INFECTION AND IMMUNITY, Oct. 1995, p. 4011–4015 Vol. 63, No. 10
0019-9567/95/$04.00+0

Endogenous Interleukin-12 Is Involved in Resistance of Mice to *Mycobacterium avium* Complex Infection

BERNADETTE M. SAUNDERS, YIFAN ZHAN, AND CHRISTINA CHEERS*

Department of Microbiology, University of Melbourne, Parkville, Victoria 3052, Australia

Received 13 April 1995/Returned for modification 9 May 1995/Accepted 11 July 1995

Acquired cellular resistance against *Mycobacterium avium* complex (MAC) infections involves the induction of Th1 type gamma interferon (IFN-γ)-producing T cells. Interleukin-12 (IL-12) is a cytokine involved in the control of IFN-γ production by T cells and NK cells. The role of IL-12 in the response to MAC infection was investigated. Depletion of endogenous IL-12 by injection of monoclonal antibody prior to and during intranasal infection with MAC resulted in an 150- to 550-fold increase in bacterial load in lung, spleen, and liver homogenates by 10 weeks postinfection. Depletion of IL-12 abrogated the ability of spleen cell cultures to produce IFN-γ in response to stimulus with live MAC. IL-12-depleted mice showed a 75% decrease in the number of inflammatory cells entering the lungs following intranasal infection with MAC, with significant reductions in cytotoxic activity and nitric oxide production by lung cells. This work suggests that IL-12 plays a major role in the activation of IFN-γ-producing cells during MAC infection.

Bacteria of the *Mycobacterium avium* complex (MAC) are facultative intracellular pathogens and the most common cause of disseminated bacterial infection in AIDS patients. Acquisition of a MAC infection significantly shortens the life span of these patients compared with that of patients with the same T-cell counts (3, 8, 20). Control of MAC infection requires the presence of activated $CD4^+$ T cells which produce an array of cytokines, including gamma interferon (IFN-γ), involved in activating macrophage bactericidal activity (1, 19). Studies involving IFN-γ gene and IFN-γ receptor gene knockout mice have shown that IFN-γ, produced by activated T cells and perhaps natural killer (NK) cells, plays an essential role in protective cellular immunity against mycobacteria (9, 21).

Interleukin-12 (IL-12), a recently described cytokine, has been shown to induce IFN-γ production by both $CD4^+$ T cells and NK cells (18, 25) and to stimulate naive T cells in vitro to promote the development of Th1 (IFN-γ-producing) cells (18). Recent work by Khan et al. (22), Tripp et al. (27), and our laboratory (29) has shown that neutralization of endogenous IL-12 before infection with *Listeria monocytogenes*, *Toxoplasma gondii*, or *Brucella abortus* leads to increases in bacterial or parasite numbers and increased animal morbidity.

In the clinical situation, lung cells are often the first to encounter invading MAC organisms (3, 7). Thus, to mimic conditions in the human host and to assess the role of IL-12 in the lung inflammatory response, we depleted mice of endogenous IL-12 by using monoclonal antibody (MAb) prior to and during intranasal infection. We report here that depletion of endogenous IL-12 exacerbated MAC infection, ablated the in vitro production of IFN-γ by cultured spleen cells from infected animals, and led to a decreased lung inflammatory response.

MATERIALS AND METHODS

Infection of mice. The MAC isolate was a virulent strain isolated at Fairfield Hospital (Melbourne, Victoria, Australia) from an AIDS patient and identified as serotype 8. It was grown in Middlebrook 7H9 broth in a stirring culture for 7 to 10 days and then frozen in 1-ml ampoules at −70°C. Before use, the suspension was thawed at 37°C and sonicated for 10 s to disperse clumps. Six- to eight-week-old male C57BL/10 mice, pedigree bred and maintained in the Department of Microbiology, University of Melbourne, Parkville, Victoria, Australia, were infected intranasally with a 60-μl suspension containing 10^5 MAC organisms inoculated onto the external nares with a micropipette (Gilson Medical Electronics, Villers-le-Bel, France) while anesthetized (Penthrane [Abbott Laboratories, North Chicago, Ill.]). The dose was standardized turbidometrically and checked retrospectively by viable counts on Middlebrook 7H11 agar (Difco Laboratories, Detroit, Mich.). MAC-infected mice were housed within a class II isolation cabinet (Gelman Sciences, Melbourne, Victoria, Australia) in cages covered with filter hoods.

