Clin Exp Immunol 1993; 91:277–281

Differential mechanisms of intracellular killing of *Mycobacterium avium* and *Listeria monocytogenes* by activated human and murine macrophages. The role of nitric oxide

L. E. BERMUDEZ *Kuzell Institute for Arthritis and Infectious Diseases, Medical Research Institute of San Francisco at California Pacific Medical Centre, San Francisco, CA, USA*

(Accepted for publication 15 September 1992)

SUMMARY

Murine peritoneal macrophages activated with interferon-gamma (IFN-γ) produce large quantities of nitric oxide and are efficient in the killing of certain intracellular pathogens. To examine the role of this mechanism in the killing of *Mycobacterium avium* by murine and human macrophages, we infected mouse peritoneal macrophages and human monocyte-derived macrophages with *M. avium* and *Listeria monocytogenes* and stimulated the cells with recombinant tumour necrosis factor (TNF), granulocyte-macrophage colony-stimulating factor (GM-CSF) or IFN-γ, in the presence or absence of N-monomethyl-L-arginine (NMA) or arginase. Neither competitive inhibition with NMA nor depletion of arginine by arginase had any effect on the inhibition of growth/intracellular killing of *M. avium* by activated human and murine macrophages. In contrast, activation of murine but not human macrophages infected with *L. monocytogenes* by IFN-γ was significantly inhibited by the addition of NMA/arginase. Furthermore, murine macrophages produced large concentrations of nitric oxide following stimulation with recombinant cytokines, although no significant increase of nitric oxide production was observed with human monocyte-derived macrophages.

Keywords intracellular bacteria intracellular killing *M. avium* macrophages *L. monocytogenes* nitric oxide

INTRODUCTION

Organisms of the *Mycobacterium avium* complex are facultative intracellular bacteria that preferentially infect macrophages [1,2]. Once ingested by macrophages *M. avium* is killed or has the growth inhibited by activated cells [3,4]. Recent studies have shown that murine macrophages activated with recombinant interferon-gamma (IFN-γ) or recombinant tumour necrosis factor (TNF) kill a number of intracellular pathogens by a non-oxidative, L-arginine-dependent generation of reactive nitrogen intermediates [5,6]. However, similar mechanisms of intracellular killing have not been demonstrated using human macrophages. Studies by Murray & Teitelbaum [7] using *Toxoplasma gondii*, *Leishmania donovani* and *Chlamydia psittaci* and Cameron and colleagues [8] using *Cryptococcus neoformans* showed that while murine macrophages kill these organisms by a mechanism dependent on the production of nitrogen intermediates, the same is not observed in human macrophages. The only possible exception was reported by Denis using *M. avium* [9].

Since our previous studies suggest an important role of non-oxidative mechanism for the killing of strains of *M. avium* by macrophages [10], we examined the possibility that production of nitrogen intermediates would be responsible by the observed killing.

Correspondence: Dr Luiz Bermudez, Kuzell Institute, 2200 Webster Street, Room 305, San Francisco, CA 94115, USA.

MATERIALS AND METHODS

Human and murine macrophages

Cultures of human macrophages were established as previously reported [11]. Briefly, monocyte-derived macrophages were cultured in 24-well plates, containing 5×10^5 macrophages/well, with RPMI 1640 + 10% fetal bovine serum and L-glutamine. Monolayers were cultured for 7 days before infection. The number of viable cells in each monolayer was followed by counting every other day as previously described [11]. No preferential detachment was seen in the experimental groups.

Cultures of mouse peritoneal macrophages were established as described [11,12]. Tissue culture plates containing 5×10^5 macrophages/well were cultured in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum and L-glutamine without antibiotics (complete medium). After 2 h of adherence, monolayers were washed with 37°C Hank's balanced salt solution (HBSS) to remove non-adherent cells and cultured in complete medium for 18 h.

Murine and human monolayers were then infected either with *M. avium* strains 101 (serovars 1) or with *Listeria monocytogenes* (clinical isolate, 2776), as previously described [3]. The number of macrophages in the monolayers was monitored every other day to ensure that a similar number of cells were present in all monolayers. Monolayers hosting more than 65% of the initial population of cells were not used for the experimental analysis.

