have demonstrated that human mononuclear phagocytes produce TNF-α and lymphotoxin (LT) in response to *P. falciparum* antigens (21). Recently, enhanced LT levels also have been observed in malaria-infected individuals (7). In this study, we have examined the human neutrophil response to living *P. falciparum* merozoites and the regulation of this response by cytokines (TNF-α, IFN-γ, LT), complement, and antibody. The results, derived from a phagocytic assay and respiratory burst response measured as chemiluminescence, suggest that complement and antimalarial antibodies are important in neutrophil-mediated killing of *P. falciparum*. The cytokines significantly increased the neutrophil respiratory burst response and phagocytosis of merozoites, particularly in the presence of antibody.

MATERIALS AND METHODS

Reagents. Human recombinant (r) LT (specific activity, 1.2×10^7 U/ml; 1.2×10^8 U/mg; >99% purity; endotoxin, $<8 \times 10^{-3}$ pg/ml), rIFN-γ (specific activity, 6×10^6 IU/ml; 2×10^7 U/mg; >99% purity; endotoxin, <0.125 pg/ml), and rTNF-α (specific activity, 3×10^7 U/ml; 6×10^7 U/mg; >99% purity; endotoxin, $<1.4 \times 10^{-4}$ pg/ml) were produced by Genentech Inc. (South San Francisco, Calif.) and kindly provided by G. R. Adolf from Boehringer, Ingelheim, Vienna, Austria. Endotoxin levels were determined by *Limulus* amoebocyte lysate assay. Murine monoclonal antibody (MAb) (immunoglobulin G [IgG]) to human LT, IFN-γ, and

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TNF-α were provided by G. R. Adolf. Goat anti-mouse IgG was obtained from Cooper Biomedical (Malvern, Pa.).

Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) (Sigma, St. Louis, Mo.), phenol-red-free RPMI 1640 (CSL, Melbourne, Australia), and Percoll (Pharmacia LKB Biotechnology, Uppsala, Sweden) were purchased. Luminol was prepared with 0.25 mg of Hanks balanced salt solution per ml, and Percoll (75%) was prepared in 10× Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline (d = 1.126). Phorbol myristate acetate (PMA) (Sigma) at 10^{-6} M, *N*-formyl-methionine-leucine-phenylalanine (FMLP) (Sigma) at 5×10^5 M, and zymosan (opsonized with human group AB^+ serum) at 20 mg/ml were prepared.

Neutrophils. Neutrophils were prepared from the peripheral blood of healthy volunteers by the rapid single-step technique (22) involving 30-min centrifugation of the blood on Hypaque-Ficoll medium (d = 1.114). These cells were 96 to 99% pure and >99% viable, as routinely determined by the trypan blue exclusion method, and contained <1% mononuclear leukocytes. Neutrophils were suspended in phenol-red-free RPMI 1640 and kept on ice until used. Direct comparisons between the effects of different cytokines or sera were made with neutrophils from the same individual.

Merozoites. Synchronized *P. falciparum* cultures were maintained under in vitro conditions (41, 42). Cultures with 3 to 5% parasitaemia were adjusted to give approximately 3×10^5 merozoites per ml and maintained as 7-ml aliquots in 75-cm^2 culture flasks (Corning Glass Works, Corning, N.Y.) in an incubator at 37°C in 5% O_2–5% CO_2 in N_2 until used. Centrifuge tubes (10 ml) containing 3-ml aliquots of 75% sterile isotonic Percoll were also maintained at 37°C. Five min before the interaction with neutrophils, merozoites were prepared by layering 7 ml of culture on 3 ml of Percoll and centrifuging the tubes at 750 × g for 5 min. Merozoites prepared in this manner produced a dark band in the liquid phase (above Percoll) and contained approximately 95% merozoites and 5% mature schizonts (with fully formed merozoites) with nearly 100% viability as detected by merozoite reinvasion assay (65).

