INFECTION AND IMMUNITY, Dec. 1990, p. 3996–4003
0019-9567/90/123996-08$02.00/0

Vol. 58, No. 12

Production of Tumor Necrosis Factors Alpha and Beta by Human Mononuclear Leukocytes Stimulated with Mitogens, Bacteria, and Malarial Parasites

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Received 16 March 1990/Accepted 13 September 1990

Tumor necrosis factors alpha and beta (TNF-α and TNF-β) are multifaceted polypeptide cytokines which may mediate some of the significant changes in cellular homeostasis which accompany the invasion of the mammalian host by viruses, bacteria, and parasites. Although it is well established that bacterial lipopolysaccharide is a potent inducer of TNF-α, there is still very little known of the types of agents which can trigger the production of TNFs in mononuclear leukocytes. Using an enzyme-linked immunosorbent assay for measuring TNF-α and TNF-β, we examined the capacity of various T-lymphocyte and B-lymphocyte mitogens as well as microbial components to stimulate production of these cytokines in culture. The mitogens phytohemagglutinin, concanavalin A, and pokeweed mitogen induced production of both TNF-α and TNF-β, while whole-killed *Staphylococcus aureus* and *Bordetella pertussis*, like lipopolysaccharide, were potent inducers of TNF-α but failed to stimulate TNF-β production. TNF-α production was detectable within 1 h after stimulation, while TNF-β production was not detected until after 8 h of culture. The bacterial products tetanus toxoid, purified protein derivative, pertussis filamentous hemagglutinin, and pertussis toxin were all able to induce TNF-α and TNF-β production. Disrupted (frozen-thawed) *Plasmodium falciparum*-infected erythrocytes were also potent inducers of TNF-α and TNF-β. The results demonstrated that a wide variety of microbial components are inducers of TNF-α. Some may not only be more effective than lipopolysaccharide but can also induce TNF-β production. Furthermore, evidence is presented showing that TNF-β but not TNF-α production correlates with lymphoproliferation.

Tumor necrosis factors alpha and beta (TNF-α and TNF-β), also known as cachectin and lymphotoxin, respectively, have a wide range of biological effects (3, 26, 47). These cytokines appear to play a central role in the profound deranged homeostastis which accompanies the invasion of the mammalian host by infectious agents such as viruses, bacteria, and parasites. TNFs are most likely the major mediators of septic shock. Their causation of major hemodynamic changes in the host is not surprising in view of their marked effects on many cell types, including fibroblasts, endothelial cells, adipocytes, hepatocytes, synoviocytes, and tissues such as bone, hypothalamus, and muscle (3, 24, 26).

Besides their role in pathophysiology, it is likely that the principal reason for their evolutionary conservation is related to their role in defense against cancer and infections. TNFs inhibit growth of malignant cells and display antiviral activity (2, 3, 26, 33, 35) and prime effector cells such as neutrophils (11, 14, 15, 18, 19, 23, 28, 29, 29a, 37, 44, 45) and macrophages (30, 49) for enhanced antimicrobial activity and increased tissue damage.

It is therefore important to gain an understanding of the ability of microbial structures to elicit production of TNFs by macrophages and lymphocytes. In this investigation, different types of lectin mitogens and microbial antigens were examined and compared for their capacity to induce the release of TNF-α and TNF-β from human mononuclear leukocytes (MNL).

MATERIALS AND METHODS

Cell stimulators. Three mitogens were used to stimulate human MNL, phytohemagglutinin (PHA), concanavalin A (ConA), and pokeweed mitogen (PWM) (Wellcome Pharmaceuticals and Reagents Pty. Ltd., Sydney, Australia; Calbiochem-Behring, Sydney, Australia; and GIBCO Laboratories, Grand Island, N.Y., respectively). These were used as 1 μg of PHA per ml, 25 μg of ConA per ml, and a 1/100 dilution of PWM (catalog no. 670-5360) unless specified otherwise. All reagents were made up in RPMI 1640 medium supplemented with heat-inactivated 2.5% human AB serum. Whole Formalin-fixed heat-killed bacteria (*Staphylococcus aureus* NCTC 6571 and *Bordetella pertussis* NCTL 10908) were prepared as described previously (4, 17), adjusted to 10^8 organisms per ml, and mixed with buffered formaldehyde (1.25% final) for 3 h at room temperature and then heat treated at 80°C for 3 min. The bacteria were washed in RPMI 1650 medium and stored at −20°C. These were used at a ratio of 10 bacteria to 1 MNL unless specified differently.

Pertussis toxin was >98% pure (by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and enzyme-linked immunosorbent assay). Tetanus toxin batch 003 (5,184 limit flocculation units [LF] per ml; 2.2 mg of protein per ml) (40) and tuberculin purified protein derivative (PPD) (batch 999.903-51) in 0.85% (wt/vol) saline at 2 mg/ml were all obtained from Commonwealth Serum Laboratories (Melbourne, Australia).