Macrophage activation

Macrophage monolayers were stimulated with recombinant human or recombinant mouse TNF (specific activity 2×10^7 U/mg; Genentech, South San Francisco, CA), 10^3 U/ml recombinant human or mouse granulocyte-macrophage colony-stimulating factor (GM-CSF) (10^2 U/ml) (specific activity of 3×10^7 U/mg and 1·7 U/mg respectively; Immunex, Seattle, WA) or recombinant human or murine IFN-γ (10^3 U/ml) (specific activity of $2{\cdot}5 \times 10^7$ U/mg for both preparations; Genentech). Cells were treated with recombinant cytokines for at least 4 days.

Infection

Murine and human macrophages were infected with *M. avium* (5×10^6 bacteria/5×10^5 cells) for 4 h and 1 h respectively. Monolayers were then washed three times with HBSS to remove extracellular bacteria. To quantify the number of viable intracellular bacteria at time zero, three monolayers per experiment were lysed as described below and the lysate plated onto 7H10 agar plates.

Treatment with N-monomethyl-L-arginine and arginase

As shown in other studies [7,13], N-monomethyl-L-arginine (NMA; Calbiochem-Behring, La Jolla, CA) was used at 100–400 μM as a competitive inhibitor of L-arginine. Bovine liver arginase (Sigma, St Louis, MO) was used at 20 U/ml to deplete arginine.

For the *in vitro* studies, both NMA and arginase were added 15 min before infection and 15 min before monolayer stimulation with recombinant cytokines. Because the experiments last 4 days, fresh NMA and arginase were replenished daily with cytokines. Therefore, both NMA and arginase were continuously present in the culture medium during the experiment.

NO_2^- production

Cell-free supernatants from human and murine macrophage cultures were tested for NO_2^- production using the Griess reaction as previously reported [14]. For these experiments, human and mouse macrophages were cultured in minimum Eagle medium (RPMI 1640 contains nitrate). Macrophage monolayers were stimulated with IFN-γ, TNF or GM-CSF in presence or absence of *M. avium* or lipopolysaccharide (LPS, 1 μg/ml, *E. coli* 0111B4, obtained from Capel). Medium from triplicate culture was mixed with an equal volume of Griess reagent (1% sulphanilamide/0·1% naphthylethylenediamine dihydrochloride/2·5% H_3PO_4). After 10 min at room temperature, absorbance at 540 nm (A_{540}) was measured and compared with standards established using 1–100 mM of $NaNO_2$ [14]. The concentration of NO_2^- obtained in the experiments was subtracted from the concentration of $NaNO_2$ obtained with medium alone.

Quantification of inhibition of growth/killing of M. avium *complex*

Inhibition of growth or killing of intracellular bacteria were concluded when the number of bacteria in stimulated monolayers was lower than in control monolayers with similar numbers of intracellular bacteria at the time zero after infection. Macrophage monolayers were infected with *M. avium* and cultured at 37°C in 5% CO_2 in moist air for several days in RPMI 1640 medium containing 10% heat-inactivated fetal calf serum. The number of bacteria per monolayer 4 h after infection (implantation inoculum) was obtained, and at 2 and 4 days after infection the wells were lysed and viable intracellular bacteria quantified. To lyse macrophages, 0·5 ml of iced sterile water was added in each well and incubated for 10 min at room temperature. Then, 0·5 ml of another lysing solution containing 1·1 ml of 7H9 medium and 0·4 ml of 0·25% SDS in phosphate buffer was added to each well for 10 more minutes (only mycobacteria-infected monolayers). The wells were vigorously scraped with a rubber policeman and the macrophage lysates were resuspended in 0·5 ml of 20% bovine albumin in sterile water to neutralize the SDS effect. The suspension was then vortex agitated for 2 min for complete lysis of macrophages. The macrophage lysate was sonicated for 10 s (power output 2·5 W/s) to avoid formation of bacterial clumps, and permit reliable pour plate quantification. As a control for osmotic stability, mycobacteria without macrophages were submitted to the same procedure and quantitative colony counts determined by pour plate technique. The viability count of the control organisms was the same after exposure to SDS as before. To ensure that macrophages were totally disrupted, samples were examined by Giemsa staining.

The macrophage lysate suspension was diluted serially and 0·1 ml of the final suspension was plated onto 7H10 agar. The plates were allowed to dry at room temperature for 15 min, and incubated at 37°C in 5% CO_2 and moist air for 2 weeks. The results are reported as mean colony-forming units (cfu) per millilitre of macrophage lysate suspension, obtained after 14 days in culture. Duplicate plates were prepared for each well.