IS and heated IS (HIS). Immune sera and plasma (IS) were obtained from individuals who were long-term residents in malaria endemic areas in Papua New Guinea and the Solomon Islands. These samples contained high titers of antimalarial antibodies (55) and variable complement (C) levels (<10 to 90 hemolytic units [hu]). In addition, the IgG fraction was purified from IS by affinity chromatography using a Protein G column (Mab Trap G; Pharmacia LKB Biotechnology, Uppsala, Sweden) and contained 1.20 g of IgG per liter. Several IS which contained >80 hu of C were heated at 56°C for 30 min in a water bath to inactivate the C activity and designated HIS.

NS and heated NS (HNS). Sera prepared from healthy Australians who had not been to a malaria endemic area is referred to as normal sera (NS). A fraction of this was heat inactivated at 56°C for 30 min and designated HNS. The mean immunoglobulin levels in IS and NS or HNS were 12.75 (IgG, 9.55) g/liter and 13.50 (IgG = 9.74) g/liter, respectively.

Chemiluminescence assay and pretreatment of neutrophils with cytokines. Neutrophils were resuspended at 10^7/ml in RPMI 1640, and 100-μl aliquots were dispensed into vials and left standing in an ice bath. The cells were preincubated for 30 min at 37°C with 100 μl of either TNF-α (100 U), LT (1,000 U), IFN-γ (100 U) or RPMI 1640 by addition at 30-min intervals so that each vial achieved exactly 30 min of preincubation with the cytokine (40). Immediately after this, the merozoites (2×10^6/100 μl) or control (malaria medium) and 100 μl of sera (IS, HIS, IgG, NS, or HNS) or RPMI 1640 were added and gently mixed. Where the effects of agonists were studied, 100 μl of PMA, opsonized zymosan, or FMLP were added to the neutrophils. Luminol (500 μl) was then added to the tubes and briefly mixed. The resulting light output was recorded for 30 min in millivolts in a luminometer with a water-jacketed incubator (37°C) (model 1250; LKB, Wallac, Turku, Finland). Data presented in figures represent peak values (maximum rate) of the response, except where chemiluminescence kinetics are presented. All steps were conducted under sterile conditions.

Measurement of phagocytosis. Merozoite phagocytosis by neutrophils was studied morphologically. After 30 min of incubation of neutrophils and parasites, the tubes were centrifuged, the pellet was resuspended in Hanks balanced salt solution, and cytocentrifuge smears were prepared by using the total pellet, stained with Giemsa (Gurr; BDH, Poole, England), and examined microscopically (×1,000). Comparisons were made between (i) merozoites opsonized with IS, HIS, NS, and HNS and (ii) neutrophils treated with TNF-α, LT, and IFN-γ. Neutrophils (1,000) were examined from each pellet to determine the percentage of neutrophils with at least one merozoite bound or ingested. In addition, the total number of ingested merozoites was counted in each pellet by using 300 neutrophils and expressed per 100 neutrophils.

Heat treatment and adsorption of cytokines. The cytokine preparations were heated at 80°C for 30 min and compared with the nonheated cytokines. This was used as a method to distinguish between the effects of the cytokines from those of lipopolysaccharide-contaminated preparations based on the fact that lipopolysaccharide activity is resistant at boiling temperatures.

Adsorption of cytokines LT, IFN-γ, and TNF-α was measured as described previously (40). Briefly, MAb for LT, IFN-γ, and TNF-α were bound to anti-mouse-immunoglobulin-coated plates, the relevant cytokines were added, and the plates were incubated at 37°C for 30 min. The supernatants were collected and compared in the chemiluminescence assay.

Sample size, expression of results, and statistical analysis. The number of experiments carried out are given in figure legends. Results are presented as mean ± standard error of the mean. Within each experiment, each test was conducted in duplicate, using neutrophils from the same donor. Statistical significance was analyzed by a paired t test (two-tailed).