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Plasmodium falciparum (FCQ 27/PNG K^- strain) was maintained in blood group O^+ erythrocytes essentially as described previously (31). The infected erythrocytes containing 4% parasitemia were enriched to 80% parasitemia by isopycnic centrifugation in Percoll (Flow Laboratories, Sydney, Australia) as previously described (41). The band containing the schizont stage of the parasite was collected, washed twice, and disrupted by freezing and thawing.

Cell cultures. MNL were prepared from blood of healthy volunteers by centrifugation on Hypaque-Ficoll (22). To 100 μl of 2×10^6 MNL per ml was added 100 μl of RPMI 1640 medium (containing 5% human group AB serum) or 100 μl of the stimulator in the same medium. These cultures were set up in U-bottom microtiter plates (Linbro; Flow Laboratories). The cells were cultured for the periods indicated in the Results in an atmosphere of 5% CO_2 and air and high humidity. In some experiments, the cultures were pulsed with 1 μCi of [^{3}H]thymidine ([^{3}H]TdR) (25 μl) (Amersham, Sydney, Australia) 6 h prior to harvesting the cells. Harvesting was performed with a Titertek cell harvester (Flow Laboratories). The radioactivity was measured in a scintillation counter (Beckman Instruments, Inc., Palo Alto, Calif.). To test for TNF-α and TNF-β production, we collected the supernatants from cell cultures just prior to harvesting. The supernatants were stored at −70°C and usually measured for TNF levels within 1 week.

TNFs. Recombinant human TNFs (rHuTNFs) were produced by Genentech Inc. (San Francisco, Calif.) and supplied by Boehringer Ingleheim. TNF-α derived from *Escherichia coli* contained a specific activity of 6×10^7 U/mg (assessed by a bioassay, murine L-M cells). The cytokine was >99% pure and contained <0.125 endotoxin units (EU) per ml by the *Limulus* amoebocyte lysate assay. rHuTNF-β was also derived from *E. coli* and had a specific activity of 1×10^8 to 2×10^8 U/mg by the same assay as above. This is an unglycosylated protein with a molecular weight of 16,000 and lacks the 23 N-terminal amino acids of the natural proteins. These were diluted in phosphate-buffered saline containing 10% sheep serum for use in the enzyme-linked immunosorbent assay.

Monoclonal antibodies and antiserum. Mouse monoclonal antibody (TNF-E) against rHuTNF-α (6,000 neutralizing units of TNF-α) was of the immunoglobulin G1 (IgG1) isotype and reconstituted to 1.7 mg/ml of PBS (Ernst-Boehringer Institute). A murine monoclonal antibody (LTX-9) was raised against human TNF-β and was of the IgGl isotype (3.5 μg/ml in phosphate-buffered saline).

The rabbit antiserum to rHuTNF-α contained approximately 3×10^5 to 10×10^5 U of TNF-α-neutralizing capacity per ml, and rabbit antiserum to rHuTNF-β contained 2.9×10^7 U of TNF-β-neutralizing capacity per ml. The mouse monoclonal antibody to TNF-α and both of the rabbit antisera were developed by Genentech (7).

Enzyme-linked immunosorbent assay for measuring TNF-α and TNF-β. The enzyme-linked immunosorbent assay used was a monoclonal capture method which has been used previously in our laboratory for measuring human IgG subclasses (16, 20). Microtiter plates (Cooke; Dynatech Laboratories, Chantilly, Va.) were coated with the IgG fraction of goat anti-mouse IgG (Cappel, Malvern, Pa.), and after incubation for 18 to 20 h at 4°C, the TNF monoclonal antibody was added to each well. The plates were then incubated at 37°C for 3 h, and then either dilutions of standard TNFs or unknown (conditioned media) was added. After a further incubation at 4°C for 18 to 20 h, rabbit anti-TNF antiserum was added and the mixture was incubated at 37°C for 3 h. This was followed by the addition of 60 μl of sheep anti-rabbit immunoglobulin–horseradish peroxidase conjugate (Silenus, Melbourne, Australia) and, after a 3-h incubation at 37°C and washing, the substrate 2,2′-azino-di-[3-ethylbenzthiazoline sulphonate-(6)] (ABTS) (Boehringer Mannheim). The color reaction was allowed to proceed at 37°C for 45 to 60 min, and the A_{414} was measured in a Titertek Multiskan spectrophotometer (Flow Laboratories).