Assessment of mice. At intervals after infection, groups of five mice were sacrificed by CO_2 narcosis. The lungs, spleen, and liver were removed from each mouse aseptically, and each organ was homogenized in 5 ml of sterile phosphate-buffered saline (PBS) with an Ultra Turrax tissue homogenizer (Janke and Kunkle K.S., Breisgau, Germany). Suitable dilutions were placed on Middlebrook 7H11 agar for viable counts, and the plates were incubated in a humidified container at 37°C for 7 days before counting. When spleen or lung cell cultures were being prepared, organ fragments were weighed, and approximately half were used for bacterial counts and half were used for preparation of cells. The numbers of cells or bacteria per organ were calculated on the basis of the weight of the fragment sampled. The use of organ fragments rather than whole tissues did not lead to any increase in the variability of bacterial counts.

In vivo depletion of IL-12. C57BL/10 mice were depleted of IL-12 with MAb C15.6 (rat immunoglobulin G1), which was kindly provided by G. Trinchieri, Wistar Institute, Philadelphia, Pa. Mice were injected intraperitoneally (i.p.) with 1 mg of ammonium sulfate-precipitated antibody in 200 μl of sterile PBS 48 and 24 h before infection with MAC, and then 1 mg of antibody was injected weekly to ensure continued depletion during the course of the experiment. YTS169.4 MAb was prepared by ammonium sulfate precipitation for use as a control antibody in initial experiments. Injecting this antibody had no significant effect upon bacterial growth or the lung inflammatory response compared with that of untreated mice.

Analysis of lung cell populations. The lungs were removed aseptically and placed in *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES)-buffered Dulbecco modified Eagle medium (DMEM) supplemented with 0.216 mg of L-glutamine per ml, 5×10^{-5} M 2-mercaptoethanol, 60 μg of penicillin per ml, 100 μg of streptomycin per ml, and 10% fetal calf serum (FCS) (DMEM–10% FCS) and washed free of blood, and the tissue was sliced finely with a multiblade slicer before individual strands were teased apart (17). Approximately 0.5 g of lung tissue was incubated for 30 min at 37°C in 10 ml of medium containing 0.25% dispase (Boehringer, Mannheim, Germany). Tissue supernatant was spun at 800 × *g* for 7 min to pellet cells. The remaining undigested tissue was subjected to digestion two more times, and any remaining tissue was then forced through a fine-mesh wire sieve. Lung cells from five mice of the same experimental group were pooled and treated with Tris-buffered 0.83% ammonium chloride to lyse erythrocytes, centrifuged, and resuspended in DMEM–10% FCS. The viability of the cells assessed by exclusion of 0.2% eosin was generally greater than 75% for normal cells and greater than 85% for infected cells. These cells were used in functional assays. In addition, to assess the cell types present, 10^4 cells per cytocentrifuge chamber (Shandon, Pittsburgh, Pa.) were spun through FCS onto slides and the preparations were stained with Diff Quik (Lab-Aids, Narrabeen, Victoria, Australia) or a Ziehl-Neelsen stain. At least 100 cells from

* Corresponding author. Phone: 61-3-9344-5708. Fax: 61-3-9344-1540.

TABLE 1. Effect of anti-IL-12 MAb treatment on bacterial growth

Mice[a]	No. of wks infected	No. of bacteria[b] in organ		
		Lung	Spleen	Liver
Intact infected	4	7.04 ± 0.58	4.16 ± 0.19	3.90 ± 0.56
Anti-IL-12-treated infected	4	7.18 ± 0.35	4.26 ± 0.41	3.92 ± 0.53
Intact infected	10	7.56 ± 0.64	5.75 ± 0.13	4.41 ± 0.34
Anti-IL-12-treated infected	10	9.74 ± 0.54[c]	8.52 ± 0.42[c]	6.80 ± 0.49[c]

[a] Mice were depleted of IL-12 by weekly i.p. injections of MAb C15.6 and infected intranasally with 10^5 MAC.

[b] Data are the mean log bacterial numbers ± standard deviations of five mice. The experiment was repeated twice with similar results.

[c] Significantly different from value obtained with intact infected mice ($P < 0.001$ by Student's t test).

every slide were counted to assess cell populations. Cells were classified on the basis of morphological characteristics as follows: neutrophils had multilobed nuclei, lymphocytes had rounded nuclei with little cytoplasm, and monocytes/macrophages were larger cells with a kidney-shaped nucleus, often with abundant foamy cytoplasm.

Culture for cytokine and nitric oxide (NO) production. Cultures of 2×10^5 viable spleen or lung cells pooled from five mice per group, with or without stimulation by 10^7 live MAC were incubated in 200 μl of DMEM–10% FCS in 96-well microtiter trays for 72 h. The supernatants were then harvested and assayed for cytokines or NO.