RESULTS

Merozoite-induced chemiluminescence response. Merozoites, in the presence of HNS, had very little effect on the neutrophil luminol-dependent chemiluminescence (Fig. 1). In the presence of NS containing C activity, the parasites induced a significant response in the neutrophils ($P < 0.001$). The response was further enhanced when the merozoites were opsonized with IS ($P < 0.001$) which contained <10 hu of C. No significant difference was observed between IS containing normal C activity and HIS or those which contained undetectable complement activity (data not presented). The rate of chemiluminescence formation over time is shown in Fig. 2. In the presence of IS, the parasites induced a response characterized by a marked increase in initial peak rate and sustained increase of the rate of chemiluminescence output, at least for a 25-min period. Table 1 compares the neutrophil chemiluminescence response of merozoites op-

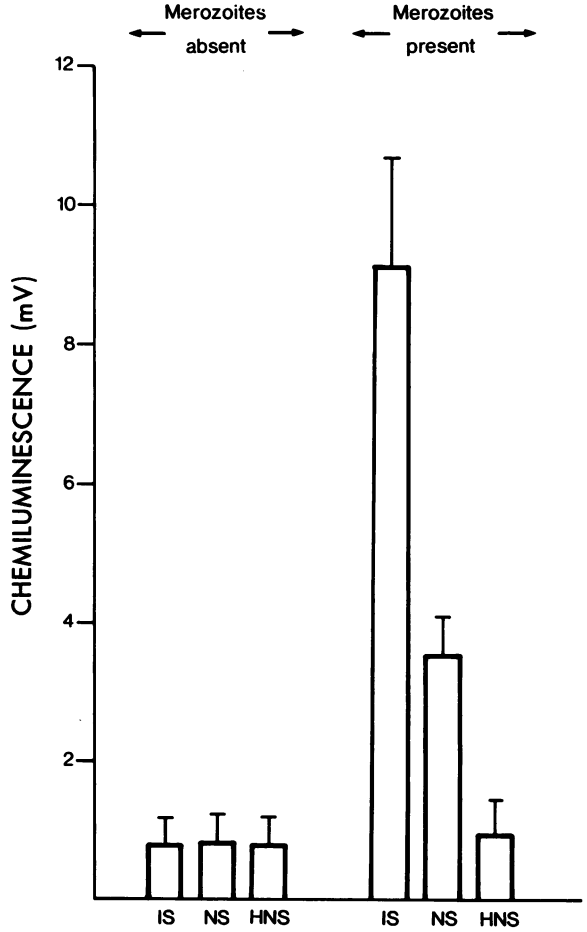

FIG. 1. Effects of IS, NS, and HNS on neutrophil luminol-dependent chemiluminescence measured in the absence and presence of *P. falciparum* merozoites. Data represent mean ± standard error of the mean from six experiments each conducted with neutrophils from different individuals. Within each experiment, comparisons were made with neutrophils from the same individual.

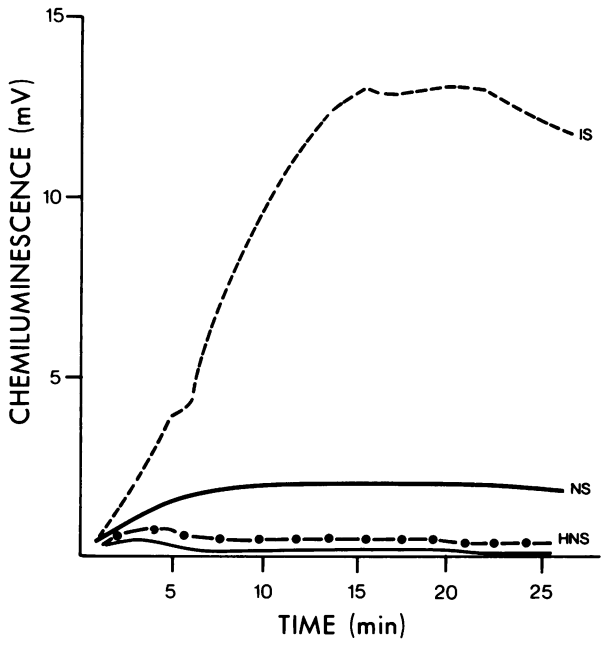

FIG. 2. Representative graph of 20 experiments showing the time-related changes in rates of luminol-dependent chemiluminescence of human neutrophils when *P. falciparum* merozoites opsonized with IS, NS, and HNS were interacted. The thin continuous line shows the basal neutrophil chemiluminescence response.

sonized with purified IgG (from IS) with IS. The purified IgG was able to enhance the merozoite-induced chemiluminescence response.

