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Lethal Tuberculosis in Interleukin-6-Deficient Mutant Mice

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Tuberculosis is a chronic infectious disease which causes major health problems globally. Acquired resistance is mediated by T lymphocytes and executed by activated macrophages. In vitro studies have emphasized the importance of macrophage activation for mycobacterial growth inhibition. In vivo, the protective host response is focused on granulomatous lesions in which *Mycobacterium tuberculosis* is contained. A cellular immune response of the T helper 1 (Th1) type is considered central for control of tuberculosis. Using interleukin-6 (IL-6)-deficient mice, we here demonstrate a crucial role of this pluripotent cytokine in protection against *M. tuberculosis* but not against *Mycobacterium bovis* BCG. Infection with *M. tuberculosis* was lethal for the IL-6-deficient mice at inocula that were still controlled by IL-6-competent mice. Spleen cells from *M. tuberculosis*-infected IL-6$^{-/-}$ mouse mutants produced elevated levels of IL-4 and reduced levels of gamma interferon compared to the control levels. Cytofluorometric analyses of spleen cells from *M. tuberculosis*-infected mice revealed more-profound alterations in T-cell ratios in IL-6$^{-/-}$ mice than in control mice. We assume that IL-6 contributes to host resistance by its proinflammatory activity and by its influence on cytokine secretion.

Tuberculosis is a chronic disease, caused by *Mycobacterium tuberculosis*, which causes enormous health problems globally (6, 32). In the infected host, mycobacteria are contained within granulomas where T lymphocytes activate antituberculous macrophage functions (26, 30). The cross talk between T lymphocytes, mononuclear phagocytes, and other host cells is mediated by cytokines. Although *Mycobacterium bovis* BCG has been widely used as a vaccine, it confers incomplete protection against tuberculosis, at least in adults (9). The insufficient efficacy of *M. bovis* BCG vaccination has been explained by differential activation of T cells and cytokines by *M. bovis* BCG and *M. tuberculosis* (18, 45).

According to their cytokine patterns, T helper (Th) cells can be separated into two groups (for reviews, see references 14 and 42). Th2 cells, characterized by potent interleukin-4 (IL-4) and IL-5 production, are primarily responsible for resistance against helminth infections. In contrast, Th1 cells, which produce IL-2 and gamma interferon (IFN-γ), are central to defense against intracellular bacteria, including *M. tuberculosis.*

IL-6 is a multifunctional cytokine with at least three major activities (1, 34, 54). First, IL-6, together with tumor necrosis factor (TNF) and IL-1, belongs to the group of proinflammatory cytokines which initiate early inflammatory responses (1, 2). Second, IL-6 is involved in the promotion of T- and B-cell responses. Third, it participates in hematopoiesis (5, 34, 54). IL-6 is produced by various cell types, including mononuclear phagocytes, fibroblasts, endothelial cells, B cells, and T cells (54). Although both Th cell types can produce IL-6, it is generally grouped as a Th2 rather than a Th1 cytokine (14).

More-recent attempts to define the role of IL-6 in the antiinfective immune response have emphasized its proinflammatory potential during acute infections. For example, IL-6-deficient mice rapidly succumb to *Listeria monocytogenes* infection (12, 35). In contrast, virtually nothing is known about the role of IL-6 in chronic infectious diseases such as tuberculosis. Phenotypic determination of IL-6 functions in tuberculosis not only was hampered by the chronicity of disease but was further impeded by chaperoning effects of an anti-IL-6 monoclonal antibody (MAb) which caused paradoxical results (27). We took advantage of mouse mutants with a disrupted IL-6 gene (IL-6$^{-/-}$) to overcome these obstacles (35). We found that IL-6$^{-/-}$ mice were highly susceptible and ultimately succumbed to tuberculosis. These data prove an essential role of IL-6 in murine tuberculosis.

