Interleukin (IL)-12 Deficiency in Susceptible Mice Infected with Mycobacterium avium and Amelioration of Established Infection by IL-12 Replacement Therapy

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Mycobacterium avium is an intracellular microorganism that infects and multiplies within macrophages. Cell-mediated immunity plays an important role in host defense, and interleukin (IL)-12, which is produced mainly by macrophages, is critical for its development. In a mouse model of disseminated M. avium infection, genetically susceptible BALB/c mice had increased mycobacterial growth and decreased IL-12 expression and developed large and numerous granulomas. In contrast, resistant DBA/2 mice exhibited reduced mycobacterial burden with increased IL-12 expression and developed fewer and smaller granulomas. In susceptible mice with established M. avium infection, IL-12 replacement therapy resulted in persistent reduction of mycobacterial burdens. IL-12 itself, however, could not inhibit mycobacterial growth in vitro. By enhancing host defenses, IL-12 exerts a potent mycobactericidal activity in vivo with low toxicity. This suggests that IL-12 replacement therapy is rational for M. avium infection in susceptible hosts.

Infections with Mycobacterium avium complex (MAC), also known as M. avium-intracellulare, are an important health problem, especially in immunosuppressed persons. Disseminated MAC infections are frequent in AIDS patients with CD4 T cell counts <100/μL and cause increased morbidity and shortened survival times [1]. In addition, most MAC strains are resistant to antituberculous drugs [2]. These bacteria are facultative intracellular pathogens and invade and multiply within macrophages. Host defense mechanisms against MAC are poorly understood.

The genetic control of susceptibility or resistance to mycobacterial infection is generally considered to be multifactorial. The influence of specific genes depends on the dose of mycobacteria, the route and the phase of infection, and the parameter of host response studied. In mice, natural resistance or susceptibility to mycobacterial infection with bacille Calmette-Guérin (BCG), Mycobacterium lepraemurium, and MAC is under genetic control [3-6]. In bred mouse strains, intravenous infection with low doses of BCG is biphasic, with an early nonimmune phase (natural resistance or susceptibility at 0-3 weeks) characterized by rapid proliferation of the bacteria in reticuloendothelial organs (liver and spleen) of susceptible strains and absence of bacterial growth in resistant strains [7]. The natural resistance or susceptibility in the early phase is thought to be controlled by the chromosome 1 gene designated Bcg/It/Lo [3], with or without other less-defined non-H-2 genes [4, 8]. However, the precise role of such genes is not known. In addition, backcross analysis experiments have shown that natural resistance to M. avium is under polygenic control [6]. The late phase (3-6 weeks) is associated with specific immune response [9, 10], leading either to clearance of the bacteria or to persistent infection of susceptible strains in reticuloendothelial organs. The late phase of infection is controlled by genes linked to H-2 [10].

Macrophages and monocytes are the first line of defense against mycobacteria and when infected, they rapidly become activated to inhibit the growth of or to kill intracellular bacteria [11]. During intracellular persistence, microbial proteins are processed and presented, thus initiating T cell activation [12]. By secreting cytokines, such as interferon-γ (IFN-γ), CD4 Th1 cells activate macrophages, converting them from a habitat to potent effector cells [11]. Protection in the host is a local event focused on granulomatous inflammation [13]. Macrophages accumulate at the site of microbial growth and become activated through the Th1 cell – cytokine – macrophage axis [11]. The development of cell-mediated immunity plays an important role in the defense against mycobacteria [11, 13].

Interleukin (IL)-12, a heterodimeric cytokine produced mainly by macrophages and monocytes, has numerous biologic functions. It promotes the differentiation and proliferation of CD4 Th1 cells from uncommitted T cells and stimulates IFN-γ production from Th1 and NK cells and, consequently, initiates cell-mediated immunity [14]. IFN-γ mediates the activation of macrophage bactericidal functions such as respiratory burst, nitric oxide synthesis, and production of numerous prote-
ases and other lysosomal enzymes. The key role of IFN-γ-mediated macrophage activation in eradication of intracellular pathogens has been established [11, 15].

In the present study, by using a mouse model of disseminated *M. avium* infection, we examined IL-12 expression in genetically susceptible (BALB/c) and resistant (DBA/2) mice in response to *M. avium* infection. We also assessed the potential efficacy of IL-12 replacement therapy for an established systemic infection with *M. avium* in susceptible mice.

