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Two Soluble Antigens of *Plasmodium falciparum* Induce Tumor Necrosis Factor Release from Macrophages

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The production of cytokines such as tumor necrosis factor (TNF) may contribute to the pathology of malaria. We showed previously that crude preparations of heat-stable exoantigens from parasite cultures induce the release of TNF in vitro and in vivo. When separated from the culture medium by affinity chromatography, in which immune immunoglobulin G was used as ligand, the mixture of exoantigens of *Plasmodium falciparum* retained the capacity to induce the secretion of TNF, both by human monocytes from Gambian children and by mouse macrophages. Two individual antigens, Ag1 and Ag7, further purified by affinity chromatography and identified by crossed immunoelectrophoresis, also stimulated TNF production by both types of cell but differed in other functional properties. Thus, the activity of Ag7, but not that of Ag1, was inhibited by polymyxin B, and antisera made against boiled exoantigens of the rodent parasite *Plasmodium yoelii* which blocked the ability of these antigens to induce the production of TNF also inhibited the activity of Ag7 without affecting Ag1. Since the prevalence of antibody against Ag7 in sera from children in endemic areas appears to correlate with the development of immunity against the manifestations of the disease, this antigen may be one cause of pathology, perhaps through its ability to induce the production of TNF. Its serological relationship with rodent exoantigens suggests that it might be a candidate for an antidisease vaccine which has the advantage that its active moiety is not subject to significant antigen polymorphism.

The clinical pictures of malaria and endotoxemia have much in common, and this, among other observations, originally led to the proposition that they were caused by common mediators, such as tumor necrosis factor (TNF) (5). The arguments have been well reviewed more recently by Clark (4). Much evidence is now emerging to support the idea that this cytokine indeed plays an important role in causing the pathological changes that are characteristic of malaria. Thus, the association of production of TNF with disease has been clearly shown recently in a study of patients suffering their first infection with *Plasmodium falciparum*, in which it was found that the amount of circulating TNF in the serum correlated directly with the severity of the disease (19). In African children, high levels of circulating TNF are found more frequently in malaria than in most other infections, and they fall in convalescence (20); the levels are significantly higher in fatal cases of malaria than in survivors (12; D. Kwiatkowski, Immunol. Lett., in press). In a mouse model of cerebral malaria in which the presence of TNF in the blood was associated with the appearance of neurological symptoms and death, treatment with antibody against TNF delayed mortality and prevented the occurrence of the cerebral symptoms (11). In humans, cerebral malaria is thought to be related to sequestration of parasites in the capillaries of the brain, and TNF has now been shown to increase the expression of the intracellular adhesion molecule 1 receptor on endothelial cells to which parasites can bind (3).

Macrophages are believed to be the major source of TNF; in the laboratory, bacterial lipopolysaccharide (LPS) has been used conventionally to trigger its release. When human blood mononuclear cells were cocultured with erythrocytes infected with *P. falciparum*, a sharp rise in the rate of secretion of TNF occurred when schizonts ruptured (20); since TNF is pyrogenic (9), this finding may help to explain why fever in patients with malaria is typically associated with schizont rupture. Parasitized erythrocytes from both cytoadherent and noncytoadherent strains have been shown to induce the production of TNF (23). Similarly, parasitized erythrocytes from mice infected with rodent malarial parasites trigger TNF release from activated mouse macrophages in vitro, and we found that heat-stable exoantigens from the blood stages of *Plasmodium yoelii* and *Plasmodium berghei* induced its production both in vitro and in vivo (1a, 2). Similar antigens in *P. falciparum* culture medium induced the production of TNF from both human monocytes (23, 29) and mouse macrophages (29).

