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Involvement of Tumor Necrosis Factor Alpha in Intracellular Multiplication of *Legionella pneumophila* in Human Monocytes

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We investigated the role of tumor necrosis factor alpha (TNF-α) in human peripheral monocytes infected with *Legionella pneumophila* in vitro. Exogenous TNF-α significantly inhibited the intracellular multiplication of the bacterium. This effect was concentration and time dependent and was abrogated by anti-TNF antibodies. TNF-α levels in the culture supernatants were low but were enhanced by the addition of gamma interferon. When monocytes were cultured and infected in the presence of pentoxyphilline, a potent inhibitor of TNF-α synthesis, the intracellular bacterial growth was enhanced. The effect of pentoxyphilline was concentration and time dependent and was due to the inhibition of TNF-α production, as shown by Northern (RNA) blot hybridization of total RNA. In addition, the pentoxyphilline partially abolished the inhibitory effect of gamma interferon on bacterial intracellular multiplication. These results suggest that gamma interferon inhibits, at least partially, the intracellular multiplication of *L. pneumophila* by enhancing TNF-α synthesis.

Tumor necrosis factor (TNF), an immunostimulatory cytokine, has multiple biological properties (antitumor effect, cytostatic and cytolytic effects, and weight loss induction) (7). TNF has been reported to enhance the phagocytic activity of human polymorphonuclear neutrophils and to mediate their adherence to endothelial cells (20), to mediate natural cytotoxic cell activity (17), and to stimulate the antiparasitic activity of eosinophils (21). TNF also participates in the cytokine cascade during the immune response (10), acting synergistically with gamma interferon (IFN-γ). TNF and IFN-γ have been found to activate macrophages and enhance their antitumor and antimicrobial activities (12).

Legionella pneumophila is a gram-negative facultative intracellular pathogen that causes pneumonia (11). *L. pneumophila* can infect human monocytes and alveolar macrophages in vitro and in vivo (15). The host may limit infection to survive. Intracellular multiplication can be inhibited by IFN-γ activation of alveolar macrophages (16). As TNF is produced in the lungs of *L. pneumophila*-infected mice (3), it may participate in limiting the infection. We therefore evaluated TNF production and its role in monocytes infected by *L. pneumophila* in vitro.

MATERIALS AND METHODS

Bacteria. *L. pneumophila* serogroup 1 was isolated from the lung of a patient who died from Legionnaires' disease. A bronchoalveolar aspirate was plated on buffered charcoal-yeast extract agar supplemented with α-ketoglutarate and antibiotics. Plates were incubated at 30°C with 2.5% CO_2 for 3 days. A single colony was then taken and plated onto buffered charcoal-yeast extract medium supplemented with α-ketoglutarate for another 3 days. The bacteria were harvested in sterile distilled water and tested for viability. The suspension was adjusted to 10^9 bacteria per ml, divided into aliquots, and stored at −70°C until use.

Reagents. Recombinant TNF-α (10^5 U/ml) and human IFN-γ (50 μg/ml), a kind gift from Roussel-Uclaf (Romainville, France), were stored at −70°C until use. Pentoxyphilline (PTX), a kind gift from Hoechst (Puteaux, France), was diluted in sterile distilled water and sterilized through a 0.22-μm-pore-size filter; it was stored at −20°C until use. Monoclonal anti-TNF-α antibodies were a kind gift from Knoll BASF Laboratories (Munich, Germany) and neutralized 1 U of TNF-α at a 5×10^{-2} dilution.

Preparation of monocytes. Venous blood was obtained from normal volunteers and diluted one-half in RPMI 1640 medium. It was then layered on MSL solution (Eurobio, Paris, France) and centrifuged for 30 min at room temperature. The band of peripheral blood leukocytes was collected and washed twice by centrifugation in RPMI 1640 medium. The pellet was resuspended in RPMI supplemented with 5% heat-inactivated fetal calf serum and 100 mM L-glutamine (culture medium). Peripheral blood leukocytes were allowed to adhere to plastic 24-well plates (Costar) for 2 h at 37°C before washes with medium to remove nonadherent cells. Adherent cells were incubated in culture medium with 10% heat-inactivated fetal calf serum at 37°C with 5% CO_2 for 3 days before *L. pneumophila* infection. Selected experiments done with standard culture medium were repeated for confirmation in medium containing heat-inactivated normal human serum.