Cytokine bioassay. IFN-γ was assayed by the ability of serial 3.15-fold dilutions of samples to inhibit proliferation of WEHI 279 cells in DMEM–10% FCS in flat-bottom 96-well microtiter trays (23). A titration of standard IFN-γ was included in each assay to calculate the amounts of IFN-γ in the samples. The specificity of the bioassay was checked by including the anti-IFN-γ neutralizing antibody R46A2 (16) in triplicate cultures.

NO_2^- assay. Nitric oxide (NO) production by lung cells was determined by the Griess reaction (14). Briefly, culture supernatants (50 μl) were mixed with 100 μl of 1% sulfanilamide (Sigma, St. Louis, Mo.) and 100 μl of 0.1% *N*-1-naphthylethylenediamine dihydrochloride in 2.5% polyphosphoric acid (Sigma) at room temperature for 5 min. A_{540} was measured. NO_2^- was quantified by comparison to $Na(NO_2)$ (Sigma) as a standard.

In vitro cytotoxic assay. P815 cells were maintained by continual passage in DMEM–10% FCS. As previously described (6), 10^4 target cells labelled with ^{51}chromium (Amersham Australia, Castle Hill, New South Wales, Australia) were added to round-bottom microtiter trays along with an appropriate number of viable effector cells. After 18 h of incubation, plates were centrifuged to pellet cells and 100 μl of supernatant was sampled. Maximum lysis was calculated by adding 100 μl of 0.05% Tween 80 (Labchem, Auburn, New South Wales, Australia) to several wells of cells. Spontaneous lysis varied from 20 to 35% of the maximum count. All cultures were assayed in triplicate, and results, expressed as mean percent specific lysis, were calculated as follows: [(test cpm − spontaneous cpm)/(maximum cpm − spontaneous cpm)] × 100, where cpm is counts per minute.

RESULTS

Effect of depletion of IL-12 on bacterial growth. To test the effect of endogenous IL-12 on resistance against MAC infection, C57BL/10 mice were depleted of IL-12 prior to and during intranasal infection with 10^5 MAC. At 4 and 10 weeks postinfection, mice were sacrificed and organs were collected for bacterial analysis. During the first 4 weeks of infection, bacterial numbers increased to the same degree in both intact and IL-12-depleted mice. By 10 weeks postinfection, a significant exacerbation of infection with MAC was seen in all organs analyzed (Table 1). The numbers of bacteria in lung, spleen, and liver cultures were 150- to 550-fold higher in anti-IL-12-treated mice than in untreated mice ($P < 0.001$). Preliminary experiments showed that injection of control immunoglobulin had no effect upon bacterial growth or the lung inflammatory response (the values for bacterial growth [mean log bacterial number ± standard deviation] in mice infected with MAC for 10 weeks were 7.29 ± 0.10 for an untreated lung and 7.50 ± 0.18 for a control, immunoglobulin-treated lung, and the numbers of cells isolated per lung were 5.8×10^6 for untreated mice and 5.3×10^6 for control, immunoglobulin-treated mice). Ziehl-Neelsen staining of lung cells from infected animals showed a large increase in intracellular bacteria in the lungs of IL-12-depleted mice (Fig. 1). Most phagocytic cells contained more than 100 bacteria, with exact numbers of bacteria per cell impossible to determine visually because of the heavy overlap of bacteria within each cell.

Lung inflammatory response. While bacterial growth at 4 weeks postinfection was unaffected by administration of anti-IL-12 MAb, depletion of endogenous IL-12 significantly altered the inflammatory response seen in mice infected for 4 weeks. The lungs of intact infected mice had enlarged to twice their normal size by 4 weeks postinfection, while the lungs of IL-12-depleted mice did not increase in size (Table 2). By 10 weeks postinfection, the lungs of IL-12-depleted animals, though larger than normal, were still significantly smaller than the lungs of intact infected mice. This reduction in lung weight was accompanied by a significant reduction in the number of inflammatory cells entering the lungs of IL-12-depleted mice from that of untreated infected mice (Fig. 2). Compared with control mice, intact infected mice showed a 10-fold increase in the numbers of phagocytic cells and lymphocytes within the lungs of mice infected for 4 weeks. In contrast, cell numbers in depleted infected mice increased only threefold compared to those of control mice. By 10 weeks postinfection, the number of cells isolated from the lungs of depleted infected mice was only 30% less than the number isolated from the intact infected mice, and the major deficiency was in macrophages/monocytes.

