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Inhibition of Tumor Necrosis Factor Alpha Alters Resistance to *Mycobacterium avium* Complex Infection in Mice

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Increased production of tumor necrosis factor alpha (TNF-α) appears to play an important role in the progression of human immunodeficiency virus disease. One treatment strategy being explored is the use of TNF-α inhibitors. TNF-α also appears to be important in conferring resistance to infections, and the inhibition of this cytokine may exacerbate the emergence of opportunistic pathogens, such as *Mycobacterium avium* complex (MAC). The present study examines the possibility that inhibition of TNF-α will increase the progression of disease in mice infected with MAC. C57BL/6 beige (*bg*/*bg*) mice have been shown to be highly susceptible to infection with MAC and are routinely used for testing of antimycobacterial drugs. However, *bg*/*bg* mice are known to exhibit impaired phagocyte and natural killer cell function. Since these cell types are important sources of TNF-α, the susceptibility of the *bg*/*bg* strain to infection with MAC was compared with those of the heterozygous (*bg*/+) and wild-type (+/+) strains of C57BL/6 mice. The susceptibilities of the *bg*/*bg* and *bg*/+ strains of mice infected with MAC were found to be comparable. The +/+ strain was the least susceptible. Mycobacterial burden and serum TNF-α levels increased over time in all the strains of mice tested. The *bg*/+ strain of C57BL/6 mice was then chosen to measure the activity of TNF-α antagonists. Treatment with dexamethasone decreased serum TNF-α levels and increased mycobacterial burden. Treatment with anti-TNF-α antibody or pentoxifylline did not significantly alter serum TNF-α levels but increased mycobacterial burden. Treatment with thalidomide neither consistently altered mycobacterial burden in the spleens or livers of infected mice nor affected serum TNF-α levels.

In human immunodeficiency virus (HIV) disease, overproduction of tumor necrosis factor alpha (TNF-α) is associated with increased viral replication in vitro (9, 23) and a wasting syndrome in humans (17, 21). One of the focuses of HIV treatment research has been to reduce TNF-α levels by using drug therapy. However, the impact of therapeutic reduction of TNF-α levels on the susceptibility of patients with AIDS to opportunistic infections and the progression of disease is not known.

Mycobacterium avium complex (MAC) is a common opportunistic infection in AIDS patients. It is well established that TNF-α is produced in response to *Mycobacterium* infections and is important in their control. Addition of TNF-α to cultures or animals infected with a *Mycobacterium* sp. has been associated with increased resistance to the infection, and inhibition of TNF-α has been reported to decrease resistance. In vitro addition of TNF-α to human or murine macrophages infected with *M. avium* resulted in increased intracellular killing of mycobacteria (4, 10, 19). Similarly, treatment of infected mice with TNF-α, with or without interleukin 2 (IL-2), resulted in a decrease in the mycobacterial burden in the spleens and livers of the animals (6, 8). In another study, however, an additive decrease in resistance as measured by an increase in mycobacterial CFU was observed in mice treated with a combination of antibodies to TNF-α and gamma interferon (IFN-γ) compared to that observed after administration of either antibody alone (1). The addition of pentoxifylline, a chemical inhibitor of TNF-α, to *M. avium*-infected human monocyte-derived macrophages also resulted in increased numbers of intracellular mycobacteria (32).

The purpose of this study was to evaluate the effect of TNF-α inhibitors on MAC disease progression in vivo. Several substances, including dexamethasone, antibody to TNF-α, pentoxifylline, and thalidomide, which have different mechanisms of action and effects on production and secretion of TNF-α, were evaluated in C57BL/6 *bg*/+ immunocompetent mice infected with *M. avium*. Although C57BL/6 beige (*bg*/*bg*) mice have been reported to be more susceptible to infection with MAC than wild-type (+/+) C57BL/6 mice (12, 14, 15, 18), *bg*/*bg* mice exhibit impaired phagocytic (13), NK (3, 29, 30), and T (2, 33)-cell functions, which constitute important mechanisms of immunoregulation and sources of TNF-α. The current study also evaluated the utility of C57BL/6 *bg*/+ littermates as a model for assessment of the effects of modulation of TNF-α on MAC infections.

MATERIALS AND METHODS

Mice. Female C57BL/6 *bg*/+ mice, 5 to 6 weeks of age, were used in all studies. Additionally, female C57BL/6 +/+ and C57BL/6 *bg*/*bg* mice (5 to 6 weeks old) were used in experiments which compared disease progression and serum TNF-α production among the different strains of mice. All animals (Jackson Laboratories, Bar Harbor, Maine) were randomized and housed in groups of no more than five in microisolator cages and were fed ad libitum.