Affinity purification of *P. falciparum* culture medium, using immunoglobulin G (IgG) from immune Liberians as ligand, followed by immunoelectrophoresis revealed the presence of at least seven separate parasite antigens (P. H. Jakobsen, E. M. Riley, S. J. Allen, S. O. Larsen, S. Bennett, S. Jepsen, and B. M. Greenwood, Trans. R. Soc. Trop. Med. Hyg., in press). This mixture of antigens is recognized by lymphocytes from Gambian adults (27) and children (26). Here we describe work done to determine whether first the mixture of purified soluble antigens and then two of the individual antigens induce the secretion of TNF in vitro. The latter antigens, numbered Ag1 and Ag7, both occur on the surfaces of trophozoites and schizonts, although Ag7 is largely schizont specific, and they are therefore likely to be released at the critical time in the developmental cycle when fever occurs. Ag7 is recognized by human immune sera from

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many parts of the world; it specifically stimulated proliferation of T cells from clinically immune adults from the Gambia and induced secretion of interleukin-1 by human monocytes, and it is pyrogenic in rabbits (P. H. Jakobsen, L. Hviid, T. G. Theander, E. M. Riley, P. Grellier, L. Bruun, K. Dalsgaard, J. Schrevel, and S. Jepsen, Acta Pathol. Microbiol. Immunol. Scand., in press). Of this series, Ag1 is the exoantigen most frequently found in the plasma of children with acute malaria (17).

MATERIALS AND METHODS

Soluble antigens of *P. falciparum*. Soluble parasite antigens (SPAG) from cultures of the F32 isolate of *P. falciparum* were purified in endotoxin-free conditions by affinity chromatography on a CNBr-Sepharose 4B column (Pharmacia Fine Chemicals, Uppsala, Sweden) containing as ligand a pool of IgG from clinically immune Liberian adults which had a high titer of precipitating antibody against soluble *P. falciparum* antigens, as described previously (13). The pooled fractions of eluted antigens were concentrated, and the different soluble antigens were identified by cross immunoelectrophoresis as before. Pure preparations of Ag1 and Ag7 were made by affinity chromatography, using Liberian IgG that precipitated each antigen only (14; Jakobsen et al., in press).

Mice. Outbred females at least 6 weeks old were used (Tuck No1; A. Tuck & Sons, Battlesbridge, Essex, United Kingdom). BALB/c nude mice were obtained from the National Institute of Medical Research, London, United Kingdom.

Rodent parasites. The YM lethal variant of *P. yoelii* (10) (obtained from D. Walliker, Edinburgh University) was used. Mice were infected intravenously with 10^4 parasitized erythrocytes, and parasitemia was determined from blood films stained with Giemsa.

Rodent parasite exoantigens. As described previously (2), mice with high parasitemia were bled by cardiac puncture, and the erythrocytes were washed twice in sterile phosphate-buffered saline and then suspended in phosphate-buffered saline at a concentration of 10^8 parasitized cells per ml in suspension on a roller for 24 h at 37°C. The next day, the suspensions were centrifuged at 500 × *g* for 10 min. The supernatant was then boiled for 5 min and centrifuged at 1,300 × *g* for 10 min; these supernatants were passed through a 0.2-μm-pore-size membrane filter (Flow Laboratories, Irvine, Ayrshire, United Kingdom) and stored at 4°C. We (1a, 2, 25, 28, 29; J. Taverne, C. A. W. Bate, and J. H. L. Playfair, Immunol. Lett., in press) and others (23) have shown previously that exoantigens that stimulate TNF production from macrophages withstand boiling and that the procedure acts as a preliminary step in their clarification.

Antisera against parasite exoantigens. Primary antisera were made as described previously (2). Briefly, groups of at least three mice were injected intraperitoneally with boiled supernatants and were bled 10 to 12 days later. Each pool of antiserum was checked to confirm that it inhibited the ability of the parasite antigens, but not of LPS, to induce the release of TNF by mouse macrophages. One pool of antiserum was made similarly by injection of boiled concentrated medium from a continuous culture system of *P. falciparum* Nf54 (29). Hyperimmune serum against *P. yoelii*, obtained from mice that had recovered from infection after vaccination with a Triton X-100 lysate of parasitized erythrocytes injected with saponin as adjuvant, was kindly provided by J. B. de Souza (24).