Activation of the bactericidal properties of phagocytic cells is essential to contain MAC infection. The production of NO is one mechanism used by infected cells to kill invading bacteria (4). Analysis of NO production by lung cells from intact and IL-12-depleted mice showed a significant increase in NO production by lung cells from infected mice compared with that of control mice at 4 and 10 weeks postinfection. NO production by lung cells from IL-12-depleted infected mice was significantly less than ($P < 0.001$) that of intact infected mice (Table 3). Depletion of IL-12 also significantly impaired the cytotoxic activity of lung cells from infected mice. Lung cells were used in an in vitro cytotoxicity assay to test their ability to lyse the macrophage-sensitive cell line P815. Lung cells from intact infected mice showed significantly enhanced cytotoxicity compared with healthy lung cells (Fig. 3). However, the lung cells from IL-12-depleted mice showed no increase in cytotoxic activity after 4 weeks postinfection and only slightly increased cytotoxicity at 10 weeks postinfection.

Effect of anti-IL-12 on IFN-γ production by spleen and lung cells during MAC infection. The production of IFN-γ by activated T cells is required to activate mycobactericidal activities of mononuclear phagocytes (12). It has been reported that IL-12 is necessary for the production of IFN-γ by NK and T cells (18, 28). Spleen cell cultures from mice infected for 4 or 10 weeks produced IFN-γ in response to stimuli with live MAC (Table 4). However, depletion of endogenous IL-12 completely abrogated the production of IFN-γ by spleen cell cultures in vitro, confirming the IL-12 dependence of IFN-γ production in MAC-infected mice.

DISCUSSION

Defense against MAC depends upon the activation of the IFN-γ-producing T cells. The results of this study provide evidence for a requirement for endogenous IL-12 to induce IFN-γ production to activate mycobactericidal activities of infected macrophages and limit bacterial growth in vivo. This