MATERIALS AND METHODS

Mice. Gene-targeted IL-6$^{-/-}$ mice and their immunocompetent counterparts, IL-6$^{+/+}$ mice, have been described elsewhere (35). The original homozygous IL-6$^{-/-}$ mouse strain on a 129 × C57BL/6 background was backcrossed to C57BL/6 mice, and the second backcross thereof was used in these experiments. Both IL-6$^{-/-}$ and IL-6$^{+/+}$ mice were derived from this second backcross. The 129 and C57BL/6 strains are BCG resistant and sensitive, respectively, and the IL-6$^{-/-}$ and IL-6$^{+/+}$ mice are of mixed *bcg* genotypes. The IL-6$^{-/-}$ mutants cannot produce IL-6 because of a disrupted IL-6 gene (35). The mice were bred and maintained in our animal facilities at the University of Ulm under specific-pathogen-free conditions. In all experiments, 7- to 10-week-old animals of either sex were employed. In a given experiment, the mice were age and sex matched.

Bacteria and infections of mice. *M. bovis* BCG and *M. tuberculosis* were cultured in Dubos broth base (Difco, Detroit, Mich.) supplemented with Dubos medium albumin (Difco) after mouse passage. A mid-log-phase culture was aliquoted and stored at −70°C until use. *M. bovis* BCG strain Chicago (ATCC 27289) was used in the range of 5×10^5 to 5×10^6 live bacteria in phosphate-buffered saline (at a fixed inoculum in a given experiment) for intravenous (i.v.) infection via the lateral tail vein. The exact doses for given experiments are provided in the figure legends. *M. tuberculosis* H37Rv was originally provided by J. K. Seydel (Forschungsinstitut Borstel, Borstel, Germany), and in the different experiments concentrations of 5×10^5 to 1×10^6 viable organisms were used for i.v. infection.

Determination of mycobacterial growth. The bacterial loads in liver, spleen, and lung were determined at different times postinfection (p.i.) by plating serial dilutions of organ homogenates obtained by homogenization with a laboratory blender (Seward Medical, London, United Kingdom). Appropriate dilutions were plated on Middlebrook agar plates (Difco) supplemented with oleic acid-albumin-dextrose-catalase enrichment (Difco). After 3 to 4 weeks of culture at 37°C, CFU were counted.

In vitro stimulation and cytokine production. At the time points indicated below, animals were sacrificed and the spleens were removed. Single-cell suspensions were prepared and resuspended in Iscove's modified Dulbecco's medium (IMDM) (Seromed, Berlin, Germany) supplemented with 10% fetal calf serum (Boehringer Mannheim, Mannheim, Germany), 2 mM glutamine, 100 U

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of penicillin-streptomycin (Gibco, Paisley, United Kingdom) per ml, and 1 μg of indomethacine (Sigma, St. Louis, Mo.) per ml (complete IMDM). Cells were seeded into 96-well plates (Nunc, Roskilde, Denmark) at a final concentration of 2×10^5 per well and stimulated with *M. tuberculosis* H37Ra lyophilisate (Difco). Positive-control cultures were stimulated with concanavalin A (Sigma) or pokeweed mitogen (Sigma) at 5 μg/ml. After 2 days of culture, supernatants were removed, 0.2-μm-pore-size sterile filtered (for *M. tuberculosis*-infected material), and frozen at −20°C until analyzed. Cytokine concentrations were determined in triplicate.

ELISA. IFN-γ concentrations were determined by a double-sandwich enzyme-linked immunosorbent assay (ELISA) using specific MAbs R4-6A2 and AN18-17.24 as described previously (39). Murine recombinant IFN-γ (rIFN-γ) (generous gift of G. Adolf, Ernst Boehringer-Institut für Arzneimittelforschung, Vienna, Austria), with a specific activity of 10^7 U/mg, was diluted in complete IMDM to obtain a standard curve. The detection limit of the ELISA was 0.05 U of IFN-γ per ml. IL-4 concentrations were determined in a sandwich ELISA using specific MAbs BVD4-1D11 and BVD6-24-G2 as described previously (55). The detection limit was 2 pg of IL-4 per ml, calculated from a standard curve with murine recombinant IL-4 (rIL-4) (Genzyme, Cambridge, Mass.) with a specific activity of $\geq 10^7$ U/mg. Concentrations of IL-12 were determined in a two-site ELISA using specific MAbs C17.8 and C15.6 (kind gift of G. Trinchieri, Wistar Institute, Philadelphia, Pa.) and murine rIL-12 (kindly provided by S. Wolf, Genetics Institute, Cambridge, Mass.) with a specific activity of 5.6×10^6 U/mg for standardization as described previously (37). The detection limit was 20 pg of IL-12 p40/p70 per ml. Determination of TNF activities was done with the TNF-sensitive L929 cell line as described elsewhere (24). Murine recombinant TNF (rTNF) (Genzyme) with a specific activity of $\geq 5 \times 10^7$ U/mg was used to calculate a standard curve. The detection limit was 2 pg of TNF per ml. TNF, IFN-γ, IL-4, and IL-12 concentrations were determined with SoftmaxPro software (Molecular Devices, Sunnyvale, Calif.) using four-parameter standard curve calculations. Optical densities were analyzed with SpectraMax equipment (Molecular Devices).