Statistical analysis

Each experiment was repeated at least three times. All data in each experiment were obtained on duplicate wells and the means were calculated. Significance of killing differences between treated *versus* control macrophage cultures at identical time-points was tested by Student's *t*-test.

RESULTS

Effect of arginase and NMA on intracellular killing by activated macrophages

Previous studies have demonstrated that human and murine macrophages stimulated with recombinant TNF or GM-CSF can inhibit the growth of or kill intracellular *M. avium* [3,4,9,11]. As shown in Table 1, incubation of macrophage monolayers with arginase (20 U/ml) and NMA (200 μM) had no effect on the ability of activated human macrophages to kill *M. avium*. Increasing the concentration of NMA to 400 μM had no effect in reversing the ability of TNF or GM-CSF to activate human macrophages (data not shown).

Murine macrophages have been described as having nitrogen-dependent bactericidal mechanism against intracellular pathogens. However, as shown in Table 2, NMA and arginase

Table 1. Intracellular killing of *Mycobacterium avium* by activated human macrophages

Treatment	Viable intracellular organisms*		
	day 0	day 4	Per cent change†
Control (no treatment)	$1{\cdot}6 \pm 0{\cdot}4 \times 10^5$	$4{\cdot}0 \pm 0{\cdot}3 \times 10^6$	—
TNF (10^3 U/ml)	$1{\cdot}6 \pm 0{\cdot}4 \times 10^5$	$8{\cdot}8 \pm 0{\cdot}3 \times 10^4$	97·8
TNF + NMA/arginase‡	$1{\cdot}6 \pm 0{\cdot}4 \times 10^5$	$8{\cdot}5 \pm 0{\cdot}4 \times 10^4$	97·9
GM-CSF (10^2 U/ml)	$1{\cdot}6 \pm 0{\cdot}4 \times 10^5$	$7{\cdot}6 \pm 0{\cdot}2 \times 10^4$	98·1
GM-CSF + NMA/arginase	$1{\cdot}6 \pm 0{\cdot}4 \times 10^5$	$7{\cdot}5 \pm 0{\cdot}3 \times 10^4$	98·1

* Mean ± s.d. of three experiments.

† Per cent of change compared with control at the same point.

‡ Monolayers were incubated with 200 μM of N-monomethyl-L-arginine (NMA) and 20 U/ml of arginase.

Table 2. Intracellular killing of *Mycobacterium avium* by activated murine macrophages

Treatment	Viable intracellular organisms*		
	day 0	day 4	Per cent change†
Control (no treatment)	$1{\cdot}7 \pm 0{\cdot}3 \times 10^4$	$2{\cdot}5 \pm 0{\cdot}4 \times 10^5$	—
TNF (10^3 U/ml)	$1{\cdot}7 \pm 0{\cdot}3 \times 10^4$	$7{\cdot}7 \pm 0{\cdot}3 \times 10^4$	69·2
TNF + NMA/arginase‡	$1{\cdot}7 \pm 0{\cdot}3 \times 10^4$	$8{\cdot}1 \pm 0{\cdot}3 \times 10^4$	67·6
GM-CSF (10^2 U/ml)	$1{\cdot}7 \pm 0{\cdot}3 \times 10^4$	$7{\cdot}1 \pm 0{\cdot}4 \times 10^4$	71·6
GM-CSF + NMA/arginase	$1{\cdot}7 \pm 0{\cdot}3 \times 10^4$	$7{\cdot}5 \pm 0{\cdot}3 \times 10^4$	70·0

* Mean ± s.d. of three experiments.

† Per cent of change compared with control at the same time point.

‡ Monolayers were treated with 200 μM of N-monomethyl-L-arginine (NMA) and 20 U/ml of arginase.

also had no effect on TNF or GM-CSF-mediated mycobactericidal/mycobacteriostatic activity in murine macrophages, suggesting a different, non-oxidative mechanism for killing of *M. avium* by activated murine macrophages.

In contrast with the above described results, IFN-γ and TNF-mediated bactericidal effects were inhibited by NMA and arginase in murine macrophages but not human macrophages infected with *L. monocytogenes* (Table 3).

Production of reactive nitrogen intermediates

Cultures of adherent human and murine macrophages were examined regarding their ability to secrete NO_2^- under a number of conditions.