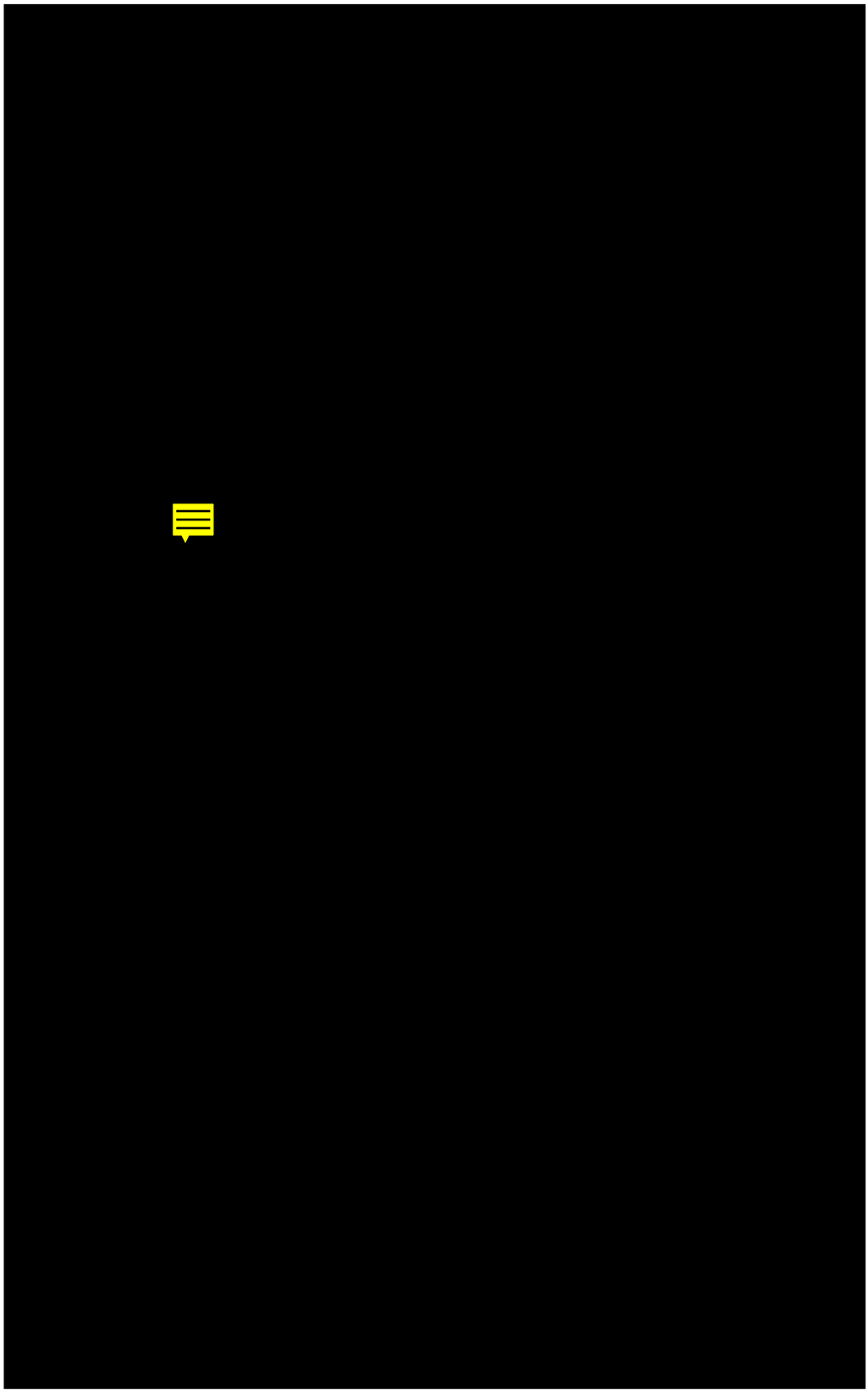

FIG. 1. Ziehl-Neelsen-stained lung cell suspensions of intact (top) and IL-12-depleted (bottom) mice infected for 10 weeks. The MAC organisms stain red. Magnification, ×1,000.

TABLE 2. Effect of anti-IL-12 MAb treatment on lung weight

Mice[a]	Lung wt (mg/lung) of mice infected for:	
	4 wks	10 wks
Control (uninfected)	230 ± 30	230 ± 30
Intact infected	390 ± 57	460 ± 40
IL-12-depleted infected	240 ± 18[b]	375 ± 30[c]

[a] Mice were depleted of IL-12 by weekly i.p. injections of MAb C15.6 and infected intranasally with 10^5 MAC.
[b] Significantly different from value obtained with intact infected mice ($P < 0.001$ by Student's t test).
[c] Significantly different from value obtained with intact infected mice ($P < 0.01$ by Student's t test).

TABLE 3. Effect of in vivo neutralization of IL-12 on NO production by lung cell cultures following MAC infection

Mice[a]	NO_2^- level (μM)[b]	
	4 wks	10 wks
Control (uninfected)	<2	<2
Intact infected	68.5 ± 2.3	188 ± 5.7
IL-12-depleted infected	14.7 ± 0.3[c]	44.5 ± 3.9[c]

[a] Mice were depleted of IL-12 by weekly i.p. injections of MAb C15.6 and infected intranasally with 10^5 MAC.
[b] Cultures of 2×10^5 lung cells were incubated for 72 h in 200-μl volumes in a 96-well microtiter tray. Culture supernatants were harvested and assayed for nitrite levels. Data are the means and standard deviations of cultures pooled from five mice (infected for 4 or 10 weeks) and prepared in triplicate. Experiments were repeated twice with similar results.
[c] Significantly different from value obtained with intact infected mice ($P < 0.001$ by Student's t test).

study extends previous studies from this laboratory and other laboratories into the role of IL-12 in response to bacterial infections such as *B. abortus* (29) and *Listeria monocytogenes* (27) and parasitic infections such as *Leishmania major* (26) and *T. gondii* (22).

The main functions of IL-12 are to induce IFN-γ production by NK cells (28) and direct $CD4^+$ T cells into a Th1 IFN-γ-producing response (18). The production of IFN-γ by NK cells is important in the response of immunocompetent mice to toxoplasmosis (22). Administration of IL-12 protected against this infection, an effect abrogated by NK cell depletion. The resistance of SCID mice, which lack functional T and B cells but have enhanced NK cell function (2), to *L. monocytogenes* (27) and *T. gondii* (15) was enhanced by administration of IL-12. IL-12 also contributes to resistance to infection by influencing T-cell responses. IL-12 enhances resistance to *Leishmania* infection by initiating an IFN-γ-producing Th1 response (26). A previous study in our laboratory has also shown an important role for IL-12 in activating IFN-γ-producing T cells following infection with *B. abortus* (29). This paper provides further evidence of the importance of IL-12 in inducing IFN-γ production following infection with MAC.

Depletion of endogenous IL-12 completely abrogated the ability of in vitro spleen cell cultures from mice infected for 4 or 10 weeks to produce IFN-γ following stimulation with live MAC. A previous study in our laboratory (24) has shown that in vitro IFN-γ production at these times was completely abrogated by depletion of $CD4^+$ T cells. Depletion of either $CD4^+$ T cells or IFN-γ from infected mice by injection of MAb led to exacerbation of bacterial growth 5 or more weeks after infection, with decreases in the inflammatory response very similar to those described here. This suggests a requirement for IFN-γ, produced by $CD4^+$ T cells, to contain MAC infection, and a requirement for IL-12 to induce this IFN-γ production.