**Materials and Methods**

**Mice.** Female specific pathogen-free BALB/c (H-2d) and DBA/2 (H-2k) mice, 6–8 weeks old, and weighing 20–25 g were purchased (Charles River Japan, Tokyo). BALB/c mice are classified as genetically susceptible, whereas DBA/2 mice are resistant to MAC infection [5, 6]. No significant changes in weights were observed during the experiment period regardless of treatment.

**Microorganism.** *M. avium* Mino (provided by T. Yamazaki and R. M. Nakamura, Division of Mycobacteriology, National Institute of Health, Tokyo), originally isolated from the sputum of a patient with nontuberculous mycobacteriosis [16], was grown in aerated Middlebrook 7H9 broth (Difco, Detroit) supplemented with albumin dextrose catalase (ADC enrichment; Difco) and 0.05% Tween 80 with shaking at 37°C. Log-phase bacteria were harvested, washed, and stored at −80°C. Before mice were infected, an ampule was thawed, vortexed for 2 min, filtered through a membrane filter (2-μm pore size; Millipore, Bedford, MA), and sonicated for 5 s (25 kHz; power output, 20 W) using a high-intensity ultrasonic processor (Handy Sonic UR-20P; Tomy Seiko, Tokyo) to obtain a single-cell bacterial suspension. After sonication, we determined the number of viable *M. avium* by plating serial dilutions of the suspension onto Middlebrook 7H10 agar, which was maintained for 8 days at 37°C. Mice were inoculated in tail veins with 10⁸ cfu/mL. Tissue was kept on ice during these procedures. Bacterial growth in the target organ was monitored against time by plating the tissue pellet was dissolved in diethyl pyrocarbonate–treated H₂O.

**RT-PCR** was done as previously described [18]. Briefly, 5 μg of total RNA from a spleen sample was reverse-transcribed into cDNA using 200 U of Moloney murine leukemia virus RT (GIBCO BRL, Grand Island, NY). The cDNA was then amplified using specific primers for murine IL-12 p40, IL-12 p35, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH): The latter was used as an internal control. The primers used were 5’-AATTGCGGTT-GGAAGCAGCG-3’ (sense) and 5’-GAACACTGCCCCACTTGG-CTG-3’ (antisense) for IL-12 p40 [19], 5’-ACCTCAGTTTGGCC-AGGTC-3’ (sense) and 5’-CAAGGCACAGGGTCATCATC-3’ (antisense) for IL-12 p35 [19], and 5’-GAGCCACAGGGTCCATCATC-3’ (antisense) for GAPDH [20]. Amplifications were done in 1.5 mM MgCl₂, 50 mM KCl, 10 mM TRIS–HCl (pH 8.3), 0.2 mM each dNTP, 2.5 μM Taq DNA polymerase (Boehringer Mannheim Biochemicals, Indianapolis), 2 μM each specific primer, and 1 μL of the reverse-transcribed cDNA samples. The cDNA was amplified in an automated thermal cycler (Perkin-Elmer Cetus, Norwalk, CT) at 95°C for 40 s, 60°C for 60 s, and 72°C for 90 s. Amplification was stopped at 30 cycles. The rate of amplification with the primer sets used was exponential for 30 cycles (as assessed in preliminary experiments). After amplification, the sample (20 μL) was separated on a 2% agarose gel containing 0.3 μg/mL ethidium bromide. The DNA was visualized and photographed using UV transillumination. The sequences of cytokine-specific primer pairs 5’ and 3’ have been reported [19].

**Aqueous spleen extracts.** Aqueous spleen extracts were prepared as described [21, 22]. In brief, homogenized tissues were centrifuged in a refrigerated unit at 2000 g for 30 min and the tissue pellet was discarded. Samples were then sterilized with a membrane (pore size, 0.45 μm; Millipore) and stored at −70°C until use. Aqueous extracts were adjusted for 1 mg/mL protein in PBS.