Supernatants of human macrophages not infected or infected with *M. avium* strain 101 (serovars 1) or *L. monocytogenes* for 72 h produced very small amounts of NO_2^-, which did not increase significantly if the cells were stimulated with TNF, GM-CSF or IFN-γ for 72 h. In contrast, murine macrophages produced increased amounts of NO_2^- when stimulated with IFN-γ, GM-CSF or TNF (Table 4).

DISCUSSION

Stimulation of murine and human macrophages with certain cytokines such as TNF and GM-CSF is associated with mycobacteriostatic/mycobacterial activity against *M. avium* [3,4,9]. In addition, stimulation of macrophages with IFN-γ is associated with intracellular killing of *L. monocytogenes* [15]. The mechanisms by which macrophages kill intracellular pathogens is not completely known, but a number of studies have demonstrated the importance of oxidative and non-oxidative pathways [16,17]. The L-arginine-dependent generation of reactive nitrogen intermediates (RNI) is an important antimicrobial pathway of activated macrophages [7] and has been shown to participate in the intracellular killing of several pathogens [6,7,13,18,19].

Recent studies, however, have suggested that generation of RNI plays no role *in vitro* as an antimicrobial mechanism of human macrophages [7,20]. Our present investigation showed that intracellular killing of *M. avium* by cytokine-activated human or murine macrophages is independent of the generation of RNI. Furthermore, RNI plays a role in the intracellular killing of *L. monocytogenes* by murine macrophages, but apparently has no effect on the *Listeria* killing of human macrophages. These results suggest that human macrophages kill *L. monocytogenes* using partially or totally different mechanisms than murine macrophages and that both human and murine macrophages stimulated with TNF or GM-CSF kill *M. avium* by an RNI-independent mechanism. Furthermore, our results confirm previous studies by James & Glaven [21],

Table 3. Intracellular killing of *Listeria monocytogenes* by human and murine macrophages

Treatment	Viable intracellular organisms*		
	day 0	day 2	Per cent change†
Murine macrophages			
Control (none)	$2{\cdot}1 \pm 0{\cdot}4 \times 10^5$	$7{\cdot}1 \pm 0{\cdot}3 \times 10^5$	—
IFN-γ (10^3 U/ml)	$2{\cdot}1 \pm 0{\cdot}4 \times 10^5$	$4{\cdot}9 \pm 0{\cdot}3 \times 10^3$	99·3
IFN-γ + NMA/arginase	$2{\cdot}1 \pm 0{\cdot}4 \times 10^5$	$3{\cdot}9 \pm 0{\cdot}5 \times 10^5$	45·1
Human macrophages			
Control (none)	$3{\cdot}8 \pm 0{\cdot}3 \times 10^5$	$1{\cdot}0 \times 0{\cdot}4 \times 10^6$	—
IFN-γ (10^3 U/ml)	$3{\cdot}8 \pm 0{\cdot}3 \times 10^5$	$5{\cdot}8 \pm 0{\cdot}3 \times 10^4$	94·2
IFN-γ + NMA/arginase	$3{\cdot}8 \pm 0{\cdot}3 \times 10^5$	$6{\cdot}2 \pm 0{\cdot}4 \times 10^4$	93·8

* Mean ± s.d. of three experiments.
† Percentage of change compared with control at day 2.

Table 4. NO_2^- production by human and mouse macrophages *in vitro*

Treatment	NO_2^- production (nmol) macrophages*	
	Human	Mouse
None	$4{\cdot}3 \pm 1{\cdot}5$	$7{\cdot}2 \pm 1{\cdot}9$
TNF (10^3 U/ml)	$11{\cdot}1 \pm 3$	79 ± 15
GM-CSF (10^2 U/ml)	13 ± 4	61 ± 11
IFN-γ (10^3 U/ml)	$6{\cdot}2 \pm 0{\cdot}9$	84 ± 7
Mycobacterium avium infection	$2{\cdot}3 \pm 0{\cdot}1$	$4{\cdot}1 \pm 0{\cdot}3$
Listeria monocytogenes infection	$3{\cdot}5 \pm 0{\cdot}3$	$6{\cdot}0 \pm 0{\cdot}2$

* Macrophages were stimulated with recombinant cytokines for 72 h or infected for the same period of time.

Cameron and colleagues [8] and Murray & Teitelbaum [7] that human monocyte-derived macrophages activated *in vitro* release no or very small amounts of NO_2^-.