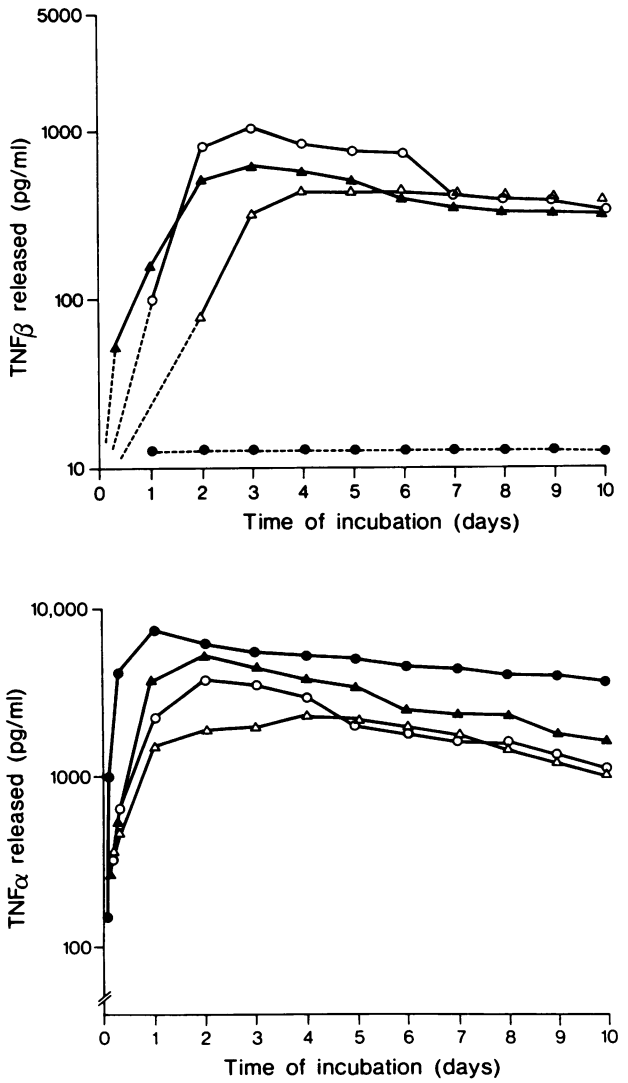

FIG. 1. Production of TNF-α and TNF-β by MNL stimulated with *S. aureus* (●), PHA (○), ConA (▲), or PWM (△). Shown is an experiment representative of two others with cells from different individuals.

The TNF-α assay was unable to detect TNF-β and vice versa. Both the TNF-α and TNF-β assays were unable to detect human interleukin-1α, human interleukin-1β, human interleukin-2, and human gamma interferon (data not presented).

The assay measured equivalently TNFs that were purified (e.g., as recombinant forms) as well as those present in conditioned medium (data not presented).

Statistics. Data were analyzed by either multiple regression or the two-tailed *t* test.

TABLE 1. Production of TNFs by mitogen-stimulated MNL[a]

Stimulant (no. of expts)	[^{3}H]TdR incorporation (dpm)	TNF-α (pg/ml) (range)	TNF-β (pg/ml) (range)
None (38)	1,813 ± 170	238 ± 40 (UD–922)[b]	UD
PHA (38)	220,295 ± 22,085	5,346 ± 539 (1,425–19,284)	1,799 ± 181 (22–5,755)
PWM (38)	96,033 ± 9,614	2,462 ± 175 (528–5,409)	669 ± 91 (480–2,488)
ConA (38)	89,756 ± 16,187	3,901 ± 371 (887–9,167)	940 ± 103 (54–3,210)
S. aureus (9)	11,873 ± 4,366	17,784 ± 1,540 (9,575–23,050)	UD

[a] Three-day cultures and results are mean ± standard deviation of the results from the number of experiments indicated in parentheses, each from a separate individual.
[b] UD, Undetectable.

RESULTS

E. coli lipopolysaccharide (LPS), as expected, was found to be a good inducer of TNF-α but failed to stimulate production of TNF-β. LPS was active in inducing TNF-α over a very wide concentration range of 1 ng to 50 μg/ml (data not presented). TNF-α production on day 3 of culture peaked at approximately 12.5 μg/ml (ranging from 2,000 to 4,000 pg of TNF-α per ml). Further increases in LPS did not increase TNF-α production.

The mitogens PHA, ConA, PWM, and *S. aureus*, which stimulate different subpopulations of lymphocytes, have been used as standard agents for stimulating lymphocyte proliferation in culture. Both PHA and ConA are T-cell mitogens which react with different subpopulations of T lymphocytes (48). PWM stimulates B-lymphocyte proliferation in a T-cell-dependent manner (48), and *S. aureus* is a B-cell mitogen which does not appear to require T-cell help (4). These were studied for their capacity to stimulate TNF-α and TNF-β production. While all four agents stimulated production of TNF-α, only PHA, ConA, and PWM stimulated production of TNF-β (Fig. 1, and Table 1). TNF-α but not TNF-β production occurred in the absence of a stimulus. Even after many days of culture, the leukocytes failed to produce TNF-β in the absence of mitogens. TNF-α production was evident within 1 h of stimulation and reached maximum levels within 1 to 2 days with only a slight decline thereafter over a culture period of 10 days (Fig. 1). This occurred with respect to all four mitogens. However, TNF-β