**Antibody-capture bioassay for IL-12 activity.** Levels of bioactive IL-12 were determined by antibody-capture bioassay as described [23]. Aqueous spleen extracts and serial dilutions of standard murine IL-12 (specific activity: 7 × 10⁶ U/mg of protein; Genetics Institute) [24] were added to 96-well flat-bottom plates coated with 5 μg/mL purified sheep IgG antibody to murine IL-12 (Sheep 7 G28A PB2; Genetics Institute). A 1:500 dilution of a 1.2 mg/mL preparation of the antibody completely neutralized 5 ng/mL murine IL-12. Human phytohemagglutinin (PHA)–stimulated lymphoblasts (peripheral blood mononuclear cells [PBMC] stimulated with 20 μg/mL PHA for 3 days and then with 50 U/mL IL-2 for 1 day) were added (2 × 10⁵ cells/well). After incubation for 48 h, proliferation was measured by incorporation of [³H]thymidine (0.5 μCi = 18.5 kBq/well for 6 h). A standard curve was plotted, and IL-12 content of samples was calculated.

**In vivo treatment with IL-12.** For administration to mice, recombinant murine IL-12 (Genetics Institute) was diluted in PBS containing 1 mg/mL (0.1%) mouse serum albumin (MSA; Sigma, St. Louis). In vivo intraperitoneal (ip) treatment with murine IL-12 (0.1, 1, or 10 μg/kg/day) 6 times a week was begun 21 days
after susceptible mice were inoculated with 10^8 cfu of *M. avium* and continued for 3 weeks. Endotoxin levels of murine recombinant IL-12 measured by the limulus amebocyte assay did not exceed 0.5–0.9 EU/mg of protein [24]. Infected control mice were given 0.1% MSA-PBS alone for 3 weeks.

**Statistical analysis.** There were >10 mice in each group. Data were analyzed by Macintosh II computer using a statistical software package (Statview II; Abacus Concepts, Berkeley, CA) and expressed as mean ± SD. Data that appeared statistically significant were compared by an analysis of variance of means of multiple groups. *P < .05 was considered significant.

**Results**

**Growth of *M. avium* in susceptible and resistant mice.** Groups of 10 mice for each time point were injected intravenously with 10^8 cfu of *M. avium* Mino. There were no differences between BALB/c and DBA/2 mice in mycobacterial growth at day 1. In BALB/c mice, active multiplication of *M. avium* in organs was observed 21–140 days after inoculation (figure 1). In contrast, the number of viable *M. avium* recovered from DBA/2 organs was significantly lower than from BALB/c mice at these stages (*P < .02*). These results are consistent with previous studies in which in vivo growth of mycobacteria, including MAC and BCG, was observed in susceptible (e.g., BALB/c) but not in resistant mice (e.g., DBA/2) [5, 7, 25].

**Histopathologic features.** At day 21, the spleens of BALB/c mice infected with *M. avium* showed many circular clusters composed of epithelioid macrophages and lymphocytes; DBA/2 mice had smaller and fewer lesions at the late stages. The cellular components of granulomatous lesions were similar to those of susceptible BALB/c mice.

**Tissue IL-12 mRNA and protein expression.** We used RT-PCR to examine IL-12 mRNA expression in the spleens of mice infected with *M. avium* (figure 3). A very mild increase in IL-12 p40 mRNA expression was detected in spleens of BALB/c mice shortly after mycobacteria were injected (3–24 h), but levels returned to baseline by day 3. In contrast, we found a marked increase in IL-12 p40 mRNA expression in spleens of DBA/2 mice shortly after inoculation: Peak intensity occurred by 3 h and returned to baseline levels by day 21. In both strains of mice, IL-12 p35 and GAPDH mRNA was expressed constitutively in the spleen during the course of *M. avium* infection and the levels were similar. We next examined bioactive IL-12 protein levels in the spleens of mice infected with *M. avium* by using an anti-IL-12 antibody-capture biosay (figure 4). IL-12 bioactivity in spleen extracts of infected DBA/2 mice reached a maximum within 1 day and gradually declined thereafter to lower (but still significant) levels by day 21. In contrast, IL-12 activity in the extracts from BALB/c mice infected with *M. avium* was considerably less and was maintained for less time than in DBA/2 mice. IL-12 p40 mRNA expression related to subsequent production or translation of bioactive IL-12 protein. Spleen extracts from uninfected mice (both DBA/2 and BALB/c) had no IL-12 bioactivity (data not shown). Resistant DBA/2 mice expressed high levels of IL-12 mRNA and protein in response to *M. avium* infection compared with susceptible BALB/c mice.