Infection of mice. MAC strain 101 (MAC 101) was cultured on Middlebrook 7H11 agar plates (Remel, Lenexa, Kan.). After 2 to 3 weeks of incubation, transparent colonies of MAC 101 were picked from the plates, suspended in sterile Middlebrook 7H9 broth (Difco Laboratories, Detroit, Mich.), aliquoted, and frozen at −70°C as the stock culture (5×10^8 to 1×10^9 CFU/ml) for all infection studies. The mice were infected intravenously with 5 to 6×10^7 CFU of MAC 101 in 7H9 broth. Control mice were sham infected with broth. Groups of five animals were sacrificed at weeks 1, 3, 5, and 8 following infection and evaluated for body weight, organ weight (spleen, liver, and lung), and microbial burden in the weighed subsections of these organs. Blood was collected for

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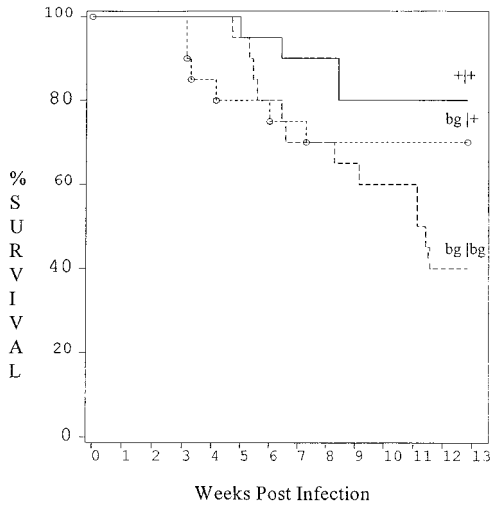

FIG. 1. Percent survival of three strains (*bg*/*bg*, *bg*/+, and +/+) of C57BL/6 mice infected with MAC 101. All the uninfected mice survived the 13-week period of observation. Analysis by the log rank test indicated that there was a statistically significant difference in time to death among the three strains of mice ($P < 0.05$). The pairwise comparison showed the susceptibility of the *bg*/+ strain of mice to be similar to that of the *bg*/*bg* strain ($P = 0.144$). The +/+ mice were the least susceptible and were statistically different from the *bg*/*bg* strain of mice ($P = 0.014$) but not from the *bg*/+ strain ($P = 0.373$).

measurement of TNF-α levels in the serum. A separate group of each strain of mice, infected ($n = 20$) and uninfected ($n = 10$), was set aside for a survival study.

Microbial burden. Weighed sections of tissues (liver, lung, and spleen) were homogenized in Middlebrook 7H9 medium (Difco), and aliquots from different dilutions were plated onto Middlebrook 7H11 agar plates (Remel) in triplicate. The cultures were incubated for 3 weeks at 37°C in 7% CO_2.

TNF-α levels. TNF-α levels in the sera were measured by an enzyme-linked immunosorbent assay with kits obtained from Genzyme Diagnostics (Cambridge, Mass.).

Treatment with TNF-α inhibitors and measurement of disease progression. C57BL/6 *bg*/+ mice were infected with MAC 101 as described above. Sufficient animals were randomized to each treatment group in every study to compensate for the expected death rate due to mycobacterial infection. The mice were treated with various doses of dexamethasone, anti-TNF-α antibody, pentoxifylline, thalidomide, or the vehicle beginning at the time of initiation of infection. All treatments were administered by the intraperitoneal route for a period of up to 8 weeks. Dexamethasone 21-phosphate (Sigma Chemical Co., St. Louis, Mo.) was administered on alternate days. Pentoxifylline (Sigma Chemical Co.) was administered daily. Thalidomide (courtesy of John Reepmeyer, Division of Drug Analysis, Food and Drug Administration [FDA], St. Louis, Mo.) was prepared daily in acidified, tissue culture grade water (pH 5.0) immediately prior to injection to minimize the potential for hydrolysis. Anti-TNF-α antibody and the isotype control (XT-11-22 and GL113, respectively; DNAX, Palo Alto, Calif.) were administered once a week. Dexamethasone, pentoxifylline, and anti-TNF-α antibodies were dissolved or diluted in phosphate-buffered saline (PBS), pH 7.2, and thalidomide was diluted in acidified water. PBS and acidified water, respectively, were used as vehicle controls in each experiment. Untreated (i.e., naive) mice, infected and uninfected, were also included in the study. Five mice from each treatment group were sacrificed at different time points, and the spleens and livers were processed for measurement of microbial burden. TNF-α levels were measured in the sera as described above.