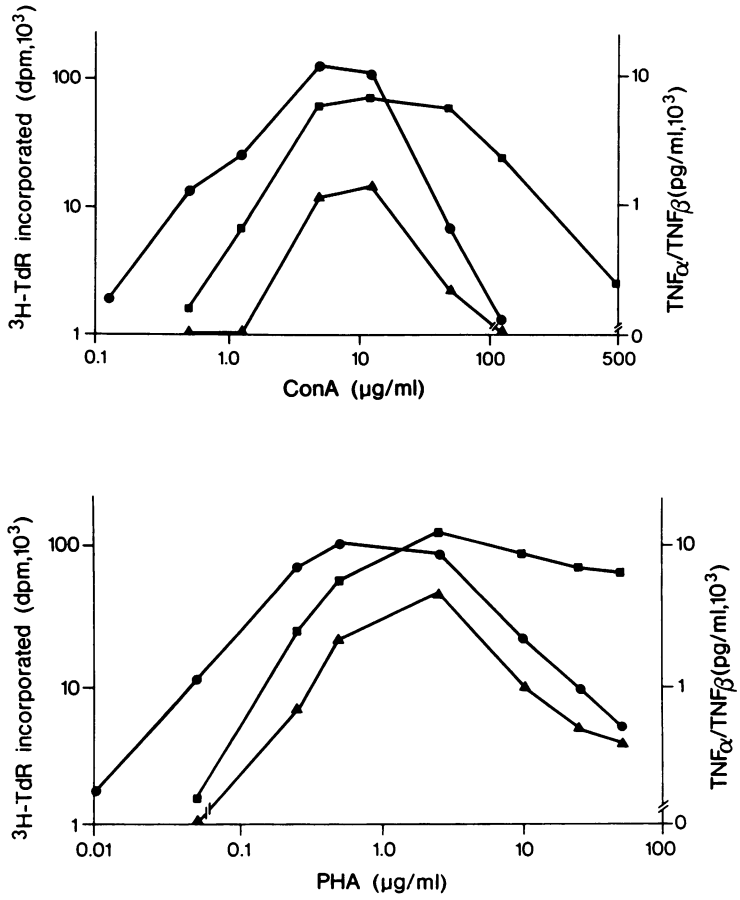

FIG. 2. Lymphoproliferation (●) and TNF-α (■) and TNF-β (▲) production by MNL stimulated with various concentrations of either PHA or ConA. Shown is an experiment representative of two to three others with cells from different individuals.

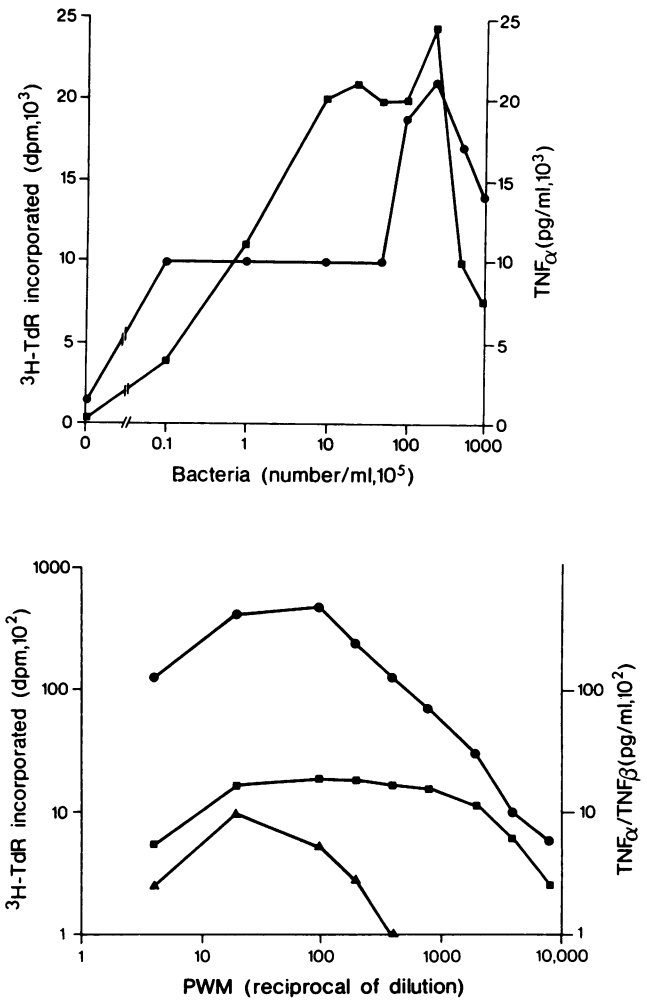

FIG. 3. Lymphoproliferation (●) and TNF-α (■) and TNF-β (▲) production by MNL stimulated with various concentrations of either PWM or *S. aureus*. Shown is an experiment representative of two others with cells from different individuals.

production, although detectable in the first few hours, required at least 24 h to reach appreciable levels (Fig. 1). The concentrations did not peak until 3 days, and these levels were essentially maintained for the 10-day period of culture.

Under the culture conditions described here, ConA and PWM produced similar concentrations of TNF-α and TNF-β (Table 1). Significantly more TNF-α was produced in the presence of PHA ($P < 0.001$). This mitogen also induced greater production of TNF-β. MNL stimulated with *S. aureus* produced much higher levels of TNF-α than cells stimulated by any of the mitogens, but no TNF-β was detected in the same cell preparation.