Effect of TNF-α, LT, and IFN-γ on the merozoite-induced neutrophil chemiluminescence responses. Previous studies from our laboratory have shown that optimal concentrations of TNF-α, LT, and IFN-γ required to stimulate an oxygen-dependent respiratory burst were 100, 1,000 and 100 U/10^6 neutrophils, respectively (19, 20, 42), and these concentrations were used to prime neutrophils for enhanced interaction with IS-opsonized merozoites. Neutrophils from a different individual were used in each experiment. Results from 20 experiments showed that TNF-α significantly ($P < 0.001$) altered the neutrophil response for merozoites with a 4.6-fold increase. The rate of chemiluminescence production as a function of incubation time is shown in Fig. 3. The effects of TNF-α are seen both as enhancement of peak rate and as a sustained response. Similar observations were made with LT and IFN-γ (data not presented). The fold increases for LT (20 experiments) and IFN-γ (15 experiments) were 3.3 and 3.2, respectively.

As shown in Table 1, in the presence of purified IgG (prepared from IS), TNF-α, LT, and IFN-γ enhanced the merozoite-induced chemiluminescence response of the neutrophils.

Comparison of the effects of TNF-α, LT, and IFN-γ. Six separate chemiluminescence experiments were carried out to compare the effects of different cytokines using neutrophils from the same individuals. In the absence and presence of IS-opsonized merozoites, the ability to stimulate neutrophils was TNF-α > LT > IFN-γ (Fig. 4). Cytokine-enhanced effects were also seen when the merozoites were interacted with neutrophils in the presence of NS and HNS (data not presented).

Cytokine concentration. When IS-opsonized merozoites were interacted with neutrophils pretreated with different concentrations of cytokines, a concentration-dependent enhancement of chemiluminescence was seen with up to 50 U

TABLE 1. Effects of cytokines on the neutrophil chemiluminescence response to *P. falciparum* merozoites opsonized with either IS or IgG

Neutrophil treatment	Chemiluminescence (mV)[a] with:	
	IgG	IS
None	0.84 ± 1.6	0.96 ± 0.4
Merozoites	3.60 ± 0.6	18.75 ± 13.2
TNF-α	21.29 ± 9.7	18.26 ± 7.1
TNF-α + merozoites	33.90 ± 1.1	45.20 ± 13.9
LT	12.42 ± 5.6	12.49 ± 6.6
LT + merozoites	24.82 ± 3.7	40.57 ± 17.8
IFN-γ	3.23 ± 2.5	1.93 ± 0.1
IFN-γ + merozoites	8.61 ± 2.9	39.0 ± 8.9
Merozoites (IgG or IS absent)	0.89 ± 0.05	

[a] Results are presented as mean ± standard error of the mean of three experiments. IgG was at 1.20 g/liter; IS contained IgG at 9.74 g/liter.

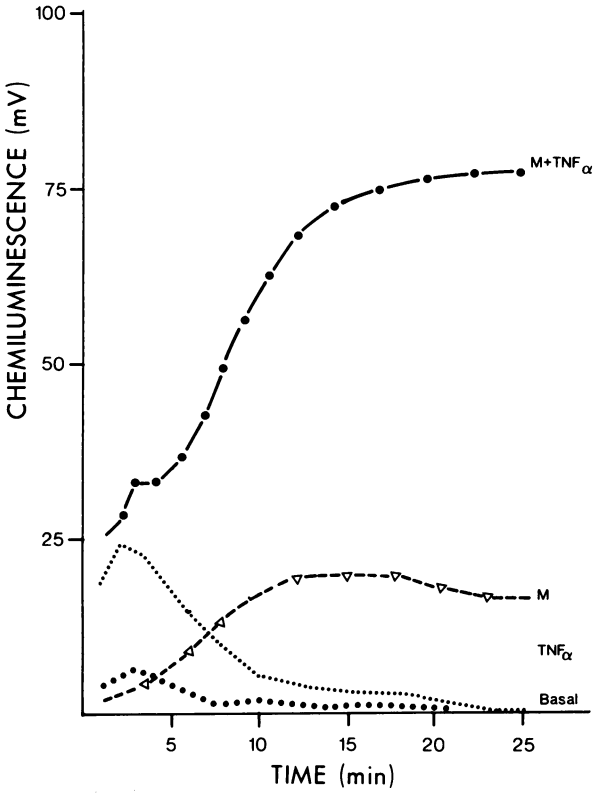

FIG. 3. Representative graph of more than 20 experiments showing the time-related changes in rate of luminol-dependent chemiluminescence of human neutrophils treated with TNF-α and challenged with IS-treated merozoites.