Human monocyte cultures. Blood was collected from Gambian children, age 2 to 6 years, none of whom had parasitemia; these children would have had malaria at some time but were not infected at the time of sampling. Mononuclear cells were separated on pyrogen-free Lymphoprep (Nyegaard), washed in pyrogen-free normal saline (Travenol Laboratories, Morton Grove, Ill.), and suspended at 10^6 cells per ml in minimal essential medium (Whittaker-MA Bioproducts) with added *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), penicillin, streptomycin, and 1% heat-inactivated serum from one European adult. Samples of 100 μl were dispensed into the wells of a microtiter plate and incubated with 100-μl volumes of various concentrations of the affinity-purified parasite antigens or LPS (*Escherichia coli* O55:B5; Sigma Chemical Co., St. Louis, Mo.) in duplicate wells, or with medium alone, for 20 h at 37°C in 5% CO_2. Supernatants were then collected and stored at −20°C.

Mouse peritoneal cells. Mouse peritoneal cells were prepared as previously described (2). Briefly, cells were collected from mice given thioglycolate intraperitoneally 3 to 5 days previously, using Hanks balanced salt solution (Flow Laboratories) containing 1 U of heparin and 5 μg of polymyxin B (Sigma) per ml. Washed cells were suspended in 5% fetal calf serum in RPMI 1640 (Flow Laboratories) containing polymyxin B and adjusted to 10^7 viable cells per ml; 0.1-ml volumes were then dispensed into wells of 96-well microtiter plates (Nunc, Roskilde, Denmark). The cells were incubated for 2 to 3 h at 37°C to allow macrophages to adhere and then for 30 min with an equal volume of medium containing 2 μg of indomethacin (Sigma) per ml. Nonadherent cells were removed, the medium was replaced by 0.2-ml volumes of RPMI 1640 containing the stimulants to be tested, and the cultures were incubated overnight. (Serial dilutions of the stimulants were always tested to establish a dose-dependent relationship, and experiments were repeated at least twice.) The next day, supernatants were collected and assayed for TNF by their cytotoxicity for L929 cells. A 1/10 dilution of each was made in medium containing 5% fetal calf serum and 1 μg of emetine (Sigma) per ml and stored at −20°C in case a titration needed to be repeated. Cultures incubated with serial dilutions of LPS or with medium alone were included in every experiment as positive and negative controls on the capacity of the macrophages to yield TNF.

TNF assays. TNF in the supernatants of monocyte cultures was measured by enzyme-linked immunosorbent assay (20), using recombinant human TNF (Amersham Corp., Arlington Heights, Ill.) as a standard; levels are therefore expressed as picograms per milliliter. TNF from mouse macrophages was assayed colorimetrically by its cytotoxicity for L929 cells (obtained from the European Collection of Animal Cell Cultures, Porton Down, Salisbury, Wiltshire, United Kingdom) seeded at 2.5 × 10^4 cells per well the day before, as described previously (2). Serial dilutions were tested in duplicate in 0.1-ml volumes per well of 96-well microtiter plates in RPMI 1640 containing 1 μg of emetine per ml. One unit is defined as the amount causing 50% cell destruction.

RESULTS

We reported previously that supernatant medium from a continuous-flow culture system of blood-stage *P. falciparum*, when boiled and then concentrated, induced the release of TNF from mouse macrophages as well as from human monocytes and that control medium from cultures of

uninfected human erythrocytes did not (29). The amounts of TNF were of the same order as obtained in cultures incubated with 0.1 to 1.0 μg of LPS per ml. Several lines of evidence indicated that heat-stable parasite exoantigens in the culture medium and not traces of contaminating endotoxin stimulated the release of TNF. In particular, TNF induction by the antigens, but not by LPS, was abolished specifically by mouse antisera made against boiled culture supernatants from both human and rodent parasites, and it still occurred in the presence of a concentration of polymyxin B, or of a monoclonal antibody, that blocked induction by 1 μg of LPS per ml.