Infection with *L. pneumophila*. Adherent cells were preincubated for various times with the test reagents and then infected with *L. pneumophila* (bacteria/cell ratio = 1:10) for 2 h at 37°C. Supernatants were then discarded, and the cells were washed thrice with culture medium. Fresh culture medium with or without the test reagents was added, and the cultures were incubated further at 37°C with 5% CO_2 for 3 days. At day 3, the supernatants were discarded, the cells were washed three times with culture medium, and 1 ml of sterile distilled water was added. Cells were then removed from the plates with a rubber policeman, and intracellular bacteria were released by ultrasonic lysis (5 s; Bioblock

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TABLE 1. Exogenous addition of TNF-α inhibits intracellular multiplication of *L. pneumophila* in human peripheral monocytes in a concentration-dependent fashion[a]

Concn of TNF-α (U/ml) added 1 h before infection	Addition of IFN-γ (0.01 μg/ml)	*L. pneumophila* CFU/ml (mean ± SD)
None	−	$(4 \pm 0.21) \times 10^6$
10^2	−	$(1 \pm 0.12) \times 10^6$
10^3	−	$(4 \pm 0.32) \times 10^4$
10^4	−	$(5 \pm 0.13) \times 10^4$
None	+	$(2.5 \pm 0.07) \times 10^3$

[a] The IFN-γ inhibitory effect is presented for comparison. The initial bacterial inoculum was 10^4 CFU/ml. Values are means of three experiments.

Scientifique 20/200 SX). CFU were counted by plating on buffered charcoal-yeast extract medium supplemented with α-ketoglutarate after 3 days of incubation at 30°C with 2.5% CO_2.

Cytokine assays. TNF activity was detected with 5×10^4 L-929 cells per well seeded in 96-well microtiter plates and suspended in 0.1 ml of RPMI 1640 medium containing 10% fetal calf serum. Actinomycin D (Sigma) at a final concentration of 1 μg/ml was added before the sample dilutions (0.1 ml). The cells were incubated at 37°C for 18 h and then extensively washed in Hanks' balanced salt solution (GIBCO). Adherent cells were stained for 10 min with crystal violet (0.2% in 2% ethanol) and washed with water, and 0.1 ml of 1% sodium dodecyl sulfate (SDS) was added to each well to solubilize the stained cells. The A_{550} of each well was read. Percent cytotoxicity was defined as the relative absorbance of the test sample versus that of the control (medium only) wells, as follows: % cytotoxicity = {[1 − absorbance of samples/absorbance of control]} × 100. The cytotoxicity titer (units per milliliter) was defined as the dilution of test sample that caused 50% cytotoxicity.

TNF-α and interleukin-6 (IL-6) production in the culture supernatants were also measured, using an enzyme immunoassay (Medgenix, Brussels, Belgium), and results were expressed as picograms per milliliter.

RNA preparation and analysis. Total RNA was extracted from the infected monocytes by the guanidium isothiocyanate method. RNA corresponding to 2×10^6 cells (about 15 μg) was size fractionated by electrophoresis through a 1% agarose gel containing 0.02 M 3-(*N*-morpholino)propanesulfonic acid buffer and 0.66 M formaldehyde and then transferred to a nylon membrane and baked. The IL-6 message was detected with a 1.1-kb *Eco*RI-*Bam*HI fragment cDNA probe (a kind gift of B. B. Aggarwal). The TNF-α message was detected with a 0.810-kb *Eco*RI fragment cDNA probe (also a gift from B. B. Aggarwal). For IL-6 and TNF-α RNA analyses, filters were prehybridized at 42°C for at least 4 h in 50% formamide solution containing 5× SSPE (1× SSPE is 150 mM NaCl, 10 mM NaH_2PO_4 [pH 7.4], 1 mM EDTA), 5× Denhardt's solution (1× Denhardt's solution is 0.1% bovine serum albumin, 0.1% Ficoll 400, 0.1% polyvinylpyrrolidone), 1% SDS, 5% dextran sulfate, and 200 μg of carrier salmon sperm DNA per ml. The filters were then hybridized in the same buffer at 42°C overnight with random hexamer-primed [α-^{32}P]dCTP-labeled cDNA probes. After 15-min washes at 65°C (2× SSC containing 0.5% SDS and then 0.5× SSC), the filters were exposed to Kodac X-Omat XAR 5 films for 7 days at −80°C.