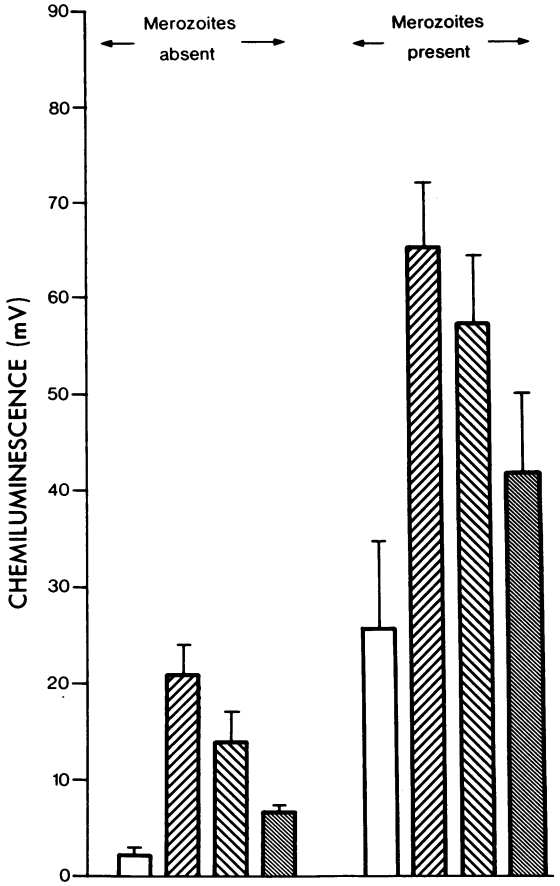

FIG. 4. Comparisons of the effects of TNF-α, LT, and IFN-γ on human neutrophil luminol-dependent chemiluminescence measured in the absence and presence of *P. falciparum* merozoites. Neutrophils were either treated with TNF-α (▨), LT (▧), or IFN-γ (□) or incubated with Hanks balanced salt solution (□). Data represent mean ± standard error of the mean from six experiments each conducted with neutrophils from different individuals. Within each experiment, comparisons were made with neutrophils from the same individual.

of TNF-α or IFN-γ or 1,000 U of LT, with no further significant increase up to 15,000 U (data not presented).

Comparisons of the merozoite-induced response with responses to other neutrophil agonists. Table 2 compares neutrophil stimulation caused by PMA, FMLP, and opsonized zymosan with that induced by IS- and HIS-opsonized merozoites. Merozoites induced a response which was greater than that induced by the optimal concentration of FMLP. The response to merozoites by neutrophils pretreated with TNF-α was much greater than that which could be induced with either PMA or opsonized zymosan, which are considered to be strong neutrophil stimulators.

Effects of IS, C, TNFα, LT, and IFN-γ on *P. falciparum* merozoite phagocytosis by neutrophils. Morphological studies on wet mounts and short-term studies based on cytocentrifuge smears showed that merozoites were very rapidly bound by neutrophils. Table 3 show that at the end of 30 min of chemiluminescence the percentage of neutrophils found with at least one merozoite bound or ingested was higher in the presence of IS or NS than with HNS ($P < 0.025$). This was also found when the neutrophils were pretreated with TNF-α, LT, and IFN-γ. A higher number of cytokine-treated neutrophils were involved in phagocytosis of merozoites in the presence of IS or NS than with HNS ($P < 0.01$) (Table 3). Of the three cytokines, TNF-α was more effective than LT and IFN-γ (Table 3). In two separate experiments, IS with and without intact C were compared and no difference was found between the groups (data not presented).