To investigate the nature and properties of the different antigens present in the culture medium that might be responsible for these findings, we tested the capacity of different preparations of affinity-purified soluble antigens of *P. falciparum* to induce TNF release both from human monocytes and from mouse peritoneal macrophages. The SPAG preparations, which contained all of the antigens that bound to IgG pooled from sera obtained from immune Liberians, were found to induce TNF release from both types of cell, as did preparations of the two individual antigens tested, defined by crossed immunoelectrophoresis as Ag1 and Ag7 (13). The buffers used in each case to elute the antigens from affinity columns did not themselves induce TNF secretion. Cells from a number of children were tested with affinity-purified but unfractionated SPAG and with Ag7 diluted 1/1,000. Others were subsequently tested with a higher dilution of Ag7 and a similar dilution of Ag1. The results are expressed as means and standard errors for TNF secretion compared with the responses to 5 ng of LPS per ml of the two groups (Fig. 1).

The results of representative titrations of the ability of the same antigens to cause TNF secretion by mouse peritoneal macrophages are shown in Fig. 2, compared with the yields of TNF obtained from cultures stimulated with a single concentration of SPAG and of LPS in each case. Each preparation induced TNF secretion from the mouse cells in a dose-dependent manner, and at higher concentrations the amounts released were equivalent to the stimulus provided by 0.1 μg of LPS per ml. The relative sensitivity of human and mouse cells to stimulation by the parasite exoantigens cannot be assessed precisely since the conditions were different in London and the Gambia and the TNF was assayed by different methods, but it is clear that both Ag1 and Ag7 stimulated TNF release from both types of cell and that both experimental systems gave yields in the nanogram range.

Effect of polymyxin B. Polymyxin B neutralizes the activity of LPS by binding to its lipid A moiety (22). It did not prevent induction of TNF by preparations of soluble antigens of rodent parasites (1a, 2) or by boiled, concentrated preparations of *P. falciparum* culture medium, although it decreased the amounts induced by the latter (29). It did, however, affect the electrophoretic mobility of Ag1 and, when incorporated into the gel before immunoelectrophoresis, totally abolished the precipitate of Ag7 (13). Experiments were therefore done to determine whether it also blocked the ability of these antigens to induce TNF production by mouse macrophages (Table 1). Polymyxin B at 10 μg/ml did not decrease the amount of TNF released in response to stimulation by Ag1, but the activities of Ag7 and SPAG (the latter at a concentration which induced an amount of TNF of the same order as that concentration of Ag1) were markedly diminished, even by the lower concentration of polymyxin tested.