TABLE 2. Exogenous addition of TNF-α (10^3 U/ml) inhibits intracellular multiplication of *L. pneumophila* in human peripheral monocytes in a time-dependent fashion[a]

Time of TNF-α addition before (−) and after (+) infection	Presence of anti-TNF-α antibody (1/10 diluted)	Addition of IFN-γ (0.01 μg/ml)	*L. pneumophila* CFU/ml (mean ± SD)
None	−	−	$(6 \pm 0.23) \times 10^5$
−24 h	−	−	$(3 \pm 0.20) \times 10^3$
−24 h	+	−	$(2.8 \pm 0.15) \times 10^5$
−1 h	−	−	$(2 \pm 0.11) \times 10^3$
−1 h	+	−	$(2 \pm 0.57) \times 10^5$
−30 min	−	−	$(1 \pm 0.01) \times 10^3$
−30 min	−	+	$(1 \pm 0.27) \times 10^3$
−30 min	+	+	$(2 \pm 0.33) \times 10^3$
+30 min	−	−	$(3 \pm 0.27) \times 10^5$
+30 min	+	+	$(2.5 \pm 0.19) \times 10^3$
+30 min	−	+	$(2 \pm 0.12) \times 10^3$

[a] The IFN-γ inhibitory effect is shown for comparison. The initial bacterial inoculum was 10^4 CFU/ml. Values are means of three experiments.

RESULTS

Exogenous TNF-α inhibits intracellular multiplication of *L. pneumophila* in human peripheral monocytes in a concentration- and time-dependent fashion. We incubated human peripheral monocytes with TNF-α (10^2 to 10^4 U/ml) for 1 h before *L. pneumophila* infection and assayed the cultures for bacterial CFU after 3 days. As shown in Table 1, *L. pneumophila* multiplication was inhibited in human peripheral monocytes treated with TNF-α at 10^3 and 10^4 U/ml, while no significant inhibition was observed at lower TNF-α concentrations (three experiments). The results obtained with different times (24 h, 1 h, and 30 min) of monocyte incubation with 10^3 U of TNF-α per ml before infection were similar, and the intracellular multiplication of *L. pneumophila* was found to be consistently inhibited. However, when the monocytes were incubated with TNF-α 30 min after *Legionella* infection, there was no significant inhibition of bacterial multiplication, even when higher TNF-α concentrations were used (10^4 U/ml; data not shown).

To examine whether the action of TNF-α on the multiplication of *L. pneumophila* was specific, we attempted to inhibit its effect with a monoclonal anti-TNF antibody. When monocytes were incubated with 10^3 U of TNF-α per ml plus a monoclonal anti-TNF antibody (diluted 1/10) 1 h before infection, the effect of TNF-α on *L. pneumophila* multiplication was significantly reduced (Table 2). A similar reduction was observed when TNF-α and the anti-TNF antibody were added to the cell culture 24 h before infection.

In all experiments, the effect of exogenous IFN-γ (0.01 μg/ml) was studied in parallel. IFN-γ was a strong inhibitor of intracellular multiplication even when added 30 min after infection. The addition of both TNF-α and IFN-γ did not potentiate the inhibitory effect of IFN-γ further (Table 2).

TNF-α production during *L. pneumophila* infection in vitro. TNF-α and IL-6 production by human monocytes infected with *L. pneumophila* was determined by biological assays and enzyme immunoassays in the culture supernatants at various periods after infection. A moderate increase in TNF-α production was observed in the culture supernatants 24, 48, and 72 h after infection, while the addition of 10 μg of lipopolysaccharide (LPS) per ml resulted in significant TNF-α production (Table 3). IL-6 (and IL-1) production was significantly increased during this period (data not shown). The enzyme immunoassay confirmed the results obtained by

TABLE 3. TNF-α production in culture supernatants of human monocytes infected with *L. pneumophila* at various times after IFN-γ (0.1 μg/ml) and LPS (10 μg/ml) addition[a]

L. pneumophila infection	Addition of:		TNF-α activity (U/ml)		
	IFN-γ	LPS	24 h	48 h	72 h
−	−	−	<10	<10	<10
+	−	−	16	20	25
−	+	−	16	16	14
−	−	+	90	70	75
+	+	−	60	50	25
−	+	+	150	120	135

[a] Values are means of three experiments.

the biological method for TNF-α production (8, 14, and 17 pg of TNF-α per ml at 24, 48, and 72 h after infection, respectively). Since the presence of TNF-α in the culture supernatants was not significantly increased during the infection, we studied TNF-α production after the addition of IFN-γ to the *L. pneumophila*-infected monocytes: TNF-α production in the culture supernatants was consistently increased (Table 3).