Results of experiments in our laboratory have shown that NK cell activity (measured by increased YAC-1 cell killing) peaks 2 to 3 weeks after intranasal infection and then declines, while T-cell activation (measured by IFN-γ production in in vitro spleen cell cultures) usually occurs in the fourth week after intranasal MAC infection (24). Depletion of endogenous IL-12 did not affect bacterial growth during the first 4 weeks of infection. This indicates either that production of IFN-γ by NK cells was unaffected by IL-12 depletion, which would be in contrast to published literature showing that IL-12 induces IFN-γ production by NK cells (15, 24), or more likely, that any IFN-γ produced by NK cells during this initial period of infec-

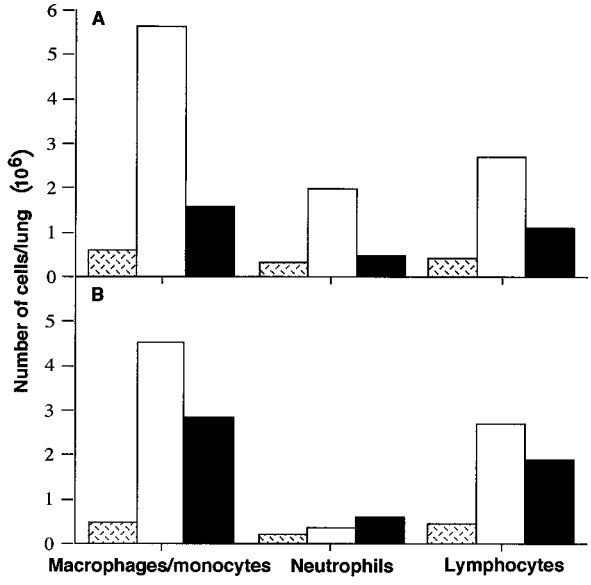

FIG. 2. Number of cells per lung in control (▨), intact infected (▭), and IL-12-depleted infected (■) mice, intranasally infected for 4 (A) or 10 (B) weeks with 10^5 MAC. Data are typical of two separate experiments.

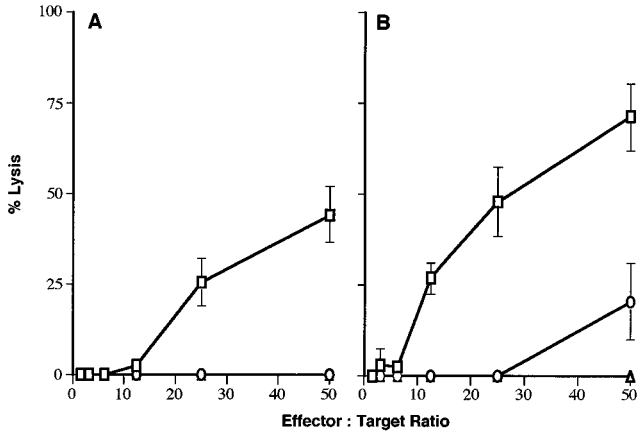

FIG. 3. Specific lysis of P815 target cells by lung cells from uninfected (△) or intact infected (□) and IL-12-depleted infected (○) mice. Groups of five mice were intranasally infected with 10^5 MAC 4 (A) or 10 (B) weeks prior to sacrifice. Data are the means and standard deviations of triplicate cultures. Experiments were repeated twice with similar results. The values for IL-12-depleted infected mice were significantly different from those for intact infected mice ($P < 0.001$ by Student's t test).

TABLE 4. IFN-γ production in intact and IL-12-depleted MAC-infected mice

Mice[a]	IFN-γ (IU/ml) produced[b]	
	4 wks	10 wks
Intact infected	26.9 ± 0.85	21.4 ± 5.4
IL-12-depleted infected	<1[c]	<1[c]
Control (uninfected)	<1	<1

[a] Mice were depleted of IL-12 by weekly i.p. injections of MAb C15.6 and infected intranasally with 10^5 MAC.

[b] Cultures of 2×10^5 spleen or lung cells were incubated with 10^7 live MAC for 72 h in 200-μl volumes in a microtiter tray. Culture supernatants were harvested and assayed for IFN-γ. Data are the means and standard deviations of cultures pooled from five mice (infected for 4 or 10 weeks) and prepared in triplicate. Cultures incubated without antigen yielded less than 1 IU of IFN-γ per ml. Experiments were repeated twice with similar results.