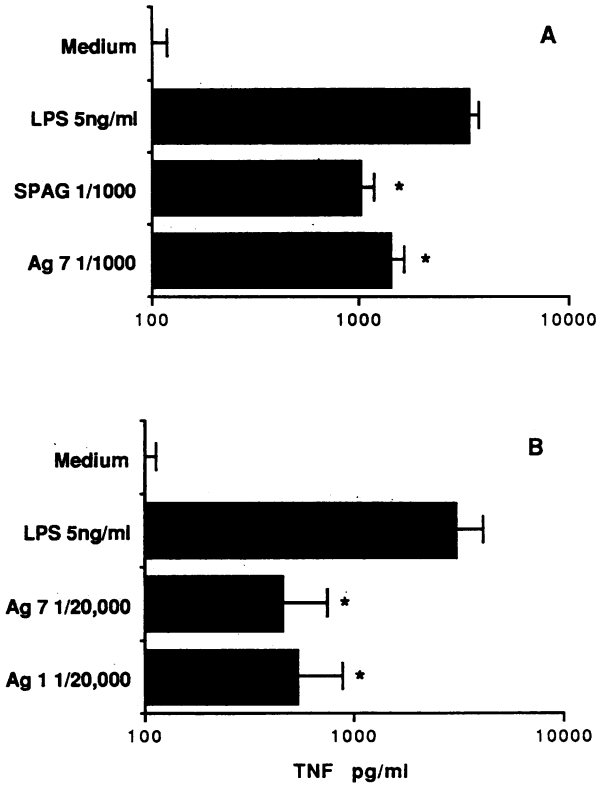

FIG. 1. TNF secretion by human monocytes in response to stimulation by *P. falciparum* exoantigens. Results are means and standard errors of yield of TNF from cells from groups of 28 (A) and 18 (B) Gambian children, as determined by enzyme-linked immunosorbent assay. *, $P < 0.001$, compared with the medium control by a paired *t* test performed on log-transformed data.

Inhibition by antiserum. Previously we observed serological cross-reactions between exoantigens of human and rodent parasites in that TNF release induced by parasite exoantigens present in supernatants of *P. yoelii* and in crude *P. falciparum* culture medium was inhibited by antisera made against the boiled supernatants from both species (1, 29). Similar antisera were therefore tested for their ability to block TNF induction in mouse macrophages in response to SPAG from the F32 isolate of *P. falciparum* to investigate the existence of any serological relationships and also to confirm that macrophage stimulation by the antigens could be distinguished from that by LPS, which was unaffected by antisera. Typical results obtained with one of two pools of antiserum against *P. falciparum* Nf54 are shown in Fig. 3. This serum cross-reacted with *P. yoelii* (Fig. 3a) and at a 1/80 dilution blocked 97% of the activity of a potent *P. yoelii* boiled supernatant (a similar pool blocked 84% of the activity at a 1/400 dilution). When diluted 1/100, it did not significantly inhibit the ability of Ag1 to induce even a few hundred units of TNF (Fig. 3b) but totally prevented TNF production by Ag7 at all antigen concentrations tested (Fig. 3c), suggesting that preparations of rodent parasite exoantigens contain an antigen that is serologically related to Ag7 but not to Ag1.

The results of some representative tests on the capacity of various other antisera to block the ability of the purified *P. falciparum* exoantigens to induce TNF production by mouse macrophages are summarized in Table 2; as a control,

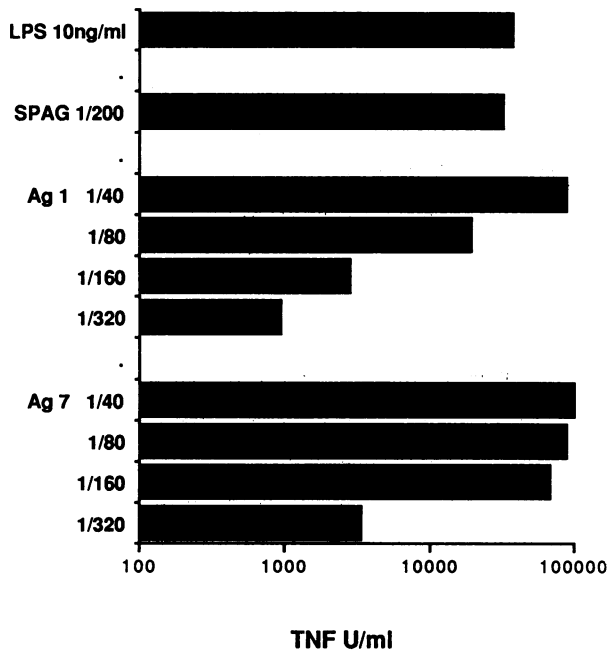

FIG. 2. TNF released by mouse macrophages in response to stimulation by *P. falciparum* exoantigens. Shown are results of typical titrations of TNF production by Ag1 and Ag7 compared with yields of TNF induced by LPS and SPAG. Values are means of duplicates, determined by cytotoxicity assays. Three different preparations of Ag1 and Ag7 and two of SPAG were tested, with similar results.