Statistical analysis. A Kaplan-Meier curve was used to demonstrate survival rates over time. Time to death among three different strains of mice was analyzed by the log rank test. The pairwise comparison was used after the overall *P* value was found to be less than 0.05; therefore, no adjustment was imposed on the pairwise comparison of *P* values.

Differences in various parameters (including body weight, organ weight, microbial burden, and serum TNF-α level) were determined by analysis of variance. Results of the microbial burden (in CFU) were analyzed after log transformation of the data. All *P* values reported are the results of two-tailed tests, with no adjustment for multiple comparisons.

RESULTS

Susceptibility of C57BL/6 (*bg*/+) mice to MAC 101. C57BL/6 mice heterozygous for the beige allele (*bg*/+) were susceptible to infection with MAC 101. Mortality rates for *bg*/+ mice were intermediate to the rates observed for beige (*bg*/*bg*) mice and C57BL/6 wild-type (+/+) mice (Fig. 1). Following infection, initial deaths among the *bg*/+ mice occurred earlier (week 3) than among the *bg*/*bg* and +/+ mice (week 5). However, by week 6, the mortality rates for *bg*/+ mice were comparable to those for the *bg*/*bg* mice. Mortality in *bg*/*bg* mice continued to increase throughout the observation period, whereas the mortality rates stabilized at week 8 for both *bg*/+ and +/+ strains.

TABLE 1. Relative organ weights in three strains of C57BL/6 mice at 3 weeks following infection with MAC 101

Strain	Infection[a]	Organ wt (g)/100 g of body wt (mean ± SE)[b]		
		Spleen	Liver	Lung
bg/*bg*	+	4.04 ± 0.10*	13.66 ± 0.37*	1.54 ± 0.08*
	−	0.53 ± 0.04	6.22 ± 0.49	1.08 ± 0.05
bg/+	+	3.74 ± 0.17*	12.01 ± 0.36*	1.26 ± 0.11
	−	0.47 ± 0.03	6.34 ± 0.20	1.06 ± 0.05
+/+	+	3.79 ± 0.18*	14.04 ± 0.28*	1.22 ± 0.08*
	−	0.55 ± 0.03	6.71 ± 0.12	1.03 ± 0.03

[a] +, infected; −, not infected.
[b] *, $P < 0.05$ compared to uninfected control.

Mycobacterial disease progression, as measured by increased organ weights (splenomegaly or hepatomegaly) and microbial burden, also developed in C57BL/6 *bg*/+ mice. Organ weights, relative to body weight, increased throughout the course of infection in all three strains of mice (Table 1). C57BL/6 *bg*/+ mice exhibited splenomegaly by week 1 and hepatomegaly by week 3 following infection with *M. avium*. Mycobacteria were recoverable from the spleens, livers (Fig. 2), and lungs, and CFU increased over time in all three strains of mice infected with MAC 101. In general, the mycobacterial burden was the greatest in *bg*/*bg* mice at most time points.

Serum TNF-α levels following infection. No consistent differences in serum TNF-α levels among the three strains of

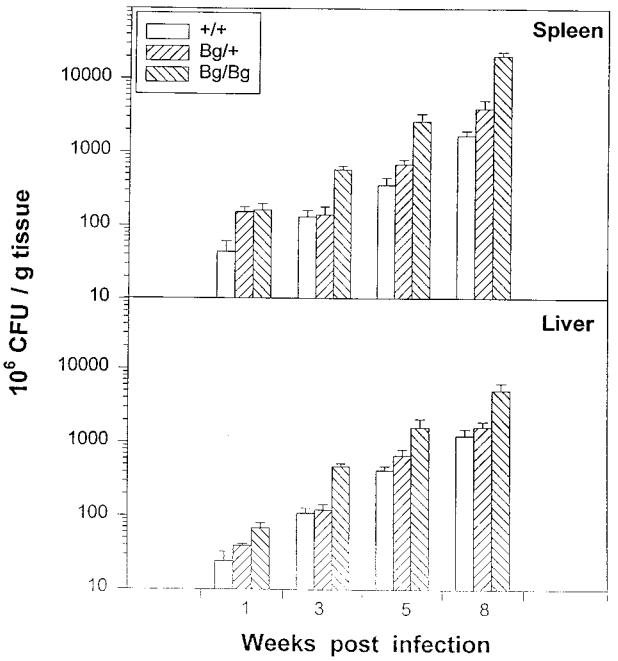

FIG. 2. Microbial burden in three strains (*bg*/*bg*, *bg*/+, and +/+) of C57BL/6 mice at weeks 1, 3, 5, and 8 following infection with MAC 101. The results are expressed as mean (+ standard error) 10^6 CFU per gram of tissue (spleen and liver).