Results of studies using different concentrations of mitogens are presented in Fig. 2 and 3. Studies with PHA showed that peak lymphocyte proliferation and peak TNF-α production did not occur coincidentally (Fig. 2). As the mitogen concentration was reduced, a related decline in TNF-α production was observed. However, when the PHA concentration was increased above the optimal concentration, there occurred a decrease in lymphocyte proliferation, but there was no drop in TNF-α production (Fig. 2). For example, at 50 μg/ml, the number of disintegrations per minute of [^{3}H]TdR incorporated by cells was 5,137 compared with 109,626 at 0.5 μg/ml, while the TNF-α production dropped only from 12,560 dpm at 2.5 μg/ml to 5,828 dpm at 50 μg/ml. TNF-β measurements showed that peak production of this cytokine occurred at 2.5 μg/ml (Fig. 2). In this case, there was a closely related decline between TNF-β production and [^{3}H]TdR incorporation at the mitogen concentrations studied.

With cells from the same individual, it was found that peak ConA-induced lymphoproliferation and TNF-α and TNF-β production occurred at 5 to 12.5 μg/ml (Fig. 2). It was evident from these results that as the lymphoproliferation sharply decreased at above the optimal ConA concentration, TNF-α continued to be produced at maximal levels, e.g., at concentrations of 50 μg/ml (Fig. 2).

Studies with PWM showed that lymphocyte proliferation correlated well with TNF-β production but not with TNF-α production (Fig. 3). Results obtained with *S. aureus* ranging from 10^4 to 10^8 bacteria per ml are presented in Fig. 3. Lymphocyte proliferation was constant between 1×10^4 and 5×10^6 bacteria and subsequently increased sharply, peaking at 2.5×10^7 bacteria, and then decreased markedly when the number of bacteria was increased to 1×10^8. In contrast, there was marked production of TNF-α, which remained at substantial levels over a bacteria concentration range of 1×10^6 to 2.5×10^7/ml. There was no production of TNF-β at all bacterial concentrations tested.

Correlations between either TNF-α or TNF-β production and the degree of lymphoproliferation and also correlations between TNF-α and TNF-β production were evaluated. Examination with PHA as a stimulus showed that there was no correlation between lymphoproliferation and TNF-α production (for 44 pairs of data, $y = x + 215{,}306$; $r = -0.01$) or between the production of TNF-α and TNF-β ($y = x + 4.4$; $r = 0.19$). However, there was a significant correlation between lymphoproliferation and TNF-β ($y = x + 116{,}000$; $r = 0.43$) ($P < 0.0036$). With MNL stimulated with PWM, again there was no correlation between lymphoproliferation and TNF-α ($y = x + 77{,}457$; $r = 0.11$) or between TNF-α and TNF-β production ($y = x + 2.3$; $r = 0.06$), but a significant correlation between lymphoproliferation and TNF-β was observed ($y = x + 69{,}300$; $r = 0.32$) ($P > 0.034$). In contrast to results with PHA and PWM, studies with ConA showed a significant correlation between production of TNF-α and TNF-β ($y = x + 1{,}900$; $r = 0.60$) ($P < 0.001$) but showed no correlation between lymphoproliferation and TNF-α ($y = x + 200{,}000$, $r = -0.12$) or TNF-β ($y = x + 166{,}000$; $r = 0.10$). There was no significant correlation between *S. aureus*-induced lymphoproliferation and production of TNF-α ($y = x + 17{,}565$; $r = 0.24$).

Whole Formalin-fixed heat-killed *B. pertussis*, like *S. aureus*, induced a marked TNF-α response in human MNL, but no TNF-β was detectable (Fig. 4). Studies with pertussis toxin showed that this molecule was a highly potent inducer of both TNF-α and TNF-β (Fig. 5). The toxin also induced marked lymphocyte proliferation. TNF-β production but not TNF-α production parallelled the lymphoproliferative activity.

Other bacterial components were also inducers of TNFs. MNL stimulated with PPD showed substantial lymphoproliferation and produced both TNF-α and TNF-β (Table 2). TNF-α production was more prominent. However, TNF-β production appeared to correspond to the degree of lymphocyte proliferation. Cells from one of the three individuals tested had a very poor TNF-α response. Tetanus toxoid