the activity of each antiserum against a *P. yoelii* antigen preparation that induced comparable yields of TNF is shown. Like antisera made against crude culture supernatants of *P. falciparum* Nf54, antisera made against heat-stable exoantigens of *P. yoelii* completely inhibited the stimulatory effects of Ag7 and of SPAG but did not block the activity of Ag1. Similarly, antiserum made against *P. yoelii* in nude mice cross-reacted with Ag7, suggesting that, like the rodent parasite antigens (1, 26), this antigen too might act

TABLE 1. Effect of polymyxin B on TNF induction in mouse macrophages by *P. falciparum* exoantigens

Sample (dilution)	TNF produced (U/ml)[a]		% Inhibition
	Control	+ Polymyxin B[b]	
SPAG (1/200)	3,438	606	81
Ag1 (1/250)	1,940	1,940	0
Ag7 (1/200)	16,889	1,838	89

[a] Representative results from three experiments.
[b] Used at 10 μg/ml with Ag1 and 5 μg/ml with the other two samples.

as a T-independent antigen. Furthermore, hyperimmune serum from vaccinated mice infected with *P. yoelii* also inhibited the activities of Ag7 and SPAG. None of three different pools of antiserum against *P. yoelii* had any inhibitory effect against Ag1; the sample shown in Table 2, for instance, showed an absence of inhibition even at a fourfold-higher concentration than that which inhibited 83% of the stimulatory effect of the homologous antigen. The batch of SPAG was diluted considerably in these tests (as indicated by the amount of TNF induced), and its inhibition by these antisera and by polymyxin B suggests that Ag1 made only a small contribution to the activity of the unfractionated preparation.

Further evidence of cross-reaction between *P. yoelii* and Ag7, but not Ag1, was obtained with a Liberian immune serum that precipitated only Ag7. At a 1/100 dilution, it reduced the amount of TNF induced by a *P. yoelii* boiled supernatant from 9,700 to 606 U/ml (94% inhibition), but even at a 1/50 dilution it had no significant inhibitory effect on stimulation by Ag1.

DISCUSSION

The ability of soluble parasite antigens known to be present in the sera of malaria patients (16) to induce the release of cytokines by human monocytes and mouse macrophages raises interesting questions, not only about the nature of the antigens and of the moiety which binds to the surface receptor that triggers TNF secretion by the cell but also about their role in pathology and the possibility that