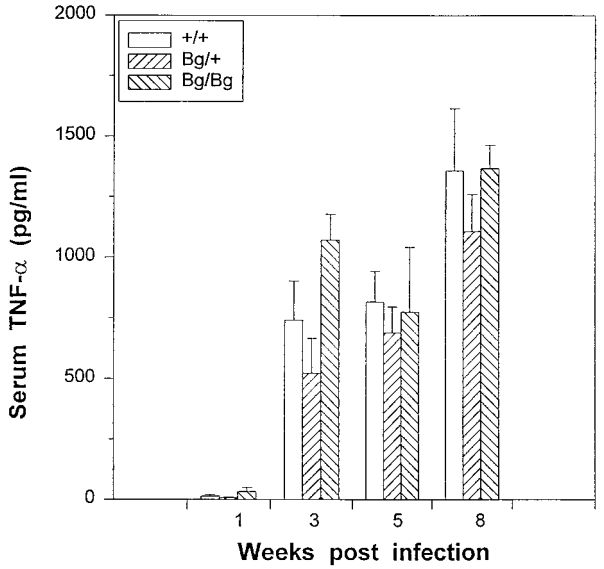

FIG. 3. Serum TNF-α levels in three strains (*bg*/*bg*, *bg*/+, and +/+) of C57BL/6 mice at weeks 1, 3, 5, and 8 following infection with MAC 101. The results are expressed as means + standard errors. The uninfected mice from all strains did not show significant levels of serum TNF-α at any of the time points when they were tested.

mice were observed following infection. TNF-α was detectable in the sera at week 1 of infection with MAC 101 (Fig. 3), and the levels of TNF-α increased over time in all infected animals. The uninfected animals demonstrated no detectable TNF-α in their sera at any of the time points when they were tested.

Effect of dexamethasone on disease progression. C57BL/6 *bg*/+ mice were treated with dexamethasone at doses of 10 or 20 mg/kg of body weight every other day up to 8 weeks. Treatment with 10 or 20 mg of dexamethasone/kg suppressed the serum TNF-α levels by two- to threefold after 3 and 8 weeks of treatment (Fig. 4). The typical mycobacterial infection-induced splenomegaly and hepatomegaly were decreased by two- to threefold in the infected mice (data not shown). In contrast, microbial burden was increased by 1 and 2 log units in the livers and spleens of mice treated with dexamethasone (Fig. 5).

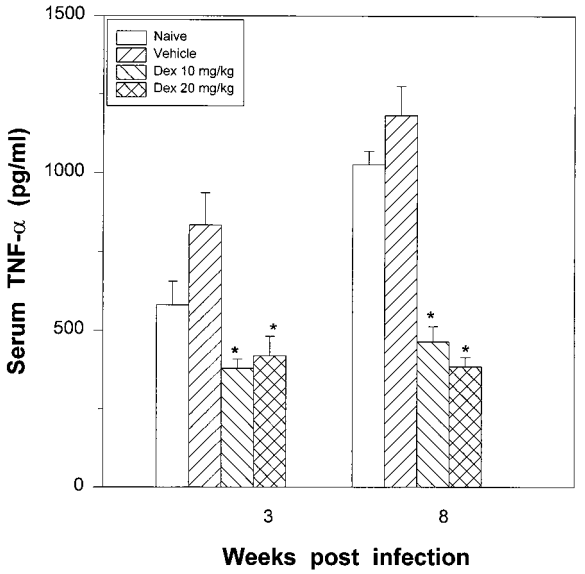

FIG. 4. Effect of treatment with dexamethasone (10 and 20 mg/kg) on serum TNF-α levels in the *bg*/+ strain of C57BL/6 mice infected with MAC 101. Dexamethasone was solubilized in PBS, pH 7.2, and administered every other day by the intraperitoneal route. The results are expressed as means + standard errors. An asterisk above an error bar indicates a statistically significant difference ($P < 0.05$) compared to the controls (naive and vehicle-treated groups). The uninfected mice from both of the treated groups did not show significant levels of serum TNF-α at any of the time points tested.