Cytofluorometry. Spleen cells were stained with the following MAbs: anti-L3T4–phycoerythrin (PE) (clone YTS 191) (MEDAC, Hamburg, Germany), anti-Lyt2–fluorescein isothiocyanate (FITC) (clone YTS 169) (MEDAC), anti-T-cell receptor alpha/beta (anti-TCRαβ)–biotin (clone H57-597) (kind gift from R. Kubo), anti-TCRγδ–biotin (clone GL-3) (kindly provided by L. Lefrançois), anti-CD3–FITC (clone 145-2C11) (kind gift of J. Bluestone), anti-NK1.1–biotin (clone PK 136) (American Type Culture Collection, Rockville, Md.), and anti-Lyt5(B220)–FITC (clone RA3-6B2) (MEDAC). Biotinylated antibodies were detected with streptavidin-PE (Gibco) or streptavidin-RED670 (Gibco). Cells were analyzed with a FACScan (Becton Dickinson, Mountain View, Calif.) using LYSIS II software (Becton Dickinson).

Histology. Lungs of *M. tuberculosis*-infected mice were fixed in formalin and paraffin embedded at different times p.i. Sections were cut 3 to 5 μm thick and stained for acid-fast bacilli (AFB) by the Ziehl-Neelsen method and counterstained with hematoxylin. Slides were analyzed under an AxioPhot microscope (Zeiss, Jena, Germany).

Statistical analysis. To assess significant differences between IL-$6^{-/-}$ and IL-$6^{+/+}$ mice, Student's *t* tests were performed. A *P* value of ≤0.05 was considered significant.

RESULTS

***M. bovis* BCG infection in IL-$6^{-/-}$ and IL-$6^{+/+}$ mice.** IL-$6^{-/-}$ and IL-$6^{+/+}$ mice were infected i.v. with *M. bovis* BCG, and bacterial growth in spleens, livers, and lungs was determined at different times p.i. As shown in Fig. 1, the course of infection did not differ significantly between IL-6-deficient mutants and their immunocompetent wild-type controls. Both mouse strains controlled infection efficiently, as indicated by the continuous decline in the numbers of *M. bovis* BCG organisms in all three organs. In particular, lungs of both IL-$6^{-/-}$ and IL-$6^{+/+}$ mice were free from mycobacteria after day 60 p.i. These results reveal efficient control of *M. bovis* BCG in the absence of IL-6, at least under the conditions employed. The small interindividual variations, despite a mixed genetic background in these mice, may suggest a minor impact of these background genes on resistance to BCG in our experimental setting.

***M. tuberculosis* infection in IL-$6^{-/-}$ and IL-$6^{+/+}$ mice.** A strikingly different result was obtained when IL-$6^{-/-}$ and IL-$6^{+/+}$ mice were infected i.v. with *M. tuberculosis* organisms (Fig. 2). The IL-$6^{-/-}$ mice succumbed to tuberculosis from day 50 p.i. onwards (median survival time [MST], 59 days). All IL-6-deficient mice had died before the end of the 120-day