[c] Significantly different from value obtained with intact infected mice ($P < 0.001$ by Student's t test).

tion does not significantly affect the ability of intranasally infected mice to contain bacterial growth during the early phase of infection.

Lung phagocytes isolated from IL-12-depleted mice recorded diminished cytotoxic activity and production of NO. Evidence suggests that reactive nitrogen intermediates produced by activated murine mononuclear phagocytes are involved in killing or inhibiting the growth of virulent *Mycobacterium tuberculosis* (5, 13) and MAC, although some NO-resistant strains of MAC have been reported (10, 11). The addition of IFN-γ is known to increase NO production by murine mononuclear phagocytes (5), and our results indicate that IL-12 is required to activate the NO production by these cells, probably through inducing IFN-γ production.

ACKNOWLEDGMENTS

This work was supported by an Australian Commonwealth AIDS research grant and a National Health and Medical Research project grant.

We thank Veronica Peros for invaluable technical assistance.

REFERENCES

1. **Appelberg, R., A. G. Castro, J. Pedrosa, R. A. Silva, I. M. Orme, and P. Minoprio.** 1994. Role of gamma interferon and tumor necrosis factor alpha during T-cell-independent and -dependent phases of *Mycobacterium avium* infection. Infect. Immun. **62:**3962–3971.
2. **Bancroft, G. J., K. C. F. Sheenan, 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. **Benson, C. A.** 1994. Disease due to the *Mycobacterium avium* complex in patients with AIDS: epidemiology and clinical syndrome. Clin. Infect. Dis. **18**(Suppl. 3)**:**S218–S222.
4. **Bermudez, L. E.** 1993. Differential mechanisms of intracellular killing of *Mycobacterium avium* and *Listeria monocytogenes* by activated human and murine macrophages. The role of nitric oxide. Clin. Exp. Immunol. **91:**277–281.
5. **Chan, J., Y. Xing, R. S. Magliozzo, and B. R. Bloom.** 1992. Killing of virulent *Mycobacterium tuberculosis* by reactive nitrogen intermediates produced by activated murine macrophages. J. Exp. Med. **175:**1111–1122.
6. **Cheers, C., and P. Wood.** 1984. Listeriosis in beige mice and their heterozygous littermates. Immunology **51:**711–717.
7. **Chin, D. P., P. C. Hopewell, D. M. Yajko, E. Vittinghoff, C. J. Horsburgh, W. K. Hadley, E. N. Stone, P. S. Nassos, S. M. Ostroff, M. A. Jacobson, C. C. Matkin, and A. L. Regingold.** 1994. *Mycobacterium avium* complex in the respiratory or gastrointestinal tract and the risk of *M. avium* complex bacteremia in patients with human immunodeficiency virus infection. J. Infect. Dis. **169:**289–295.
8. **Chin, D. P., A. L. Reingold, E. N. Stone, E. Vittinghoff, C. J. Horsburgh, E. M. Simon, D. M. Yajko, W. K. Hadley, S. M. Ostroff, and P. C. Hopewell.** 1994. The impact of *Mycobacterium avium* complex bacteremia and its treatment on survival of AIDS patients—a prospective study. J. Infect. Dis. **170:** 578–584.
9. **Cooper, A. M., D. K. Dalton, T. A. Stewart, J. P. Griffin, D. G. Russell, and I. M. Orme.** 1993. Disseminated tuberculosis in interferon gamma gene-disrupted mice. J. Exp. Med. **178:**2243–2247.
10. **Denis, M.** 1991. 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. Leukocyte Biol. **49:**380–387.
11. **Doi, T., M. Ando, T. Akaike, M. Suga, K. Sato, and H. Maeda.** 1993. Resistance to nitric oxide in *Mycobacterium avium* complex and its implication in pathogenesis. Infect. Immun. **61:**1980–1989.
12. **Flesch, I., and S. H. Kaufmann.** 1987. Mycobacterial growth inhibition by interferon-gamma-activated bone marrow macrophages and differential susceptibility among strains of *Mycobacterium tuberculosis*. J. Immunol. **138:** 4408–4413.
13. **Flesch, I. E., and S. H. Kaufmann.** 1991. Mechanisms involved in mycobacterial growth inhibition by gamma interferon-activated bone marrow macrophages: role of reactive nitrogen intermediates. Infect. Immun. **59:**3213–3218.