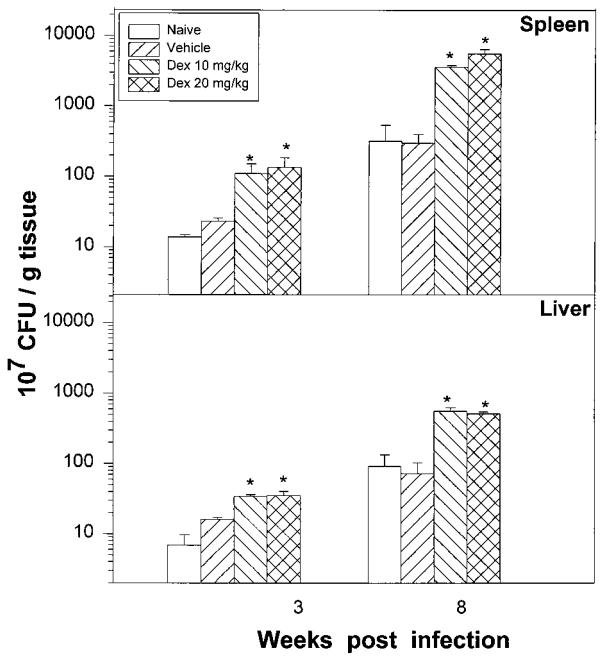

FIG. 5. Effect of treatment with dexamethasone (10 and 20 mg/kg) on microbial burden (10^7 CFU/g of tissue of spleen and liver) in the *bg*/+ strain of C57BL/6 mice infected with MAC 101. Dexamethasone was solubilized in PBS, pH 7.2, and administered every other day by the intraperitoneal route. The results are expressed as means + standard errors. An asterisk above an error bar indicates a statistically significant difference ($P < 0.05$) compared to the controls (naive and vehicle-treated groups).

Effect of anti-TNF-α antibody on disease progression. Previous studies (1) have demonstrated that administration of anti-TNF-α antibody results in an increase in mycobacterial CFU in the livers and spleens of BALB/c mice. To confirm the role of TNF-α in mycobacterial resistance in the present model, C57BL/6 *bg*/+ mice were treated with anti-TNF-α antibody at doses of 0.75 to 7.5 mg/kg once weekly for 3 weeks. Treatment with anti-TNF-α antibody for a period of 3 weeks increased the microbial burden in the spleen over the isotype control group at all doses tested. Splenic CFU ranged from 2.0×10^8 to 3.1×10^8 per g of tissue in the isotype control groups and were increased to 8.7×10^8 to 11.2×10^8 per g of tissue in the anti-TNF-α antibody-treated group. The microbial burden in the liver showed a similar trend; however, the changes observed were not statistically different (1.6×10^8 to 2.1×10^8 CFU per g of liver in the control group to 2.2×10^8 to 3.3×10^8 CFU per g of liver in the treatment group). Neither the weight of the spleen, liver, or lung nor serum TNF-α levels (as measured by enzyme-linked immunosorbent assay) were altered by treatment with anti-TNF-α antibody.

Effect of pentoxifylline on disease progression. C57BL/6 *bg*/+ mice were treated with pentoxifylline daily at doses of 30, 100, or 300 mg/kg for up to 8 weeks. Treatment with pentoxifylline up to a dose of 100 mg/kg did not significantly alter the organ weights or serum TNF-α levels in infected or uninfected mice (data not shown). Mycobacterial burden, however, was in-