Studies concerning mechanisms of intracellular killing of pathogens such as leishmania, toxoplasma, *M. leprae*, *M. tuberculosis* [22] and trypanozome using the mouse system *in vivo* and *in vitro* have suggested that production of RNI may be a chief pathway of killing by macrophages stimulated with IFN-γ. Our results using *L. monocytogenes* agree with these findings. However, our studies with *M. avium* suggest that activated murine macrophages kill *M. avium* by a different mechanism than that used to kill *M. leprae*. In fact, it has been demonstrated that human and murine macrophages can inhibit intracellular growth of *M. leprae* when stimulated with IFN-γ, which is not observed with *M. avium* infection [3,23,24]. Furthermore, recent studies using murine macrophage cell lines [22] and *M. tuberculosis* may represent either a different behaviour of *M. avium* or differences in mechanisms of killing between peritoneal macrophages and an immortalized cell line.

A number of other cytokines such as TNF and GM-CSF can also induce killing of Listeria, leishmania, and trypanozome by macrophages [25–29]. It is not known if RNI are also involved in cytotoxicity induced by cytokines other than IFN-γ.

Finally, it is possible that human macrophages can be stimulated *in vitro* and *in vivo* to produce RNI, although we could not observe it using our experimental system. It is certainly important to evaluate NO_2^- production and bactericidal mechanisms related to L-arginine-dependent RNI using human macrophages from lung and peritoneal cavity.

ACKNOWLEDGMENTS

This work was supported by the Contract no. 5U01-FD01431-03 of the Food and Drug Administration, by the Grant no. 5R01 AI 25769-05 of the National Institute of Allergy and Infectious Diseases and by the Scientific Support Fund at MRI.

REFERENCES

1 Collins FM. Mycobacterial disease, immunosuppression and acquired immunodeficiency syndrome. Clin Microbiol Rev 1989; **2**:360–74.
2 Collins FM, Stokes RW. *M. avium* complex infection in normal and immunosuppressed mice. Tubercle 1987; **68**:127.
3 Bermudez LE, Young LS. Tumor necrosis factor alone or in combination with IL-2, but not IFNγ is associated with macrophage killing of *Mycobacterium avium* complex. J Immunol 1988; **140**:3006–13.
4 Bermudez LE, Young LS. Recombinant granulocyte macrophage colony stimulating factor activates human macrophages to kill *Mycobacterium* complex. J Leuko Biol 1990; **48**:67–72.
5 Liew FY, Li Y, Milott S. Tumor necrosis factor in leishmaniasis: II-TNFα induced macrophage leishmanicidal activity is mediated by nitric oxide from L-arginine. Immunology 1990; **71**:556–61.
6 Liew FY, Yun L, Milott S. Tumor necrosis factor α with IFNγ in mediating killing of *Leishmania major* through the induction of nitric oxide. J Immunol 1990; **145**:4306–10.
7 Murray HW, Teitelbaum RF. L-arginine-dependent reactive nitrogen intermediates and the antimicrobial effect of activated human mononuclear phagocytes. J Infect Dis 1992; **165**:513–7.
8 Cameron ML, Granger DL, Weinberg JB, Kozumbo WJ, Koren HS. Human alveolar and peritonal macrophages mediate fungistasis independently of L-arginine oxidation to nitrite and nitrate. Am Rev Resp Dis 1990; **142**:1313–7.

9 Denis M. Tumor necrosis factor and granulocyte macrophage colony stimulating factor stimulate human macrophages to restrict growth of virulent *Mycobacterium avium* and to kill avirulent *M. avium*: killing effector mechanism depends on the generation of reactive nitrogen intermediates. J Leukoc Biol 1991; **49**:380–6.

10 Bermudez LE, Young LS. Oxidative and non-oxidative intracellular killing of *Mycobacterium avium* complex. Microbial Pathogenesis 1989; 7:289–98.

11 Bermudez LE, Stevens P, Kolonoski P, Wu M, Young LS. Treatment of experimental disseminated *Mycobacterium avium* complex infection in mice with recombinant IL-2 and tumor necrosis factor. J Immunol 1989; **143**:2996–3000.