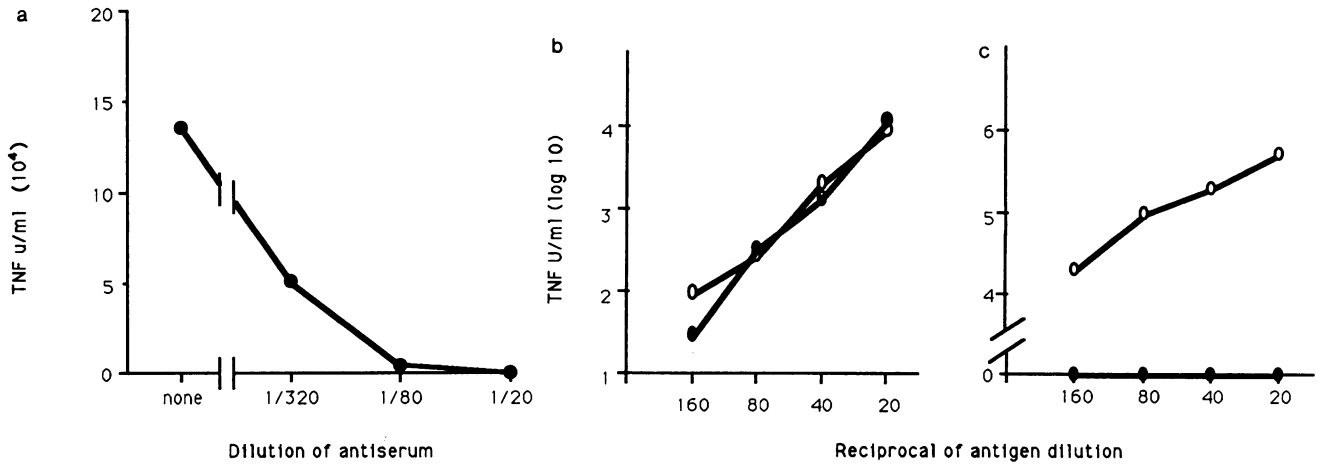

FIG. 3. Effect of antiserum against *P. falciparum* on induction of TNF release from mouse macrophages by parasite exoantigens. (a) Titration of inhibitory effect of antiserum made against boiled culture medium from *P. falciparum* Nf54 on TNF induction by a concentration of *P. yoelii* exoantigens that induced 14,000 U of TNF per ml. (b and c) Effect of a 1/100 dilution of this antiserum on TNF induction by serial dilutions of Ag1 (b) and Ag7 (c). Values are means of duplicates. Similar results were obtained with another antiserum. Symbols: ○, Without antibody; ●, with antibody.

TABLE 2. Inhibition by various mouse antisera of stimulatory activity of *P. falciparum* exoantigens on mouse macrophages compared with *P. yoelii* activity[a]

Stimulating antigen	Antiserum against:	Dilution	TNF (U/ml)		% Inhibition
			Alone	+ Antibody	
P. yoelii	*P. falciparum*	1/80	135,120	4,220	97
	P. yoelii	1/200	102,400	16,889	83
	P. yoelii[b]	1/160	16,889	2,111	87
	P. yoelii[c]	1/40	102,400	3,200	97
Ag1	*P. falciparum*	1/80	117,627	89,144	24 (NS)
	P. yoelii	1/50	33,780	33,780	0
	P. yoelii[c]	1/40	>51,200	>51,200	0
Ag7	*P. falciparum*	1/80	135,120	0	100
	P. yoelii	1/40	16,889	100	99
	P. yoelii[b]	1/160	9,700	400	99
	P. yoelii[c]	1/40	16,889	3,200	81
SPAG	*P. yoelii*	1/40	3,438	0	100
	P. yoelii[c]	1/40	3,438	400	8

[a] Values are means of duplicates. NS, Not significant.
[b] From nude mice.
[c] Hyperimmune serum from vaccinated mice that had recovered from infection. Values for primary antisera against boiled supernatants from parasite cultures are shown except in one case; the sera do not block TNF induction by LPS. At least two samples of antiserum were tested in each case.

they might be used as vaccines to prevent clinical disease (25).

It is now clear that more than one exoantigen purified from *P. falciparum* culture medium can induce the release of TNF. Ag1 and Ag7 are both glycoproteins of 60 and 77 kilodaltons, respectively. The antigenicity of Ag1 appears to depend largely on its carbohydrate component, since treatment with α-D-galactosidase diminished its precipitation by antibody from immune serum; this enzyme also abolished the ability of the mixture of affinity-purified antigens to induce lymphocyte proliferation (15). There is evidence that the TNF-inducing component of the heat-stable antigens of the rodent parasites is not protein, since it is not destroyed by digestion with trypsin or papain, but may be glycolipid in nature (our unpublished work), and it appears to act as a T-independent antigen (1, 28).

Apart from their ability to stimulate the production of TNF, both Ag1 and Ag7 share some other properties with LPS. For example, they both react with *Limulus* amoebocyte lysate (LAL): incubation with LAL before crossed immunoelectrophoresis diminished the precipitate observed with the Ag1 complex, whereas that of Ag7 completely disappeared (13). Similarly, preparations of exoantigens of *P. yoelii* and of *P. berghei* reacted strongly in a chromogenic LAL assay, giving values equivalent to >10 μg of LPS per ml, whereas control preparations from normal erythrocytes were completely negative (C. A. W. Bate, unpublished data).