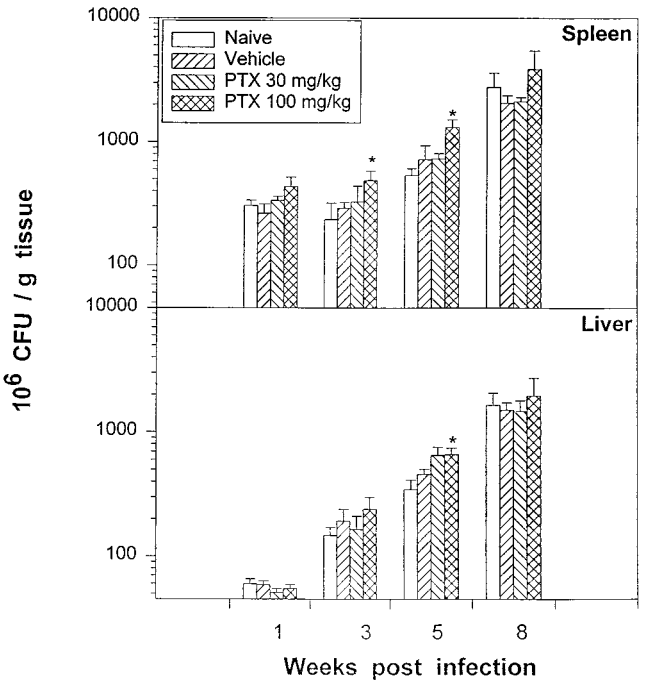

FIG. 6. Effect of treatment with pentoxifylline (PTX; 30 and 100 mg/kg) on microbial burden (10^6 CFU/g of tissue of spleen and liver) in the *bg*/+ strain of C57BL/6 mice infected with MAC 101. Pentoxifylline was solubilized in PBS, pH 7.2, and administered every day by the intraperitoneal route. The results are expressed as means + standard errors. An asterisk above an error bar indicates a statistically significant difference ($P < 0.05$) compared to the controls (naive and vehicle-treated groups). The highest dose of pentoxifylline tested (300 mg/kg) was found to be lethal.

creased significantly in the spleen after 3 and 5 weeks of treatment, and in the liver after 5 weeks of treatment, with 100 mg of pentoxifylline/kg (Fig. 6). These differences were not observed at 8 weeks postinfection. The lowest dose (30 mg/kg) had no significant effect on disease progression. The highest dose of pentoxifylline tested (300 mg/kg) was found to be toxic (all the mice died within a few hours of drug administration).

Effect of thalidomide on disease progression. C57BL/6 *bg*/+ mice were treated with thalidomide at doses of 10, 30, or 100 mg/kg daily for up to 8 weeks. Thalidomide was prepared daily in acidified water to reduce the potential for hydrolysis. Serum TNF-α levels, microbial burden, and weights of spleens and livers were not altered consistently by treatment with thalidomide. Treatment with thalidomide had no appreciable effect on mycobacterial burden following 3 weeks of infection and treatment. At 5 weeks, splenic CFU were modestly increased in the thalidomide-treated groups; the increase was statistically significant only in the 30 mg/kg/day group (Fig. 7). By 8 weeks, the treatment effect was abrogated. No changes in CFU were observed in the livers at any time point when they were tested.

DISCUSSION

One of the strategies for treatment of HIV disease has been the use of TNF-α antagonists, which have been shown to inhibit TNF-α-induced HIV replication in vitro (11, 22, 27). However, cytokines play an important role in resistance to infections. Thus, therapy that produces an imbalance or defect in one of the cytokines may produce an alteration in susceptibility to infection. TNF-α is an important cytokine in conferring protection, either directly or indirectly, against opportunistic infections due to agents such as *M. avium*. In this regard, stimulation or activation of human monocyte-derived macrophages in vitro with TNF-α was associated with increased intracellular killing of *M. avium* (4, 36). Pourshafie and Sonnenfeld (28) and Gomez-Flores et al. (16) demonstrated an increased in vitro killing of *M. avium* by murine macrophages in the presence of exogenous TNF-α. Mycobacterial killing by macrophages was enhanced if the cells were activated with IFN-γ before infection (compared to killing by resident macrophages) and was correlated with a fivefold-increased production of TNF-α (16). Finally, treatment of mice with TNF-α, IL-2, or a combination of TNF-α and IL-2 1 week after infection with *M. avium* resulted in decreased CFU in the spleen and liver (6), and treatment with anti-TNF-α antibody resulted in increased CFU in the spleens and livers of infected mice (1).

In the present study, we examined the impact of treatment with TNF-α antagonists on the susceptibility of C57BL/6 *bg*/+ mice to MAC infection. Treatment with dexamethasone at the time of infection significantly increased mycobacterial burden in the spleen and liver. Such an effect was observed within 3 weeks of infection and persisted at 8 weeks of infection. Treatment with anti-TNF-α antibody and pentoxifylline also resulted in significant increases in splenic CFU but not dependably in liver CFU. Treatment with thalidomide did not consistently alter mycobacterial burden in either the spleens or the livers of infected mice, but a trend towards an increase in splenic CFU was observed at 5 weeks postinfection. Increases in microbial burden following treatment did not result in any overt decrease in the survival of infected mice compared to that of the infected control groups; however, specific survival studies of treated mice were not conducted.