PTX, a potent inhibitor of endogenous TNF-α production, enhanced intracellular multiplication. When a monoclonal anti-TNF-α antibody (diluted 1/10) was added to the monocyte cultures before or after infection, bacterial growth was not modified after 3 days of culture (data not shown). We therefore studied the effect of PTX, a potent inhibitor of endogenous TNF-α production (but not of other cytokines such as IL-1β and IL-6), on bacterial multiplication. Cultures were infected in the presence or absence of PTX and were assayed for *Legionella* CFU after 3 days. In the presence of PTX (10 and 10^2 μg/ml, added to the culture 2 h before infection), *L. pneumophila* multiplication was consistently increased. At lower PTX concentrations (1 μg/ml) and when PTX was added to the monocyte culture after *Legionella* infection, the bacterial multiplication was similar to that in control human monocytes (culture medium only) (Table 4). When total RNA was extracted from the cells infected in the presence of PTX and assessed by Northern (RNA) blot hybridization with probes specific for TNF-α (and IL-6 as a control), we found significant inhibition of TNF-α RNA synthesis. The synthesis of IL-6 RNA was not modified (Fig. 1).

TABLE 4. Addition of PTX to infected monocytes and interference with the inhibitory effect of IFN-γ on bacterial multiplication[a]

Addition of IFN-γ	Addition of PTX[b]		*L. pneumophila* CFU/ml (mean ± SD)
	Amt (μg/ml)	Time (h)	
−	None		$(6 \pm 0.19) \times 10^5$
+	None	−2	$(8 \pm 0.37) \times 10^3$
−	+100	−2	$(2 \pm 0.21) \times 10^6$
−	+10	−2	$(3 \pm 0.12) \times 10^6$
−	+1	−2	$(1 \pm 0.23) \times 10^6$
+	+100	−2	$(8 \pm 0.21) \times 10^4$
+	+10	−2	$(2 \pm 0.31) \times 10^5$
+	+1	−2	$(1 \pm 0.08) \times 10^4$
+	+100	+1	$(1 \pm 0.11) \times 10^4$
+	+10	+1	$(1 \pm 0.02) \times 10^4$

[a] The initial bacterial inoculum was 10^4 CFU/ml. Values are means of three experiments.
[b] +, Before infection; −, after infection.

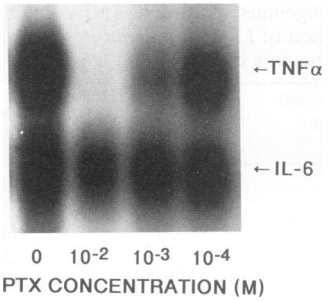

FIG. 1. TNF-α and IL-6 messages of the total RNA from *L. pneumophila*-infected monocytes were detected by Northern blot hybridization, using specific cDNA probes. The addition of various concentrations of PTX to monocyte cultures infected in the presence of IFN-γ resulted in a significant inhibition of TNF-α RNA expression but not IL-6 RNA expression.

IFN-γ inhibits intracellular multiplication of *L. pneumophila* by enhancing endogenous TNF-α production. Infection was performed in the presence of PTX plus IFN-γ, and the cultures were assayed for *Legionella* CFU after 3 days. In the presence of IFN-γ (200 U) and PTX (10 and 10^2 μg/ml), added to the cell culture 2 h before infection, *L. pneumophila* multiplication was increased relative to cultures infected in the presence of IFN-γ alone. PTX at 10 μg/ml was a more potent inhibitor of the IFN-γ effect than at the higher concentration (10^2 μg/ml). At a lower PTX concentration (1 μg/ml), we found no interference with the IFN-γ effect on bacterial multiplication (Table 4). The addition of PTX to monocyte cultures infected in the presence of IFN-γ resulted in inhibition of TNF-α RNA expression but not of IL-6 RNA expression.