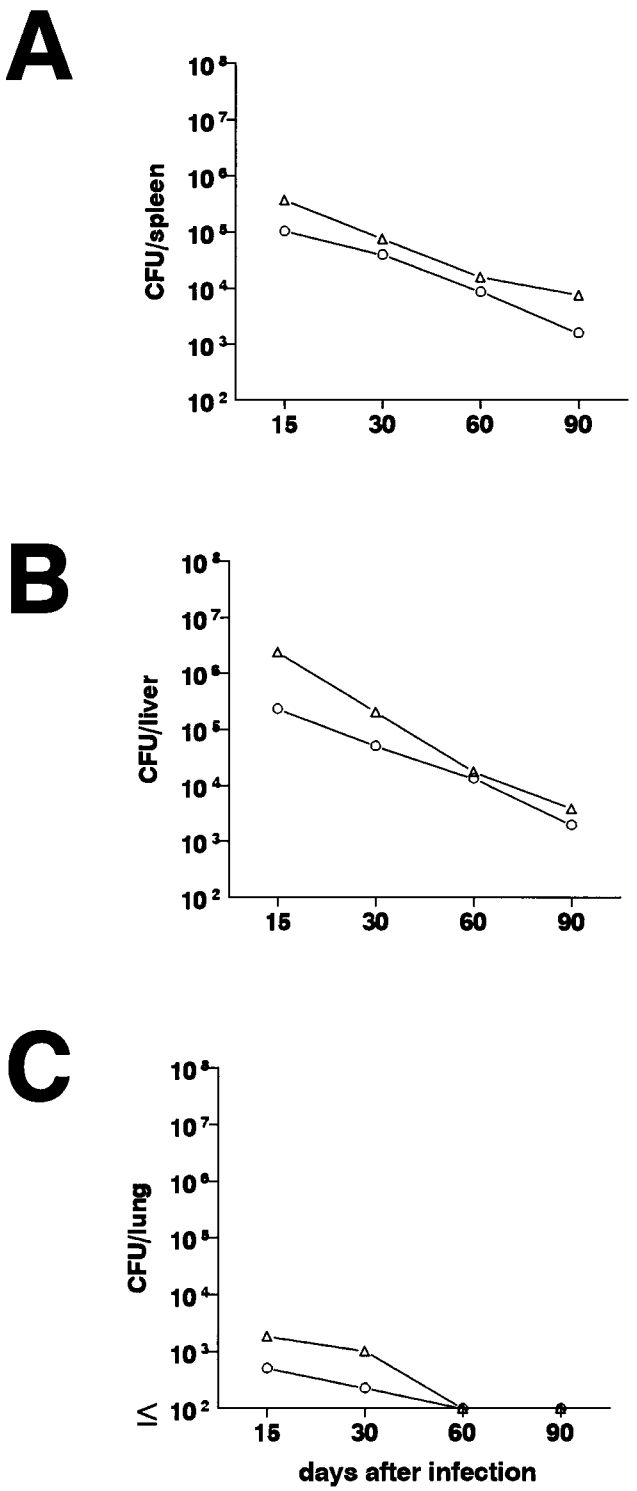

FIG. 1. Growth of *M. bovis* BCG in the spleen (A), liver (B), and lung (C) of IL-$6^{-/-}$ (△) and IL-$6^{+/+}$ (○) mice. In the experiment whose results are shown, mice were infected with 10^6 BCG organisms i.v. Organs were removed at the indicated times and homogenized, serial dilutions were plated on Middlebrook agar plates, and CFU were determined 3 to 4 weeks later. The data are means for four mice per time point (SD were <10%) and are from one representative experiment repeated twice with similar results.

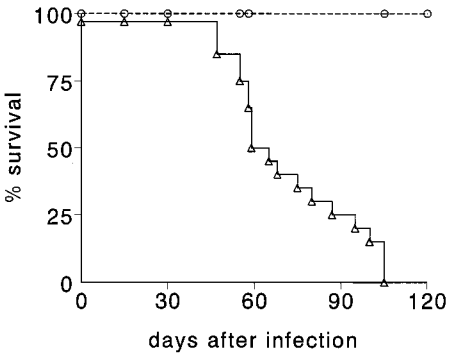

FIG. 2. Survival of *M. tuberculosis*-infected IL-$6^{-/-}$ (△) and IL-$6^{+/+}$ (○) mice. The mice were infected with 10^6 live mycobacteria i.v. Survival was monitored daily, and moribund mice were killed. Accumulated data for 20 mice per group from two different experiments are shown. MST for IL-$6^{-/-}$ mice, 59 days; MST for IL-$6^{+/+}$ mice, >120 days.