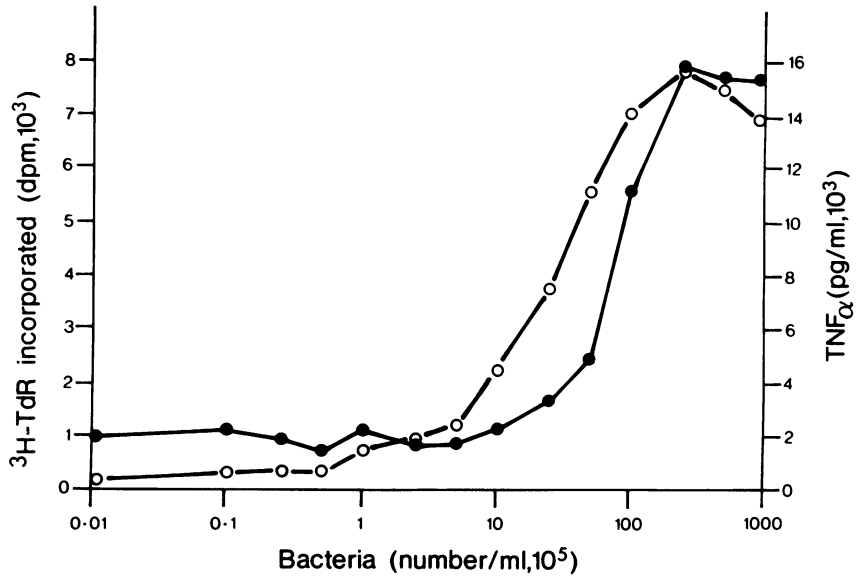

FIG. 4. Stimulation of lymphoproliferation (●) and TNF-α production (○) by *B. pertussis*. Culture supernatants were sampled for cytokine measurements after 3 days of culture. Shown is an experiment representative of two others with cells from different individuals.

antigen was mitogenic for human lymphocytes but caused very little production of TNF-α and TNF-β (Table 3).

P. falciparum stimulated production of both TNF-α and TNF-β (Fig. 6). In these studies, MNL were cultured in the presence of frozen-thawed *P. falciparum*-infected erythrocytes. The parasite preparation was also mitogenic, inducing substantial lymphoproliferation.

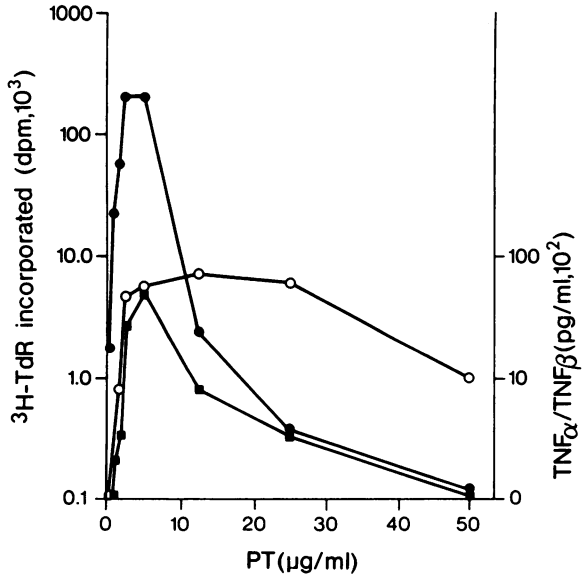

FIG. 5. Stimulation of lymphoproliferation (●) and TNF-α production (○) and TNF-β production (■) by pertussis toxin (PT). Culture supernatants were sampled for cytokine measurements after 3 days of culture. Shown is an experiment representative of two others with cells from different individuals.

DISCUSSION

A comparison of the abilities of various MNL stimulators to induce TNFs is summarized in Table 4. The data demonstrate that the mitogens PHA, ConA, and PWM are potent inducers of production of TNFs from MNL and that *S. aureus* induces TNF-α from MNL. These stimulators induced persistent production of TNFs in culture. While PHA, ConA, and PWM induced production of both TNF-α and TNF-β, *S. aureus* induced TNF-α but not TNF-β. It is interesting that the first three mitogens stimulate T cells, while *S. aureus* is exclusively a B-cell mitogen (4). This may be consistent with the view that TNF-β is mainly produced by activated T lymphocytes (26). The results suggest that

TABLE 2. Stimulation of MNL with PPD and production of TNF-α and TNF-β[a]

PPD conc (μg/ml)	[^{3}H]TdR incorporation (dpm)	TNF-α (pg/ml)	TNF-β (pg/ml)
Expt 1			
0	1,409 ± 239	430 ± 28	UD[b]
1	13,124 ± 5,889	1,046 ± 50	244 ± 33
10	24,592 ± 2,015	1,124 ± 16	102 ± 42
100	19,458 ± 2,896	735 ± 71	41 ± 22
Expt 2			
0	1,992 ± 897	12 ± 12	55 ± 47
1	35,612 ± 6,401	83 ± 73	290 ± 37
10	40,315 ± 10,724	128 ± 49	197 ± 20
100	32,876 ± 4,226	87 ± 41	148 ± 33
Expt 3			
0	2,875 ± 1,052	98 ± 27	59 ± 36
1	4,290 ± 1,289	863 ± 29	25 ± 20
10	7,262 ± 3,871	741 ± 100	35 ± 39
100	3,794 ± 1,361	209 ± 28	89 ± 7

[a] Measurements were made day 6 of culture, and results are expressed as mean ± standard deviation of six cultures.
[b] UD, Undetectable.