**IL-12 therapy for established *M. avium* infection in BALB/c mice.** We previously reported that ip treatment of BALB/c mice with IL-12 (10 μg/kg/day for 5 weeks), beginning at the time of *M. avium* infection, induced protective immunity...
Figure 2. Histopathologic features of spleens of susceptible (BALB/c) and resistant mice (DBA/2). 4-μm-thick sections of formalin-fixed, paraffin-embedded tissues stained with hematoxylin-eosin. At day 21, BALB/c mice infected with *M. avium* had many circular clusters composed of epithelioid macrophages and lymphocytes but DBA/2 mice had rare and smaller epithelioid granulomas. At day 140, BALB/c mice infected with *M. avium* had many randomly distributed granulomas and large organized lesions composed of foamy cells, epithelioid macrophages, and lymphocytes. BALB/c mice infected with *M. avium* had more and larger granulomas than did DBA/2 mice. Normal = uninfected mice.
of mice (BALB/c, DBA/2) infected with M. avium
in susceptible mice. IL-12 administration (ip) to infected
mice, although granulomas in mice given IL-12 at therapeutically
effective doses were often fused in association with de­
creased numbers of acid-fast bacilli in tissue stained by the
Ziehl-Neelsen technique (figure 6).

IL-12 toxicities in susceptible BALB/c mice with established
M. avium infection. IL-12 toxicities, including fluid leak,
skeletal muscle necrosis, hematotoxicity, and hepatotoxicity
have been reported at high dosages (≥50 μg/kg/day) [27]. We
assessed IL-12 toxicities (clinical manifestations and serum
chemistry) in M. avium–infected susceptible mice given IL-
12 at 1 and 10 μg/kg/day for 3 weeks. These doses exerted a
potent antimycobacterial effect (figure 5).

In IL-12–treated and -untreated BALB/c mice with estab­
lished M. avium infection, we could not detect fluid leak, in­
cluding pleural effusion and ascites. No nephrotoxicity (as­
essed by blood urea nitrogen and creatinine levels) was
observed in infected mice regardless of IL-12 therapy (table
1). However, we found moderate myopathy and hepatotoxicity
in infected BALB/c mice without IL-12 therapy (infected con­
trols) evaluated by serum creatine kinase (CK), and alanine
aminotransferase (ALT). The elevated serum enzymes may
have been due to M. avium infection. CK levels of IL-12­
treated mice were decreased significantly at day 63 but not at
day 42 when compared with infected controls, suggesting that
IL-12 induced myopathy is negligible and the reduction may
be caused by eradication of M. avium by IL-12 therapy. ALT
levels were higher at day 42 but lower at day 63 in infected
mice given IL-12 than in controls. This suggests that there is
mild hepatotoxicity during IL-12 therapy, which disappears
within 3 weeks after therapy ends. Similar results were ob­
served at day 140 (data not shown) and in serum levels of
aspartate aminotransferase and lactate dehydrogenase that
might be derived from multiple organs and tissues. Thus, ad­
ministration of IL-12 at therapeutically effective dosages (1–10
μg/kg/day) for 3 weeks was tolerated well with mild toxicity.

Lack of direct antimycobacterial activity of IL-12 in vitro.
To determine whether the growth inhibition of M. avium by in
vivo IL-12 treatment was exerted directly on mycobacterial
multiplication or mediated indirectly by augmentation of anti-
mycobacterial host defense mechanisms, M. avium was cul-

Figure 3. Detection of mRNA for IL-12 p40 and p35 by reverse
transcriptase–polymerase chain reaction (PCR). RNA from spleens
of mice (BALB/c, DBA/2) infected with M. avium at different times
after infection was transcribed into cDNA and amplified by PCR with
primers specific for IL-12 p40, IL-12 p35, and glyceraldehyde-3-
phosphate dehydrogenase (GAPDH). PCR products were visualized
by staining with ethidium bromide. Arrowheads indicate expected
fragment size of PCR products. MM, molecular size marker (1-kb
ladder).

IL-12 levels of spleen extracts from mice infected with M. avium. Bioactivity was measured by anti–IL-12 antibod­
cyte-capture assay. Spleens were removed at various intervals after
infection. Standard curve of recombinant IL-12 was plotted and IL-
12 content of samples was calculated. Data are mean ± SD (n = 10/
group). * P < .02 vs. susceptible BALB/c mice.
Figure 5. IL-12 replacement therapy for established *M. avium* infection in susceptible BALB/c mice. IL-12 (0.1–10 μg/kg/day) was administered intraperitoneally to infected mice for 3 weeks, beginning 21 days after inoculation with 10⁸ cfu of *M. avium*. Mycobacterial growth in organs was monitored at different times after infection. Data are mean ± SD (n = 10/group). *P* < .02 vs. susceptible BALB/c mice.