observation period, whereas all wild-type control animals survived *M. tuberculosis* infection (MST > 120 days). Already at day 15 p.i., the bacterial load was significantly increased in all three organs (mean $\log_{10}$ CFU ± standard deviations [SD] for IL-$6^{-/-}$ and IL-$6^{+/+}$ mice, respectively, were as follows: spleen, 7.47 ± 0.11 and 6.20 ± 0.35; liver, 6.30 ± 0.17 and 5.78 ± 0.15; and lung, 6.98 ± 0.15 and 5.76 ± 0.14 [ΔCFU in all organs was statistically significant at a P of <0.01; $n = 4$ mice per group]). At day 60 p.i., 100- to 1,000-fold more *M. tuberculosis* CFU were detected in IL-$6^{-/-}$ mutants than in the controls (mean $\log_{10}$ CFU ± SD for IL-$6^{-/-}$ and IL-$6^{+/+}$ mice, respectively, were as follows: spleen, 7.12 ± 0.16 and 5.14 ± 0.40; liver, 6.66 ± 0.13 and 4.70 ± 0.32; and lung, 7.36 ± 0.28 and 4.00 ± 0.30 [ΔCFU in all organs was statistically significant at a P of <0.001; $n = 4$ mice per group]). Consistent with this marked difference in lung CFU, large numbers of AFB within areas of cellular infiltrates were detected in the lungs of IL-$6^{-/-}$ mice, whereas only a few AFB, contained within distinct parenchymal lesions, were found in IL-$6^{+/+}$ mice (Fig. 3). These data demonstrate a central role of IL-6 in control of murine tuberculosis and suggest a minor impact of the genetic background in this system.

IFN-γ and IL-4 secretion by spleen cells from infected IL-$6^{-/-}$ and IL-$6^{+/+}$ mice. The immune response against infectious agents is determined by the types of cytokines which are produced during infection (14). The central role of IFN-γ in control of *M. tuberculosis* infection has been clearly established (11, 23), whereas the potential role of IL-4 in antimycobacterial immunity is less well understood (for a review, see reference 14). In order to determine the influence of IL-6 on IFN-γ and IL-4 secretion during *M. bovis* BCG or *M. tuberculosis* infection, spleen cells from infected IL-$6^{-/-}$ and IL-$6^{+/+}$ mice were stimulated in vitro with mycobacterial antigens (*M. tuberculosis* H37Ra lyophilisate) and cytokine concentrations were determined (Fig. 4). As control stimuli, the mitogens concanavalin A and pokeweed mitogen were used. Spleen cells from