TABLE 3. Stimulation of MNL by tetanus toxoid (TT) and production of TNF-α and TNF-β[a]

Treatment[b]	[3H]TdR uptake (dpm)	TNF-α (pg/ml)	TNF-β (pg/ml)
Expt 1			
None	1,409 ± 239	430 ± 28	UD[c]
TT	16,797 ± 1,572	381 ± 25	UD
Expt 2			
None	1,992 ± 897	12 ± 12	55 ± 47
TT	17,400 ± 4,013	UD	132 ± 53
Expt 3			
None	2,875 ± 1,052	98 ± 27	59 ± 36
TT	31,083 ± 4,117	257 ± 28	191 ± 16

[a] Studies represent 6-day cultures, and results are expressed as mean ± standard deviation of triplicate cultures.
[b] Cells were treated with 10 LF of tetanus toxoid.
[c] UD, Undetectable.

polyclonal B-cell activators such as *S. aureus* are unlikely to induce production of TNF-β. Both monocytes and B lymphocytes could be the source of TNF-α after stimulation with *S. aureus*. While it is clearly recognized that purified macrophages respond to *S. aureus* in producing TNF-α (20), B cells have also been shown to be capable of being stimulated to produce TNF-α (46). However, since TNF-α levels did not correlate with lymphoproliferation even when a B-cell mitogen such as *S. aureus* was used, it is most likely that the monocyte-macrophage is the major contributor of TNF-α, particularly in view of the rapid nature of the response. TNF-α was detectable within 30 to 60 min after the addition of *S. aureus*. Production of TNF-α is unlikely to be associated with LPS contamination of these preparations. Mitogen concentration-related effects on TNF-α production showed that the higher concentrations of mitogens were less effective in inducing TNF-α. If LPS contamination was a major contributor of the peak response, then at the higher

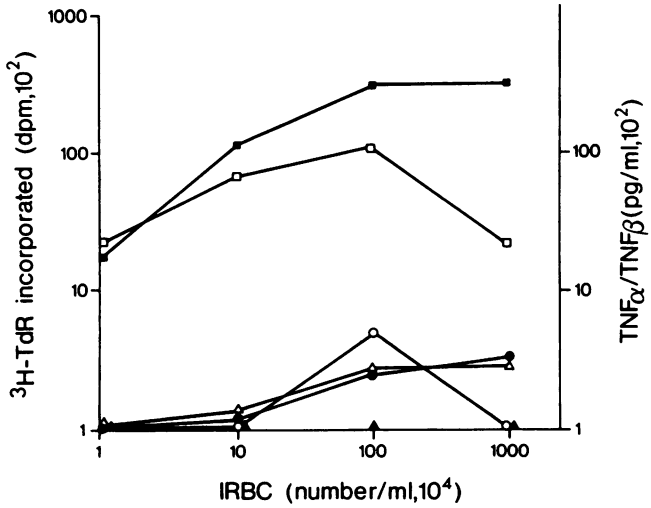

FIG. 6. Stimulation of lymphoproliferation (■) and TNF-α (□) and TNF-β (○) production by *P. falciparum*. Equivalent concentrations of erythrocytes affected lymphoproliferation (△), TNF-α (○, and TNF-β (▲) as shown. Culture supernatants were sampled for, cytokine measurements after 3 days of culture. Shown is an experiment representative of two others with cells from different individuals.

TABLE 4. Comparisons of abilities of various MNL stimulators to induce production of TNFs

Stimulator	TNF produced[a]	
	TNF-α	TNF-β
Lectins		
PHA	++++	++±
PWM	+±	+±
ConA	++	++
Bacteria or products		
S. aureus	+++++	−
B. pertussis	+++++	−
LPS	++	−
Pertussis toxin	+++	++±
PPD	+	±
Tetanus toxoid	±	±
P. falciparum	+++	++

[a] Arbitrary score of the relative production of TNFs: the highest (+++++) to undetectable (−).

mitogen concentrations, sustained production would have been expected. Indeed, comparisons of the stimulatory effects of LPS and *S. aureus* showed that the latter was a much more potent stimulus in inducing TNF-α. Since LPS was unable to stimulate TNF-β, it is feasible that LPS contamination of our stimuli is unlikely to be responsible for their capacity to induce production of this cytokine.

The data showed that the production of TNF-α precedes production of TNF-β irrespective of the stimuli. In addition, the levels of TNF-β produced were much lower than those of TNF-α. These findings are consistent with the view that TNF-α and TNF-β are mainly products of different cell types and that when production of these occurs in the same cell type the cytokines are independently regulated (1, 9). Since ConA is a stimulator of a T-cell subset which has suppressor function, the results suggest that T suppressor cells, which probably play a cytotoxic effector function, produce TNF-β. Therefore, TNF-β could be one mediator of T cells mediating cytotoxicity against tumor cells and virus-infected cells.