DISCUSSION

The alveolar macrophage is the principal target of *L. pneumophila*, which destroys these cells. Since *L. pneumophila* is a facultative intracellular bacterium, the host controls the infection by cell-mediated immune responses and by activating macrophages and rendering them resistant to intracellular multiplication. Macrophages can be activated by various lymphokines, among which IFN-γ is considered the most important. Activation of human resident macrophages by IFN-γ and enhanced antimicrobial activity against *L. pneumophila* have been described previously (16). This enhanced antimicrobial activity can also concern many other intracellular pathogens (12, 14). Production of TNF has been reported to occur in lung lavage fluid during *L. pneumophila* infection (3). In addition, synergy and a cooperative effect between IFN-γ and TNF have been described (12). We tested the hypothesis that TNF production during *L. pneumophila* infection confers enhanced antimicrobial activity to macrophages and participates in controlling the infection.

We found that TNF-α can be produced during monocyte infection by *L. pneumophila* in vitro. Significant induction of TNF-α production, however, was detected only after addition of IFN-γ to the infected monocyte culture. It is unclear why significantly increased TNF-α activity was not detected in the culture supernatants without IFN-γ addition even though IL-6 was present. The low sensitivity of the methods used for detection of TNF-α and the existence of TNF inhibitors and/or receptors may explain our results (6, 19).

Adherent mononuclear cells from three different blood donors were used in our study, and the results obtained were

not statistically different. We cannot exclude the possibility, however, that there exist individual variations in TNF production during *L. pneumophila* infection, and a good diversity of donors would enhance the biological relevance of our findings.

Legionella LPS may account for the induction of TNF in monocyte cultures. However, other investigators have reported that infection of C3H/HeJ mice with *L. pneumophila* resulted in equivalent levels of TNF in lung lavage fluid, suggesting that factors other than LPS may be involved in this induction (3).

The results obtained with the PTX showed that TNF-α participates in the inhibition of intracellular *L. pneumophila* multiplication. PTX has been reported to suppress TNF biosynthesis by acting to prevent processing of the primary transcript (8). PTX and dexamethasone inhibit TNF synthesis by macrophages at various points in the signaling pathway; PTX blocks TNF mRNA transcription, and dexamethasone blocks the TNF mRNA transcription and translation (8, 9). In contrast to the inhibition of TNF production by PTX, it was reported that this agent has no effect on IL-1β or IL-6 production (5). In our study, PTX did not affect IL-6 RNA levels.

Our results suggest an interaction between TNF-α and IFN-γ, since PTX partially abolished the inhibitory effect of IFN-γ. The synergy between TNF-α and IFN-γ has been reported in several other studies (10, 12). IFN-γ increases the transcription of TNF-α mRNA in macrophages (4) and increases the number of TNF-α receptors (1, 18). TNF-α has also been reported to be involved in IFN-γ production by natural killer cells (2). The mechanism by which IFN-γ (and TNF-α) inhibits intracellular bacterial multiplication is not clear, but recent studies have shown that it may be mediated by nitric oxide production (12, 14). TNF, IFNs, and endotoxin stimulate nitric oxide production by different cells (endothelial cells, leukocytes, macrophages, neutrophils, Kupffer cells, and hepatocytes). The formation of nitric oxide by macrophages was studied essentially in animal models (mouse and rat), but nitric oxide production by human macrophages has been described recently (13); we used human macrophages in the present study.

Taken together, our results show that TNF-α is produced during infection of human monocytes by *L. pneumophila* in vitro and participates in the inhibition of bacterial multiplication in synergy with IFN-γ. Whether TNF-α has an immunomodulating role during human infection remains to be established.