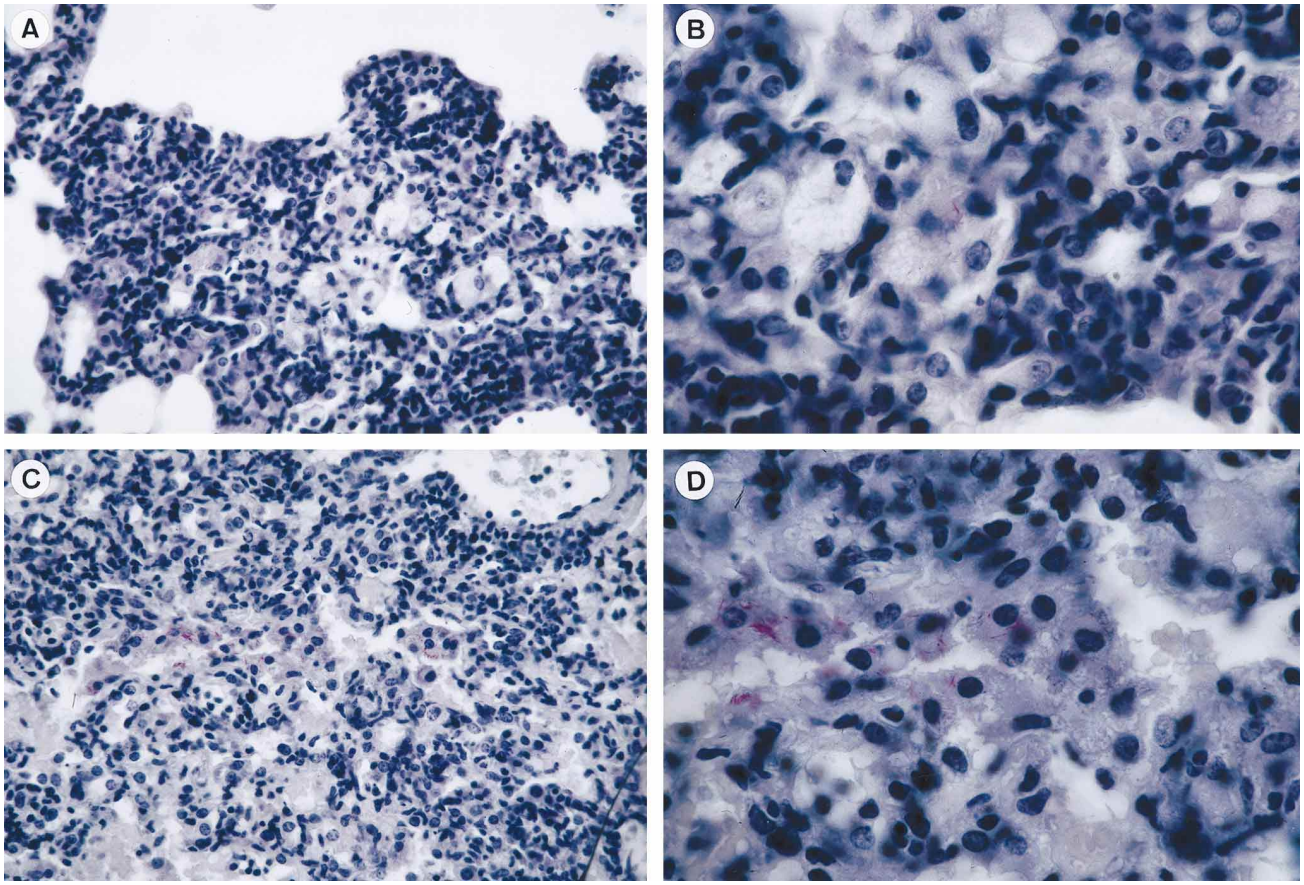

FIG. 3. Histopathology of lungs from *M. tuberculosis*-infected IL-$6^{-/-}$ and IL-$6^{+/+}$ mice. (A) Lung granulomas containing very few AFB in IL-$6^{+/+}$ mice; (B) detail from panel A; (C) lung granulomas containing abundant AFB and large cellular infiltrates in IL-$6^{-/-}$ mice; (D) detail from panel C. Original magnifications, ×100 (A and C) and ×400 (B and D). Ziehl-Neelsen stain was used for AFB, and cells were counterstained with hematoxylin.