The numbers of ingested merozoites ranged from 1 to 8 per neutrophil. IS with or without cytokine treatment significantly increased the total number of merozoites phagocytosed by 100 neutrophils over results with NS ($P < 0.05$) or HNS ($P < 0.01$). For example, for each 100 neutrophils treated with TNF-α the total numbers of merozoites ingested were 400 ± 65 (IS) and 123 ± 15 (NS) ($P < 0.01$); the numbers of merozoites ingested by neutrophils treated with LT were 226 ± 33 (IS) and 102 ± 20 (NS) ($P < 0.05$); and the numbers of merozoites ingested by neutrophils treated with IFN-γ were 256 ± 65 (IS) and 59 ± 16 (NS) ($P < 0.01$).

Effects of heat treatment and adsorption of cytokines. To exclude a role of possible endotoxin contamination, TNF-α, LT, and IFN-γ were heated at 80°C for 1 h and then tested for their ability to prime neutrophils. Heating completely abolished the cytokine-induced increase of chemiluminescence response. For example, heating reduced the TNF-α-induced chemiluminescence response to merozoites from 78.97 ± 3.92 to 12.95 ± 1.56 mV, which was essentially the response observed with the diluent-primed neutrophils (12.41 ± 1.00 mV).

The cytokine preparations were also adsorbed out with murine anti-TNF-α, anti-LT, and anti-IFN-γ MAbs. The results showed that adsorbed cytokines, in contrast to the

TABLE 2. Comparison of neutrophil chemiluminescence responses induced by merozoites in the presence of IS, PMA, opsonized zymosan, and FMLP

Neutrophil treatment	Peak chemiluminescence (mV)[a]
Hanks balanced salt solution	1.0 ± 0.71
Merozoites + IS	14.9 ± 2.1
Merozoites + HIS	13.8 ± 1.8
TNF-α	13.2 ± 4.3
TNF-α + IS	10.1 ± 3.7
TNF-α + HIS	9.7 ± 2.2
TNF-α + M + IS	172.7 ± 9.5
TNF-α + M + HIS	159.2 ± 12.5
PMA	65.2 ± 31.3
Zymosan	118.5 ± 43.9
FMLP	6.5 ± 2.5

[a] Results are presented as mean ± standard error of the mean of three experiments, except where HIS (IS heated at 56°C for 30 min) was used in two experiments.

mock-adsorbed preparations, lacked the ability to stimulate the basal and merozoite-induced responses. The chemiluminescence in millivolts was 23.30 ± 1.0 for merozoites (IS) + neutrophils, 70.63 ± 1.25 for TNF-α treated neutrophils + merozoites (IS) and 22.73 ± 2.65 for MAb-adsorbed TNF-α-treated neutrophils + merozoites (IS). The results for LT and IFN-γ also followed the same trend.

DISCUSSION

The data demonstrate that purified *P. falciparum* merozoites induced a chemiluminescence response in human neutrophils, which is an index of the production and release of oxygen-derived reactive species (ODRS). The response was promoted by anti-*P. falciparum* antibodies and was poor in the presence of HNS (56°C, 30 min). Normal serum lacking these antibodies but containing complement activity also promoted significantly the chemiluminescence response against the parasite, although it was not as effective as that obtained in the presence of antimalarial antibodies. The response was smaller in the presence of purified IgG (1.2 g/liter) than that of IS (IgG, 9.74 g/liter), most likely because of the concentration of IgG available for opsonization. When IS containing C was compared with HIS (56°C, 30 min) and IS containing negligible amounts of C, no significant difference was seen in the chemiluminescence response. These findings suggest that antimalarial antibodies play an important role in immunity against *P. falciparum* merozoites by enhancing the neutrophil ODRS which have been shown to be microbicidal and likely to be mediators, damaging adhered merozoites at the plasma membrane level or within the phagolysosomes. This is in agreement with previous findings that *P. falciparum* merozoite-induced neutrophil chemiluminescence was highest in the presence of IS (35).