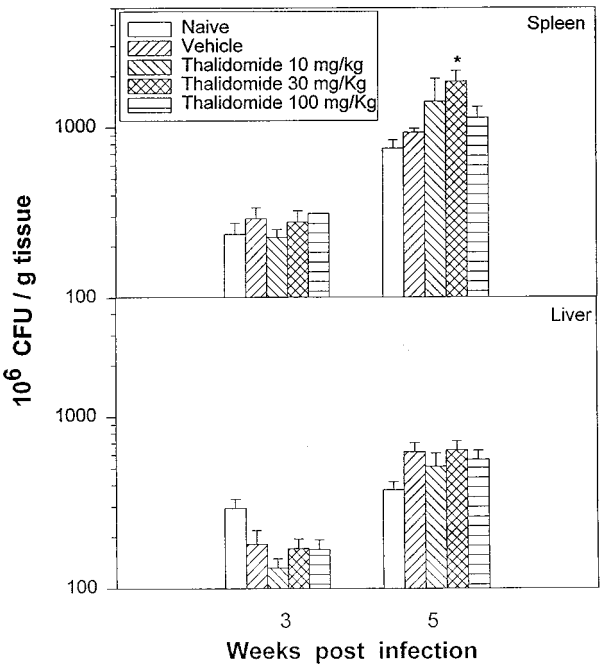

FIG. 7. Effect of treatment with thalidomide (10, 30, and 100 mg/kg) on microbial burden (10^6 CFU/g of tissue of spleen and liver) in the *bg*/+ strain of C57BL/6 mice infected with MAC 101. Thalidomide was suspended in water and administered every day by the intraperitoneal route. The results are expressed as means + standard errors. An asterisk above an error bar indicates a statistically significant difference ($P < 0.05$) compared to the controls (naive and vehicle-treated groups).

The results presented here are compatible with those of several other studies which demonstrate that inhibition of TNF-α can dysregulate the cytokine cascade, leading to a reduction in resistance to MAC infections. Treatment of *M. avium*-infected SCID and BALB/c mice with anti-TNF-α antibody was shown to increase mycobacterial CFU in the spleens and livers (1, 8) and reduce the protective effect of immunization with BCG (1). Antibody to TNF-α inhibited in vitro killing of MAC in human monocyte-derived macrophages activated with vitamin D (5), and treatment of ex vivo- or in vitro-infected human monocyte-derived macrophages with pentoxifylline or dexamethasone enhanced MAC growth (31, 32). Similarly, treatment of MAC-infected murine peritoneal macrophages with pentoxifylline or anti-TNF-α antibody suppressed the anti-mycobacterial response induced by activation of the macrophages with IFN-γ and TNF-α (16).

In the present study, dexamethasone was more potent than either pentoxifylline or thalidomide in reducing serum TNF-α levels and enhancing mycobacterial burden in infected mice. The increase in mycobacterial burden following dexamethasone treatment may be attributable to the substantive reduction of systemic TNF-α levels. Alternatively, general corticosteroid effects on multiple immune or inflammatory effector mechanisms, including neutrophil migration, phagocytosis, lymphocyte apoptosis and function, and the disruption or inhibition of several cytokines in addition to TNF-α, may contribute substantially to the mechanism of inhibition.

Clearly, TNF-α is somewhat involved in resistance to MAC infections in the present model. Anti-TNF-α antibody, unlike dexamethasone, is a neutralizing antibody which specifically inhibits the function of TNF-α. In our study, immunoreactive TNF-α levels were not reduced in the serum following administration of anti-TNF-α antibody; however, mycobacterial CFU were significantly increased in the spleen and were higher (although the increase was not statistically significant) in the liver. These effects on CFU levels are consistent with the increase in mycobacterial levels in BALB/c mice following anti-TNF-α antibody treatment reported by Appleberg et al. (1). The lack of a reduction in detectable serum TNF-α levels does not preclude the possibility that TNF-α function was altered following antibody treatment (10) or that localized production of TNF-α was reduced. Moreover, the lack of a measurable effect on serum TNF-α levels, as detected by immunoassay, may simply be a limitation of the methodology resulting from competition between the antibodies used for neutralization in vivo and detection in vitro. Alternatively, mycobacterial infections are potent inducers of TNF-α, and doses of anti-TNF-α antibody substantially higher than those used in the present study may have been needed to alter serum TNF-α levels and to further reduce CFU in the spleen and liver. The modest increase in CFU observed following administration of anti-TNF-α antibody may reflect the fact that TNF-α is not the only cytokine or immunologic mechanism contributing to overall resistance to *M. avium* infections.