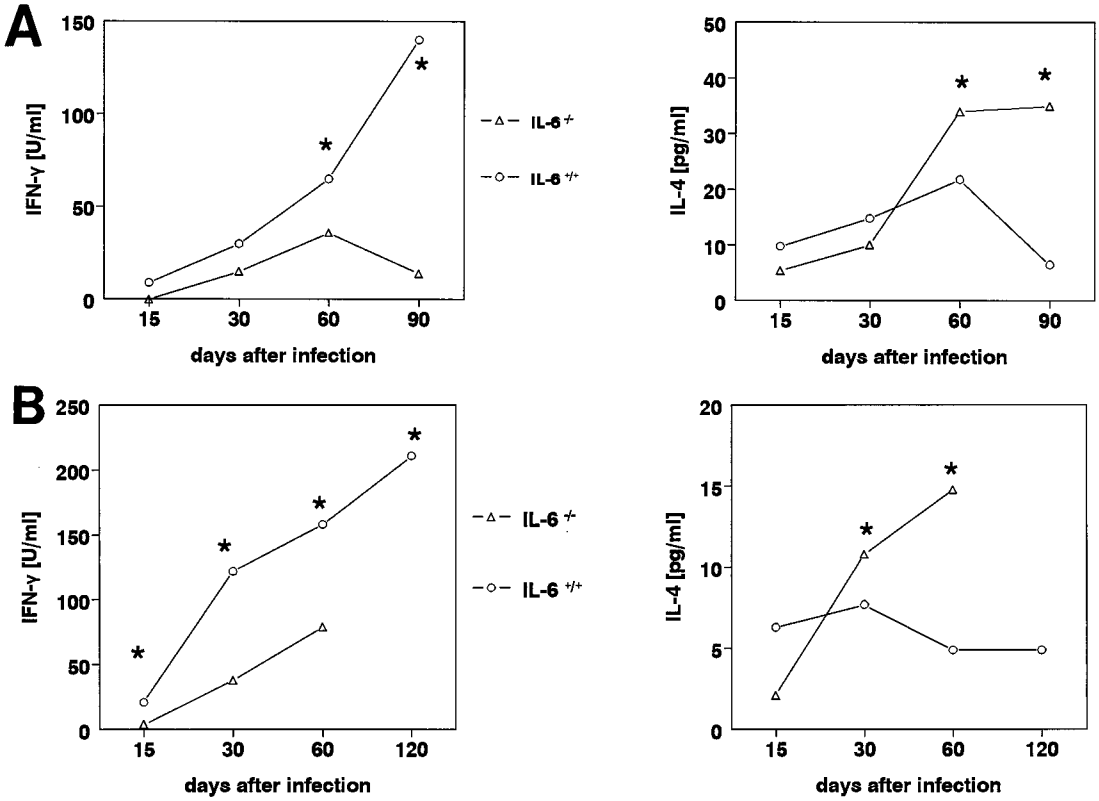

FIG. 4. IFN-γ and IL-4 secretion by spleen cells from IL-6$^{-/-}$ and IL-6$^{+/+}$ mice infected with *M. bovis* BCG (A) or *M. tuberculosis* H37Rv (B). Spleen cells were cultured with 5 μg of killed and lyophilized *M. tuberculosis* H37Ra organisms per ml for 2 days. Supernatants were analyzed for IFN-γ and IL-4 activities by ELISA. Note that *M. tuberculosis*-infected IL-6$^{-/-}$ mice died from day 50 onwards. The results are means of triplicates from one representative experiment repeated twice. SD were ≤15%. *, significance ($P \leq 0.05$ as determined by Student's *t* test).

infected IL-6$^{-/-}$ and IL-6$^{+/+}$ mice produced comparable concentrations of IFN-γ and IL-4 in response to these nonspecific stimuli (data not shown). By contrast, antigen-specific stimulation of splenic cells from *M. bovis* BCG-infected mice showed that IL-6$^{-/-}$ mice produced less IFN-γ than wild-type controls, in particular at late stages of infection (days 60 to 90 p.i.) (Fig. 4A). Conversely, IL-4 production by splenic cells from IL-6$^{-/-}$ mice increased significantly from days 30 to 90 but declined continuously in controls from days 60 to 90. Hence, although IL-6$^{-/-}$ and IL-6$^{+/+}$ mice controlled *M. bovis* BCG infection equally well, the IL-6 deficiency resulted in decreased IFN-γ and increased IL-4 levels. Spleen cells from *M. tuberculosis*-infected IL-6$^{-/-}$ and IL-6$^{+/+}$ mice produced appreciable levels of IFN-γ after stimulation with mycobacterial antigens; however, the IL-6 deficiency led to reduced IFN-γ secretion (Fig. 4B). Conversely, IL-4 secretion by splenocytes from *M. tuberculosis*-infected IL-6$^{-/-}$ mice was increased compared to that of the immunocompetent animals, although IL-4 was detectable in cell cultures from both mouse strains. Our findings therefore suggest that IL-6 deficiency favored IL-4 secretion and impaired IFN-γ production during mycobacterial infection.

IL-12 and TNF secretion by spleen cells from *M. tuberculosis*-infected IL-6$^{-/-}$ and IL-6$^{+/+}$ mice. Next, we determined IL-12 and TNF secretion by spleen cells from *M. tuberculosis*-infected IL-6$^{-/-}$ and IL-6$^{+/+}$ mice after in vitro restimulation with mycobacteria (Fig. 5). IL-12 stimulates the development of a Th1 immune response, and rIL-12 administration has been shown to improve resistance against tuberculosis (10, 52). TNF synergizes with IFN-γ in the activation of tuberculostatic macrophage functions and is central to resistance against *M. tuberculosis* and *M. bovis* BCG (24, 28). At the times tested, stimulated spleen cells from *M. tuberculosis*-infected IL-6$^{-/-}$ mice produced slightly increased IL-12 concentrations compared to those of IL-6$^{+/+}$ mice (Fig. 5). The level of TNF secretion by stimulated spleen cells from IL-6$^{-/-}$ mice was lower than that of IL-6$^{+/+}$ controls initially (Fig. 5). TNF secretion by IL-6$^{+/+}$ splenocytes, however, declined after day 30, whereas TNF production by IL-6$^{-/-}$ cells increased steadily. Accordingly, at day 60 p.i., higher TNF levels were produced in the absence of IL-6.