Following a 20- to 30-min chemiluminescence study, the neutrophils were pelleted and cytocentrifuge smears were prepared. Morphological examinations revealed that most merozoites were either bound to neutrophils or phagocytosed by the neutrophils. Both IS (with or without C) and NS containing C promoted phagocytosis. The total number of merozoites found phagocytosed by neutrophils was significantly higher in the presence of IS than in the presence of NS or HNS, with or without the cytokine treatment. This observation suggests that the presence of C is not critical, provided antimalarial antibodies are available. Both morphological and chemiluminescence studies showed that IS or purified IgG not only promoted phagocytosis but also enhanced ODRS generation and thereby provided a more efficient killing mechanism. Although the major mechanism believed to operate against the parasite is that of antibodies blocking merozoite invasion of erythrocytes (44, 49, 51, 54), collaborative mechanisms are required to complete the defense mechanism. Efficient phagocytosis and destruction of the antibody-coated merozoite is a means of ensuring that parasites which may evade the blocking effects of antibody are removed. Recent in vivo and in vitro studies have shown that the antibodies which protect humans against *P. falciparum* blood stages do not on their own inhibit merozoite invasion or parasite growth but act in cooperation with phagocytic cells (3).

Recently it has become evident that macrophages and T lymphocytes release cytokines which have the ability of enhancing the neutrophil responses to various stimuli (2, 11, 19, 20, 25, 37, 38, 50). Since cytokines such as IFN-γ, LT, and TNF-α have been shown to be elevated during the course of a malaria infection (5, 7, 26, 28, 53, 58), we consider the neutrophil-activating property of these cytokines to be relevant to immunity to malaria. Our results have demonstrated that TNF-α, LT, and IFN-γ were all capable of markedly enhancing the neutrophil response to merozoites. This enhancement was particularly evident in the presence of serum containing antibodies to *P. falciparum* or with purified IgG. The results from these studies suggest that the two major pathways for phagocytosis of merozoites and the

TABLE 3. Morphological studies on the effects of TNF-α, LT, and IFN-γ on merozoite-neutrophil interaction

Treatment of neutrophils	% of neutrophils with merozoites adhered or ingested[a]								
	HNS			NS			IS		
	Adhered	Ingested	Total	Adhered	Ingested	Total	Adhered	Ingested	Total
Hanks balanced salt solution	9 ± 1.0	7 ± 3.9	16 ± 8.5	9 ± 1.8	30 ± 8.5	39 ± 6.7	7 ± 1.4	37 ± 9.0	44 ± 7.9
TNF	12 ± 5.3	11 ± 5.6	23 ± 8.7	20 ± 1.2	42 ± 11.7	62 ± 12.0	8 ± 2.4	63 ± 13.1	71 ± 15.2
LT	17 ± 11.5	9 ± 3.4	26 ± 11.5	12 ± 3.0	34 ± 10.5	46 ± 12.4	9 ± 0.7	44 ± 12.3	53 ± 13.1
IFN-γ	13 ± 5.6	18 ± 8.5	31 ± 12.3	18 ± 1.8	31 ± 11.8	49 ± 10.5	11 ± 2.5	45 ± 9.8	56 ± 10.5

[a] Results are expressed as mean ± standard error of the mean of three experiments. The neutrophils were pretreated with 100 U of TNF-α, 1,000 U of LT, and 100 U of IFN-γ. Statistically significant differences were seen between the neutrophils found with merozoites adhered + ingested in the following: cytokine-absent (Hanks balanced salt solution) versus cytokine-treated groups (TNF-α, LT, IFN-γ) ($P < 0.05$) and HNS versus NS, IS with or without cytokine treatment (TNF-α, LT, IFN-γ) ($P < 0.05$).

associated biochemical response involves either complement or antibody opsonization of the parasite, thus implicating an important role of complement receptors and Fc receptors on neutrophils, in which case interaction with the latter results in more effective phagocytosis and chemiluminescence production. TNF-α, LT, and IFN-γ most likely mediate their enhancing effects by increasing Fc and complement receptor expression, which promotes the neutrophil interaction with complement- or antibody-opsonized merozoites. It has been shown that TNF-α, LT, and IFN-γ increase the expression of complement receptor type 3 (CR3) and Fc receptors on neutrophils (24, 30, 37, 50). Attempts have been made to elucidate the mechanisms behind TNF-α-mediated increased phagocytosis by neutrophils. It has been shown that TNF-α increases the surface expression of the C3bi receptor (adherence-promoting glycoprotein CD11b/CD18), possibly in part because of translocation from intracellular stores (24). The increase in complement-mediated phagocytosis of merozoites may occur as a consequence of the increased expression of CD11b/CD18, an event associated with activation of protein kinase C (29, 31). However, ingestion of IgG-coated targets such as merozoites probably occurs independent of TNF-α-induced activation of protein kinase C (29, 31).