IL-12, which acts as an initiation cytokine for cell-mediated immunity [14], is produced mainly by macrophages and monocytes and stimulates production of IFN-γ from Th1 and NK cells [14]. IL-12 also stimulates the production of granulocyte-macrophage colony-stimulating factor (GM-CSF) and tumor necrosis factor-α (TNF-α) [14]. These cytokines, including IFN-γ [30, 31], GM-CSF [32], and TNF-α [33, 34], enhance murine antimycobacterial defense in vivo by stimulating macrophages to inhibit or kill nontuberculous mycobacteria such as MAC. Mice with targeted disruption of the IFN-γ gene or the gene for the IFN-γ receptor show increased susceptibility to *Mycobacterium tuberculosis* and BCG [35–38]. In such animals, intravenous or aerosolic challenge with a normally sublethal number of tubercle bacilli leads to death with extensive tissue necrosis and increased mycobacteria burdens. Thus, IL-12 may augment host defense against *M. avium* infection by its ability to activate Th1 and NK cells and to induce antimycobacterial cytokines, such as GM-CSF, TNF-α, and IFN-γ, which in turn activate macrophages to express antimycobacterial activity.

Evidence in support of this concept has come from recent studies demonstrating that endogenously produced IL-12 is required for protection against *M. avium* infections in mice [39]. BALB/c mouse macrophages express low levels of IL-12 p40 mRNA when stimulated in vitro with BCG [40]. These findings are consistent with our present findings that spleens of resistant (but not susceptible) mice express high levels of the inducible IL-12 p40 mRNA and bioactive protein within 24 h after infection. Therefore, the defective IL-12 expression in susceptible BALB/c mice may result in uncontrolled replication of *M. avium*. Although a variety of cell types may produce this cytokine, macrophages appear to be the primary producers of IL-12 in response to intracellular pathogens [14, 27]. IL-12 may...
Figure 6. Morphology of spleens of infected BALB/c mice given IL-12 replacement therapy. IL-12 (10 μg/kg/day) was given intraperitoneally for 3 weeks beginning 21 days after inoculation with 10⁸ cfu of M. avium. Lesion is primarily composed of foamy cells, epithelioid macrophages, and lymphocytes. No striking differences were seen in lesions of untreated controls and IL-12-treated mice, although granulomas in mice given IL-12 were often fused in association with decreased numbers of acid-fast bacilli in tissue stained by Ziehl-Neelsen (ZN) technique. HE, hematoxylin-cosin.
participate in natural resistance to *M. avium* infection; early pathogen-macrophage interactions induce IL-12 expression to act as an initiation cytokine for protective Th1 responses. Therefore, IL-12 represents a functional bridge between early nonspecific innate resistance and subsequent antigen-specific adaptive immunity [41].

In the present study, the involvement of natural resistance-associated genes (e.g., *Bcg/lty/Lsh* and non-*H-2*) in IL-12 expression and protection remained uncertain, because the precise role of such genes has not been fully clarified [3]. Natural resistance to *M. avium* is under polygenic control [6]. Protection of the infected host is initially achieved by a local tissue reaction with granulomatous inflammation, which is the orchestrated expression of mononuclear cells such as macrophages, NK and Th1 cells, and secreted cytokines in response to mycobacteria [13, 21, 42]. In the process, cell-mediated immune responses to mycobacteria play a key role [13], although the response is two-edged and may contribute to both clearance of infecting agents and tissue damage [43].

Although compelling evidence supports a protective role of IL-12 in intracellular infections with *Leishmania major* [24, 44], *Toxoplasma gondii* [45], *M. avium* [26], and *M. tuberculosis* [46] when exogenous IL-12 is administered from the time of infection, there is only limited information concerning delayed IL-12 therapy for established infections. IL-12 exerts therapeutic activity in an established *Leishmania donovani* systemic intracellular infection [47]. Our results suggest that IL-12 exerts therapeutic activity in susceptible mice with established *M. avium* infection by augmenting host defense and provide a rationale for IL-12 replacement therapy for susceptible mice with deficient IL-12 expression. Administration of IL-12 at dosages of 0.1–10 μg/kg/day, and administration of IL-12 for 3 weeks at effective dosages of 1 and 10 μg/kg/day had minimal toxicity as assessed by clinical manifestations and blood chemistry (table 1). Thus, IL-12 may prove to be of therapeutic value with relatively few toxicities by promoting cell-mediated immunity to MAC infection, which is usually resistant to most antimycobacterial agents and to other antinfectives.