The effect of pentoxifylline appears to be more modest than that of dexamethasone or antibody to TNF-α on host resistance to MAC infections. Pentoxifylline (100 mg/kg/day) increased splenic CFU at weeks 3 and 5 postinfection and increased liver CFU at week 5 postinfection. Higher doses of pentoxifylline were toxic and could not be evaluated. Although a trend towards increased splenic CFU was observed at 5 weeks postinfection in the present study, thalidomide treatment had no consistent effect on mycobacterial burden or TNF-α levels. While thalidomide has been reported to be a selective inhibitor of TNF-α production (22, 24) in vitro, demonstration of in vivo inhibition of TNF-α has been inconsistent. Thalidomide has been shown not to inhibit endotoxin-induced TNF-α production in mice, but it did reduce TNF-α plasma levels in nonneutropenic mice after injection with *Candida albicans* (26). This may be a reflection of the potency of thalidomide with respect to inhibition of TNF-α production in murine models: TNF-α levels induced by endotoxin were 5- to 10-fold greater than the levels induced by *C. albicans*. In the present study, MAC infections are strong inducers of TNF-α production; thus, thalidomide may not be a sufficient inhibitor of TNF-α in this system to alter resistance. It should also be noted that by week 8, approximately 30% of the animals had died irrespective of treatment, and the effect on CFU may be abrogated. The abrogation of the effect may have been the result of censoring of data as the result of the level of mortality at week 8 or simply that TNF-α inhibitors were less effective at reducing TNF-α levels in this model.

Studies with TNF-α inhibitors have shown that TNF-α plays a role in resistance to MAC infections. Multiple mechanisms, however, clearly operate in conferring susceptibility or resistance to *M. avium* infection. For example, in immunocompromised C57BL/6 *bg*/*bg* mice, in vivo treatment with IFN-γ (which enhanced the production of TNF-α) did not decrease the mycobacterial burden in the spleen and liver but showed an inhibitory effect on the mycobacterial count from peritoneal macrophages (16). These seemingly contradictory actions on different cell types may be the result of differences in cell maturation and/or activation in different tissues or compartments of the lymphoid system. Multiple cytokines are also important in resistance to MAC infections. Addition of IFN-γ (19, 34), granulocyte-macrophage colony-stimulating factor (19), or anti-transforming growth factor β antibodies in vitro (7) and treatment of mice with IL-12 (20) have been reported to reduce mycobacterial burden. In contrast, IL-3, IL-6, and macrophage colony-stimulating factor have been reported to increase mycobacterial burden in vitro (34). It is of interest to note that dexamethasone and pentoxifylline showed opposite effects on IL-6 production; dexamethasone was shown to suppress IL-6 production, whereas pentoxifylline enhanced it (31). The relevance of these findings to in vivo situations is unclear.

Chemical inhibition of TNF-α decreases resistance to MAC infections in vivo and in vitro. However, most inhibitors not only inhibit different steps in the cytokine biosynthesis pathway (24) but also are not truly specific for TNF-α. For example, pentoxifylline, in addition to inhibiting TNF-α, may suppress the production of IFN-γ, IL-10, and other immune functions (25, 35). Dexamethasone inhibits multiple cytokines. Thus, suppression of a specific cytokine may disrupt the cascade effect of the cytokine network and alter susceptibility to infection. The extent of such an effect may be influenced by the time of onset of treatment, the concentration of the inhibitor used, and the immune status of the host. This could be taken to indicate a difference in cell maturation and/or activation in different tissues or compartments of the lymphoid system.

C57BL/6 *bg*/*bg* mice, known to have impaired phagocytic, NK, and T-cell functions (2, 3, 13, 29, 30, 33), have been routinely used for testing the activity of antimycobacterial drugs (14). In our studies, C57BL/6 *bg*/+ mice were chosen for measuring the activity of immunomodulatory agents. The susceptibility to MAC infection of the *bg*/+ strain was comparable to that of the *bg*/*bg* strain. The wild-type strain was less susceptible. All three strains of mice tested were shown to produce substantial amounts of TNF-α in response to MAC infection. C57BL/6 *bg*/+ mice may provide a useful model for the assessment of other immunomodulators on the progression of disease following MAC infection.

In summary, our results suggest that substances which in-

hibit cytokine production following MAC infection may reduce resistance to *M. avium* in vivo. The clinical relevance of this finding is unknown. However, it may be worthwhile to consider that treatment of AIDS patients with immunomodulatory drugs may ultimately impact their resistance to opportunistic infections.

ACKNOWLEDGMENTS

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