In addition to the involvement of neutrophils, merozoite phagocytosis by peripheral blood monocytes has been reported (13, 36). Importantly, the rate of antibody-dependent merozoite phagocytosis by monocytes is closely related to the protective status of individuals (13). It is therefore likely that both cell types play a role in killing merozoites in blood, although it is evident that this activity is highly dependent on antibody and complement (13, 36). These opsonins may be limiting during various phases of the malaria infection. When these are limiting, it is likely that the splenic macrophages may play an important role since it has been shown that splenic trapping of bacteria and other particulate matter is primarily important when antibody level is low or absent (67). Removal of IgG-opsonized particles by the liver is considered to be less efficient than by the spleen (23). When the particles are opsonized with IgM and C3bi, clearance of particles is most efficiently achieved by the liver (32, 57, 67). Therefore, in studies on macrophage mediated killing of malaria parasites, the availability of the antibody type and cytokines needs to be taken into consideration. Previously it has been shown that IFN-γ enhances killing of the intraerythrocytic stage of *P. falciparum* by macrophages (48) but interleukin 4 suppresses the monocyte- and macrophage-mediated killing of the parasite (39). In the light of the latter finding it is likely that neutrophils may play an important role in controlling the parasite since interleukin 4 does not seem to alter their antiparasitic activity (39).

The findings described in this paper further support the role of neutrophils and their activation by TNF-α, LT, and IFN-γ in immunity to *P. falciparum*. Previously we have demonstrated that phagocytosis and subsequent killing of intraerythrocytic asexual blood stages of *P. falciparum* by human neutrophils was significantly enhanced by these cytokines, particularly in the presence of IS (41, 42). Although TNF-α may be beneficial to the host by augmenting the phagocytic cells' ability to destroy merozoites and erythrocytic stages of *P. falciparum*, this cytokine may promote cerebral malaria by enhancing the sequestration of activated macrophages and parasitized erythrocytes (27, 58). However, a recent study based on 72 Zairian children with malaria showed that although TNF-α level increased with parasite density it was not associated with cerebral malaria or fatal outcome (5). In contrast to studies with neutrophils, the monocyte-dependent killing of erythrocytic stages appeared to be independent of short-term cytokine activation (18) and anti-*P. falciparum* antibodies from immune individuals in Papua New Guinea (18). Thus, our data suggest that a major role of TNF-α, LT, and IFN-γ in immunity to *P. falciparum* is to increase neutrophil-mediated killing of the parasite, and that this effect is best achieved in the presence of anti-*P. falciparum* antibodies.

This study shows that sera from "immune" individuals contain antibodies which are able to opsonize merozoites and such merozoites are able to stimulate a marked neutrophil chemiluminescence response associated with enhanced merozoite phagocytosis. This is significantly enhanced in neutrophils pretreated with cytokines. It has been shown that pretreatment of neutrophils with the cytokines TNF-α, IFN-γ, and LT enhances their responses to bacteria, fungi, and protozoan parasites (11, 12, 14, 17, 25). Evidently, direct neutralization effect of antibodies alone is not sufficient to provide protection against malaria, and the importance of other mechanisms (e.g., cytotoxic T cells against sporozoite stage, soluble factors such as crisis formation factor, and mechanisms whereby antibodies interact with cellular components to provide efficient killing of parasites) has been emphasized (1, 3, 6, 13, 33, 36, 43, 52, 63, 68). Our findings fall within the last category, although the success of these mechanisms will depend on how rapidly merozoites are coated with antibody, the affinity of the antibody (56), and the availability of activated phagocytic cells in the vicinity of merozoites. The data also support a role for T lymphocytes in neutrophil-mediated immunity to *P. falciparum*, further emphasizing the recent view that T lymphocytes activate neutrophils (4, 9, 10, 16, 60, 69).

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