Although there appeared to be a similar degree of lymphoproliferation with the mitogens ConA and PHA, there was 2.5-fold-more TNF-β production when PHA was used. The results suggest that PHA stimulates subsets of T cells which are better producers of TNF-β than those activated by ConA. Alternatively, ConA may induce T-cell suppressor activity which may regulate production of TNF-β. There was no correlation between lymphoproliferation and TNF-α production in MNL stimulated with any of the mitogens (PHA, PWM, ConA, and *S. aureus*). Thus, although these mitogens stimulate T lymphocytes and B lymphocytes, it is evident that these cells make a minor contribution to the TNF-α produced compared with the monocytes or macrophages. In contrast, there was a strong correlation between lymphoproliferation and TNF-β production for PHA and PWM. Since PHA is a strong T-cell stimulator and TNF-β is predominantly a T-cell product, this finding is not surprising. PWM is a T-cell-dependent B-cell mitogen, and presumably considerable T-cell stimulation occurs which results in significant production of TNF-β. Surprisingly, there was no correlation between lymphoproliferation and TNF-β production in MNL stimulated with the T-cell mitogen ConA. However, it is well known that PHA and ConA stimulate different subpopulations of T cells. Thus, the result suggests that ConA stimulates a proportion of T cells which either do

not produce TNF-β or suppress TNF-β production. This finding could be relevant to the murine system, in which the activation of the T helper 1 subset leads to production of TNF-β, while activation of the T helper 2 subset does not.

Further evidence that the two cytokines are produced by different cell populations in MNL is shown by the lack of correlation between levels of TNF-α and TNF-β produced in response to PHA and PWM. In contrast, there was a marked correlation between the TNF-α and TNF-β levels produced in response to ConA. This may be due to the fact that ConA is also a stimulus for macrophages.

Microbial products which are polyclonal T-cell activators have been described previously (10, 21, 43). The capacity of these microbial mitogens to stimulate TNF-β production could contribute significantly to the pathophysiology of various parasitic diseases. The importance of TNF-α in the pathophysiology of malaria infections is widely accepted (8, 42). Recently, Bate et al. (5, 6) have demonstrated that *Plasmodium yoelii*- and *Plasmodium berghei*-infected erythrocytes and soluble malarial antigens induce release of TNF-α from normal mouse macrophages. We now report that the human malarial parasite *P. falciparum* is also capable of inducing TNF-α and TNF-β from human MNL. The stimulus was parasitized erythrocytes. In the studies by Bate et al. (5), mouse macrophages which had been activated with gamma interferon were better releasers of TNF-α when stimulated with malarial parasites. Thus, it is conceivable that activated human macrophages release greater amounts of TNF-α in response to *P. falciparum* than observed in the present study. It is not surprising that *P. falciparum* was able to induce production of TNF-β from MNL of individuals not exposed to malaria, since mitogenic activity with *P. falciparum* preparations has previously been identified (25, 50). Of great interest are recent findings from our laboratory which showed that TNF-α (31) and TNF-β (L. M. Kumaratilake, A. Ferrante, and C. Rzepczyk, J. Immunol., in press) augmented the neutrophil-mediated killing of *P. falciparum*.

Antigens from other bacteria were also capable of stimulating production of TNFs. PPD stimulated proliferation of human lymphocytes and production of TNF-α and TNF-β in a concentration-dependent manner. There was a good correlation between lymphocyte proliferation and TNF-β production. This shows that for this antigen, TNF-β measurements may be a good marker for T-cell activation.

Studies with tetanus toxoid antigen showed that different individuals gave various responses to the antigen. However as with PPD, there was no correlation between the lymphoproliferative response and TNF-α production.

Although one of three individuals tested failed to produce detectable TNF-β levels, studies with the other two individuals demonstrated enhanced production of TNF-β in the presence of tetanus toxoid. Pertussis toxin was found to be a better inducer of TNF-α than either PPD or tetanus toxoid. In fact, pertussis toxin also induced a marked lymphoproliferative response which paralleled a substantial production of TNF-β This preparation was highly pure, with the main contaminant being the filamentous hemagglutinin. However, we found this agglutinin to be a poor stimulator of TNF-α, TNF-β, and lymphocyte proliferation (unpublished data) compared with the pertussis toxin. It is also unlikely that pertussis LPS is responsible for these effects since the activity of the toxin was much greater than that of LPS. Furthermore, the preparation induced TNF-β production and was mitogenic for human lymphocytes. Pertussis toxin is a major virulence factor of *B. pertussis*. Our finding that it can induce both TNF-α and TNF-β production may be pertinent to its role in the systemic manifestation of the disease, which includes weight loss, hyperinsulinemia, and hypoglycemia (32, 38, 39).

Other investigators have reported the capacity of other microbial structures or toxins to induce TNF-α production by human MNL. This includes toxic shock syndrome-associated staphylococcal and streptococcal exotoxins (12, 13, 27, 36) and the liporabinomannan of *Mycobacterium tuberculosis* (34). Our findings extend the significance of these reports by showing that a range of unrelated microbial components induce TNF-α production but that in addition these can also induce TNF-β production. This suggests that when T cells are prominent at inflammatory sites, release of TNF-β is of major importance.

TNFs have a wide range of effects on components of the inflammatory response. TNFs increase neutrophil adherence, inhibit neutrophil migration, and activate neutrophil antimicrobial or tissue-damaging properties. Since microbial components besides LPS can readily release TNF-α even in the absence of specific immunity and to the same degree as LPS, the present findings have wide implications in the pathophysiology of infections.

ACKNOWLEDGMENTS

This work was supported by grants from the National Health and Medical Research Council of Australia, the UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases, and the Adelaide Children's Hospital Research Foundation.

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