The interactions of these antigens with polymyxin B present a paradox. They do not necessarily indicate the presence of a lipid A-like structure, as polymyxin is highly charged and may combine with any molecules that have an opposite charge and so influence their behavior. Thus, the presence of polymyxin B during immunoelectrophoresis altered the mobility of Ag1, a molecule that has a strong negative charge (13), but it did not affect its capacity to interact with macrophages and induce them to secrete TNF. Similarly, rodent parasite exoantigens induce TNF release in the presence of polymyxin B, but they nevertheless can bind to polymyxin because their activity is removed by passage through a polymyxin-Sepharose column, though not if they are first digested with a protease (Bate, unpublished data). It appears that the enzyme treatment affects the charge but not the TNF-inducing component of the molecule. On the other hand, the TNF-stimulating capacity of the serologically related Ag7 was blocked by polymyxin, which also caused the disappearance of the Ag7 precipitin line (13). It would seem that reaction with polymyxin B is not always an indicator of contamination with endotoxin, though it provides another useful distinction between Ag1 and Ag7.

The conformations of the active sites on Ag1 and Ag7 that trigger both human and mouse cells also appear to be antigenically different, as the activity of the molecules can be distinguished by the use of inhibitory antisera. Furthermore, the serological cross-reactivity observed between Ag7 and rodent parasite antigens, using antiserum against a different isolate of *P. falciparum*, a human immune serum in which precipitating antibody against only Ag7 could be detected, and antisera made against rodent parasite antigens, suggests that the TNF-triggering site of Ag7 is not subject to enough antigenic polymorphism to affect this function.

We had remarked previously that, like those for LPS, receptors for human and rodent parasite antigens are present on both human monocytes and mouse macrophages and that the binding sites on these antigens must be antigenically similar since their capacity to induce TNF secretion was blocked reciprocally by antisera against parasites of the different species (which did not block the effect of LPS) (29). Our findings with these two purified antigens of *P. falciparum* confirm this suggestion. However, there is reason to believe that the antigens may act via a different cell receptor from LPS since, for example, C3H/HeJ mice, which are hyporesponsive to LPS, secreted TNF normally in response to the antigens (26).

It should be noted that not all parasite exoantigens induce TNF. Another antigen from culture supernatants of *P. falciparum*, protein 126, a product of the parasitophorous vacuole (8), was inactive in our hands when tested as a recombinant protein (our unpublished work with P. Delplace). That it is protein in nature does not in itself preclude it from having activity, since although many TNF-inducing molecules have polysaccharide components, (e.g., LPS, zymosan, and the lipoarabinomannan of *Mycobacterium tuberculosis* [21]), one protein, the toxic shock syndrome toxin 1 of *Staphylococcus aureus*, is also effective (18). We have no information about the activity of other heat-stable soluble antigens, including the heterogeneous assortment referred to as S antigens, some of which have been cloned as recombinant proteins (6). It would seem that the latter are unlikely to be of great importance as inducers of TNF since they are highly polymorphic, a property that is inconsistent with the results of our cross-inhibition experiments between exoantigens of the different *Plasmodium* species. Some S antigens have been described as poorly immunogenic, however, since antibody against them is rarely found in epidemiological surveys, perhaps because they only induce short-lived IgM responses (7).

Apart from these antigens in culture medium of *P. falciparum* that have already been identified (16) but not yet tested, we should point out that still others may not have been selected by the IgG-based affinity purification system we used, which would exclude antigens that induce mainly T-independent antibody of the IgM isotype (1), and it is possible that some of them could in fact be important stimulators of cytokine production and a major source of pathology. There is evidence that the appearance of precip-

itating antibody against Ag7 in the sera of individuals in the Gambia may be transient, suggesting that like a T-independent antigen, it may not be capable of inducing memory and that its appearance in the sera of Gambian children coincides with their development of clinical immunity against malaria (Jakobsen et al., Trans. R. Soc. Trop. Med. Hyg., in press). This antigen would therefore seem to be an interesting candidate for use in an antitoxic or antidisease vaccine (25). It will be necessary to explore and, if necessary, develop the interaction of this antibody with T cells (25), and its serological cross-reaction with rodent exoantigens may provide a useful tool for further investigation of its significance in the field.

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