In the present experiments with immunocompetent mice, IL-12 may have induced antimycobacterial cytokine production from CD4 T cells and NK cells. Although disseminated *M. avium* infections are frequent in AIDS patients with few CD4 T cells (~100/μL) [1], the potential use of IL-12 for treatment of *M. avium* infection in such patients is not easily predictable. In persons infected with the human immunodeficiency virus (HIV), IL-12 production by PBMC is impaired [48]. IL-12 restores IFN-γ and IFN-γ production by PBMC in HIV-positive persons, which results in expression of cell-mediated immune responses in vitro [49]. In vivo treatment with IL-12 protects mice from immune abnormalities observed during murine AIDS [50]. Exogenous IL-12 augments natural resistance of T cell–dependent hosts (scid mice) to intracellular parasites such as *T. gondii* [45]. IL-12 also appears to mediate its function through IFN-γ–independent mechanisms [51]. Thus, the potential use of IL-12 immunotherapy in *M. avium*–infected AIDS patients who retain some CD4 T cells, NK cells, or both may be considered. To establish the design of rational IL-12 immunotherapy for MAC infection, further studies are needed to determine the mechanism of action, timing of treatment, optimization of doses, and side effects.

**Table 1.** Serum chemistry of infected BALB/c mice treated with IL-12.

<table>
<thead>
<tr>
<th>Mice</th>
<th>Blood urea nitrogen</th>
<th>Creatinine kinase</th>
<th>AST</th>
<th>ALT</th>
<th>LDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected, naive</td>
<td>39 ± 9.8</td>
<td>0.7 ± 0.2</td>
<td>61 ± 8</td>
<td>51 ± 9</td>
<td>31 ± 3</td>
</tr>
<tr>
<td>Infected</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control, day 21</td>
<td>22 ± 0.7</td>
<td>0.3 ± 0.1</td>
<td>1149 ± 318</td>
<td>192 ± 23</td>
<td>22 ± 18</td>
</tr>
<tr>
<td>day 42</td>
<td>28 ± 4.9</td>
<td>0.5 ± 0.2</td>
<td>1639 ± 198</td>
<td>249 ± 52</td>
<td>181 ± 40</td>
</tr>
<tr>
<td>day 63</td>
<td>29 ± 9.6</td>
<td>0.7 ± 0.1</td>
<td>1895 ± 321</td>
<td>349 ± 53</td>
<td>205 ± 79</td>
</tr>
<tr>
<td>IL-12, day 42 (1 μg/kg/day)</td>
<td>33 ± 8.3</td>
<td>0.7 ± 0.2</td>
<td>1534 ± 216</td>
<td>324 ± 27*</td>
<td>198 ± 16</td>
</tr>
<tr>
<td>day 63</td>
<td>27 ± 9.5</td>
<td>0.6 ± 0.1</td>
<td>651 ± 96*</td>
<td>198 ± 27*</td>
<td>89 ± 17*</td>
</tr>
<tr>
<td>IL-12, day 42 (10 μg/kg/day)</td>
<td>28 ± 7.8</td>
<td>0.8 ± 0.2</td>
<td>1488 ± 198</td>
<td>320 ± 63*</td>
<td>253 ± 29*</td>
</tr>
<tr>
<td>day 63</td>
<td>29 ± 5.3</td>
<td>0.7 ± 0.2</td>
<td>506 ± 163*</td>
<td>107 ± 23*</td>
<td>56 ± 11*</td>
</tr>
</tbody>
</table>

NOTE. Serum chemistry was assessed by autoanalyzer (model 7150; Hitachi, Tokyo). BALB/c mice were given IL-12 for 3 weeks beginning 21 days after inoculation with 10⁸ cfu of *M. avium*. Data are mean ± SD (*n* = 10/group). AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase. Creatine kinase, AST, ALT, and LDH measurements are IU/L; others are mg/dL.

*P < .05 vs. controls.*
helpful discussions, and Hiroko T. Takeuchi for excellent technical help.

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