14. **Fortier, A. H., T. Polsinelli, S. J. Green, and C. A. Nacy.** 1992. Activation of macrophages for destruction of *Francisella tularensis*: identification of cytokines, effector cells, and effector molecules. Infect. Immun. **60:**817–825.
15. **Gazzinelli, R. T., S. Hieny, T. A. Wynn, S. Wolf, and A. Sher.** 1993. Interleukin 12 is required for the T-lymphocyte-independent induction of interferon gamma by an intracellular parasite and induces resistance in T-cell-deficient hosts. Proc. Natl. Acad. Sci. USA **90:**6115–6119.
16. **Havell, E. A.** 1986. Purification and further characterization of an anti-murine interferon-gamma monoclonal neutralizing antibody. J. Interferon Res. **6:**489–497.
17. **Holt, P. G., A. Degebrodt, T. Venaille, C. O'Leary, K. Krska, J. Flexman, H. Farrell, G. Shellam, P. Young, J. Penhale, T. Robertson, and J. M. Papadmitriou.** 1985. Preparation of interstitial lung cells by enzymatic digestion of tissue slices: preliminary characterization by morphology and performance in functional assays. Immunology **54:**139–147.
18. **Hsieh, C. S., S. E. Macatonia, C. S. Tripp, S. F. Wolf, A. O'Garra, and K. M. Murphy.** 1993. Development of TH1 $CD4^+$ T cells through IL-12 produced by Listeria-induced macrophages. Science **260:**547–549.
19. **Hsu, N., L. S. Young, and L. E. Bermudez.** 1995. Response to stimulation with recombinant cytokines and synthesis of cytokines by murine intestinal macrophages infected with the *Mycobacterium avium* complex. Infect. Immun. **63:**528–533.
20. **Jacobson, M. A., P. C. Hopewell, D. M. Yajko, W. K. Hadley, E. Lazarus, P. K. Mohanty, G. W. Modin, D. W. Feigal, P. S. Cusick, and M. A. Sande.** 1991. Natural history of disseminated *Mycobacterium avium* complex infection in AIDS. J. Infect. Dis. **164:**994–998.
21. **Kamijo, R., J. Le, D. Shapiro, E. A. Havell, S. Huang, M. Aguet, M. Bosland, and J. Vilcek.** 1993. Mice that lack the interferon-gamma receptor have profoundly altered responses to infection with Bacillus Calmette-Guerin and subsequent challenge with lipopolysaccharide. J. Exp. Med. **178:**1435–1440.
22. **Khan, I. A., T. Matsuura, and L. H. Kasper.** 1994. Interleukin-12 enhances murine survival against acute toxoplasmosis. Infect. Immun. **62:**1639–1642.
23. **Reynolds, D. S., W. H. Boom, and A. K. Abbas.** 1987. Inhibition of B lymphocyte activation by interferon-gamma. J. Immunol. **139:**767–773.
24. **Saunders, B. M., and C. Cheers.** 1995. Inflammatory response following intranasal infection with *Mycobacterium avium* complex: role of T-cell subsets and gamma interferon. Infect. Immun. **63:**2282–2287.
25. **Sypek, J. P., C. L. Chung, S. E. Mayor, J. M. Subramanyam, S. J. Goldman, D. S. Sieburth, S. F. Wolf, and R. G. Schaub.** 1993. Resolution of cutaneous leishmaniasis: interleukin 12 initiates a protective T helper type 1 immune response. J. Exp. Med. **177:**1797–1802.
26. **Sypek, J. P., S. Jacobson, A. Vorys, and D. J. Wyler.** 1993. Comparison of gamma interferon, tumor necrosis factor, and direct cell contact in activation of antimycobacterial defense in murine macrophages. Infect. Immun. **61:** 3901–3906.
27. **Tripp, C. S., M. K. Gately, J. Hakimi, P. Ling, and E. R. Unanue.** 1994. Neutralization of IL-12 decreases resistance to Listeria in SCID and C.B-17 mice. Reversal by IFN-gamma. J. Immunol. **152:**1883–1887.
28. **Tripp, C. S., S. F. Wolf, and E. R. Unanue.** 1993. Interleukin 12 and tumor necrosis factor alpha are costimulators of interferon gamma production by natural killer cells in severe combined immunodeficiency mice with listeriosis, and interleukin 10 is a physiologic antagonist. Proc. Natl. Acad. Sci. USA **90:**3725–3729.
29. **Zhan, Y., and C. Cheers.** 1995. Endogenous interleukin-12 (IL-12) is involved in the resistance to *Brucella abortus* infection. Infect. Immun. **61:** 1387–1390.