REFERENCES

1. **Agarwall, B. B., T. E. Eesallu, and P. E. Hass.** 1985. Characterization of receptors for human tumor necrosis factor and their regulation by γ-interferon. Nature (London) **318**:665–667.
2. **Bancroft, G. J., K. C. F. Sheehan, R. D. Schreiber, and E. R. Unanue.** 1989. Tumor necrosis factor is involved in the T cell-independent pathway of macrophage activation in *scid* mice. J. Immunol. **143**:127–130.
3. **Blanchard, D. K., J. Djeu, T. W. Klein, H. Friedman, and W. E. Stewart.** 1987. Induction of tumor necrosis factor by *Legionella pneumophila*. Infect. Immun. **55**:433–437.
4. **Collart, M. A., D. Belin, J. D. Vassali, S. de Kossodo, and P. Vassali.** 1986. γ-Interferon enhances macrophage transcription of the tumor necrosis factor/cachectin, interleukin 1, and urokinase genes, which are controlled by short-lived repressors. J. Exp. Med. **164**:2113–2118.
5. **Endres, S., H. J. Fuelle, B. Sinha, D. Stoll, C. A. Dinarello, R. Gerzer, and P. C. Weber.** 1991. Cyclic nucleotides differentially regulate the synthesis of tumor necrosis factor-alpha and interleukin-1 beta by human mononuclear cells. Immunology **72**:56–60.
6. **Engelman, H., D. Aderko, M. Rubinstein, D. Rotmann, and D. Wallach.** 1989. A tumour necrosis factor-binding protein purified to homogeneity from human urine protects cells from TNF toxicity. J. Biol. Chem. **264**:11974–11979.
7. **Gray, P. W., B. B. Aggarwal, C. V. Benton, T. S. Bringman, W. J. Henzel, J. A. Jarret, D. W. Leung, N. Moffat, P. Ng, L. P. Svedersky, M. A. Palladino, and G. E. Nedwin.** 1984. Cloning and expression of cDNA for human lymphotoxin, a lymphokine with tumour necrosis activity. Nature (London) **312**:721–724.
8. **Han, J., G. Huez, and B. Beutler.** 1991. Interactive effects of the tumor necrosis factor promoter and 3′-untranslated regions. J. Immunol. **146**:1843–1848.
9. **Han, J., P. Thompson, and B. Beutler.** 1990. Dexamethasone and pentoxiphylline inhibit endotoxin-induced cachectin/tumor necrosis factor synthesis at separate points in the signaling pathway. J. Exp. Med. **172**:391–394.
10. **Hori, K., M. J. Ehrke, K. Mace, and E. Mihich.** 1987. Effect of recombinant tumor necrosis factor on tumoricidal activation of murine macrophages: synergism between tumor necrosis factor and γ-interferon. Cancer Res. **47**:5868–5874.
11. **Horwitz, M. A., and S. C. Silverstein.** 1980. The Legionnaires' disease bacterium (*Legionella pneumophila*) multiplies intracellularly in human monocytes. J. Clin. Invest. **66**:441–450.
12. **Liew, F. Y., Y. Li, and S. Millot.** 1990. Tumor necrosis factor-α synergizes with IFN-γ in mediating killing of *Leishmania major* through the induction of nitric oxide. J. Immunol. **145**:4306–4310.
13. **Martin, J. H. J., and S. W. Edwards.** 1993. Changes in mechanisms of monocyte/macrophage-mediated cytotoxicity during culture. J. Immunol. **150**:3478–3486.
14. **Mayer, J. M., M. L. Woods, Z. Vavrin, and J. B. Hibbs.** 1993. Gamma interferon-induced nitric oxide production reduces *Chlamydia trachomatis* infectivity in McCoy cells. Infect. Immun. **61**:491–497.
15. **Nash, T. W., D. M. Libby, and M. A. Horwitz.** 1984. Interaction between the Legionnaires' disease bacterium (*Legionella pneumophila*) and human alveolar macrophages: influence of antibody, lymphokines, and hydrocortisone. J. Clin. Invest. **74**:771–778.
16. **Nash, T. W., D. M. Libby, and M. A. Horwitz.** 1988. IFN-γ-activated human alveolar macrophages inhibit the intracellular multiplication of *Legionella pneumophila*. J. Immunol. **140**:3978–3981.
17. **Ortaldo, J. R., L. H. Mason, B. J. Mathieson, S. M. Liang, D. A. Flick, and R. B. Herberman.** 1986. Mediation of mouse natural cytotoxic activity by tumor necrosis factor. Nature (London) **321**:700–702.
18. **Ruggiero, V., J. Tavernier, W. Fiers, and C. Baglioni.** 1986. Induction of the synthesis of tumor necrosis factor receptors by interferon-gamma. J. Immunol. **136**:2445–2450.
19. **Seckinger, P., S. Issaz, and J. M. Dayer.** 1989. Purification and biologic characterization of a specific tumor necrosis factor α inhibitor. J. Biol. Chem. **264**:11966–11973.
20. **Shalaby, M. R., B. B. Aggarwal, E. Rinderknecht, L. P. Svedersky, B. B. Finkle, and M. A. Palladino.** 1985. Activation of human polymorphonuclear neutrophil functions by interferon-gamma and tumor necrosis factors. J. Immunol. **135**:2069–2073.
21. **Silberstein, D. S., and J. R. David.** 1986. Tumor necrosis factor enhances eosinophil toxicity to *Schistosoma mansoni* larvae. Proc. Natl. Acad. Sci. USA **83**:1055–1059.