12 Bourgeois L, Beaman BL. Probable L-forms of *Nocardia asteroides* induced in cultured mouse peritoneal macrophages. 1974

13 Adams LB, Hibbs JB Jr, Taintor RR, Krahenbuhl JL. Microbistatic effect of murine-activated macrophages for *Toxoplasma gondii* role for synthesis of inorganic nitrogen oxides from L-arginine. J Immunol 1990; **144**:2725–9.

14 Ding AJ, Nathan CF, Stuehr DJ. Release of reactive nitrogen intermediates and reactive oxygen intermediates from mouse peritoneal macrophages: comparison of activating cytokines and evidence for independent production. J Immunol 1988; **144**:2407–13.

15 Buchmeier NA, Schreiber RD. Requirements of endogenous interferon-gamma production for resolution of *Listeria monocytogenes* infection. Proc Natl Acad Sci USA 1985; **82**:7404–8.

16 Murray HW, Rubin Y, Carriero SM, Harris M, Jaffe EA. Human mononuclear phagocyte antiprotozoal mechanisms: oxygen-dependent *vs.* oxygen independent activity against intracellular *Toxoplasma gondii*. J Immunol 1985; **134**:1982–7.

17 Rothermel CD, Rubin BY, Murray HW. Oxygen-independent inhibition of intracellular *Chlamydia psittaci* growth by human monocytes and interferon-gamma activated macrophages. J Immunol 1986; **137**:689–96.

18 Green SJ, Meltzer MS, Hibbs JB, Nacy CA. Activated macrophages destroy intracellular *Leishmania major* amastigotes by an L-arginine-dependent killing mechanism. J Immunol 1990; **144**:278–84.

19 Green SJ, Crawford RM, Hockmeyer JT, Meltzer MS, Nacy CA. *Leishmania major* amastigotes initiate the L-arginine dependent killing mechanisms in IFNγ-stimulated macrophages by induction of tumor necrosis factor α. J Immunol 1990; **145**:4290–6.

20 Granger DL, Hibbs JB, Perfect JR, Durack DT. Specific amino acid (L arginine) requirement for the microbiostatic activity of murine macrophages. J Clin Invest 1988; **81**:1129–34.

21 James SL, Glaven J. Macrophage cytotoxicity against schistosomula of *Schistossoma mansoni* involves arginine-dependent production of nitrogen intermediates. J Immunol 1989; **143**:4208–15.

22 Chan J, Xing Y, Maglliozzo RS, Bloom BR. Killing of virulent *Mycobacterium tuberculosis* by reactive nitrogen intermediates produced by activated murine macrophages. J Exp Med 1992; **175**:1111–22.

23 Kaplan G, Nathan CF, Gandhi R, Horwitz MS, Levis WR, Cohn ZA. Effect of recombinant interferon-gamma on hydrogen peroxide-releasing capacity of monocyte-derived macrophages from patients with lepromatous leprosy. J Immunol 1986; **137**:983–8.

24 Nathan CF, Kaplan G, Levis WR *et al.* Local and systemic effects of intradermal recombinant interferon-gamma in patients with lepromatous leprosy. N Engl J Med 1986; **315**:6–10.

25 Sibley LD, Krahenbuhl JL. *Mycobacterium leprae*-burdened macrophages are refractory to activation by gamma-interferon. Infect Immun 1987; **55**:446–52.

26 Reed SG, Grabstein KH, Phil DL, Morrissey PJ. Recombinant granulocyte macrophage colony stimulating factor restores deficient immune responses in mice with chronic *Trypanozoma cruzi* infections. J Immunol 1990; **145**:1564–9.

27 DeTitto EH, Catterall JR, Remington JS. Activity of recombinant tumor necrosis factor on *Toxoplasma gondii* and *Trypanosoma cruzi*. J Immunol 1986; **138**:491–5.

28 Weiser WY, Yan Niel A, Clark SC, David JR, Remold HG. Recombinant human granulocyte macrophage colony stimulating factor activates intracellular killing of *Leishmania donovani* by human monocyte-derived macrophages. J Exp Med 1987; **166**: 1436–45.

29 Nakane A, Minagawa T, Kato K. Endogenous tumor necrosis factor is essential to host resistance against *Listeria monocytogenes* infection. Infect Immun 1988; **56**:2563–37.

30 Roll JT, Young KM, Kurtz RS, Czuprynski CJ. Human TNFα augments anti-bacterial resistance in mice: potentiation of its effects by recombinant human IL-1α. Immunology 1990; **69**:316–20.