Phenotypes of spleen cells from infected IL-6$^{-/-}$ and IL-6$^{+/+}$ mice. Phenotypic characterization of spleen cells from IL-6$^{-/-}$ and IL-6$^{+/+}$ mice by microfluorometry revealed differential effects of *M. bovis* BCG and *M. tuberculosis* infections (Table 1). In response to *M. bovis* BCG, neither the B-cell/T-cell nor the CD4/CD8 T-cell ratio was altered by the IL-6 deficiency. Only an initial increase in the NK1.1^{+} population was apparent at an early time (day 15 p.i.). In contrast, the IL-6 deficiency caused alterations in the lymphocyte ratios during *M. tuberculosis* infection. In the IL-6$^{-/-}$ mice, *M. tuberculosis* infection decreased the CD4 T-cell population slightly and the CD8 T-cell population more profoundly. As a result, the CD4/CD8 ratio was increased in *M. tuberculosis*-infected IL-6$^{-/-}$ mice more profoundly than in immunocompetent controls. In the IL-6$^{+/+}$ mice, the CD8 T-cell population was enlarged in the initial phase of tuberculosis. After an initial decline compared to B cells in noninfected mice, the proportion of B cells increased from days 15 to 60 p.i. in *M. tuberculosis*-infected IL-6$^{-/-}$ mice. Finally, *M. tuberculosis* infection resulted in the expansion of the NK1.1^{+} population in IL-6$^{-/-}$ mice, whereas the proportion of NK1.1^{+} cells was only transiently increased

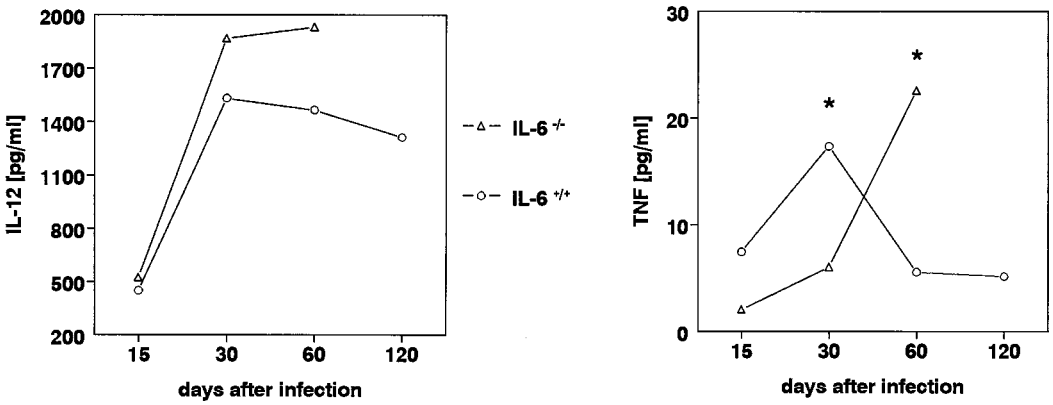

FIG. 5. IL-12 and TNF secretion by spleen cells from *M. tuberculosis*-infected IL-6$^{-/-}$ and IL-6$^{+/+}$ mice. Spleen cells were cultured as described in the legend to Fig. 4. Supernatants were analyzed by ELISA (for IL-12) or bioassay (TNF). Note that *M. tuberculosis*-infected IL-6$^{-/-}$ mice died after day 50. The results are means of triplicates from one representative experiment repeated twice. SD were ≤15%. *, significance ($P \leq 0.05$ as determined by Student's *t* test).

in *M. tuberculosis*-infected IL-6$^{+/+}$ mice. Thus, the absence of IL-6 caused more-pronounced changes in T-cell ratios in infection with *M. tuberculosis* than with *M. bovis* BCG.

DISCUSSION

Our experiments reveal a striking role of IL-6 in tuberculosis. *M. tuberculosis* is an intracellular bacterium which survives in resting macrophages (8, 30). Macrophage activation by cytokines, in particular IFN-γ, is considered the principal mechanism underlying acquired resistance (11, 15, 21–23, 30, 49). However, even activated macrophages fail to fully eradicate *M. tuberculosis* (15, 49). Accordingly, even in immune individuals, *M. tuberculosis* persists and disease generally develops after reactivation as a consequence of impaired immunity (7, 50). Experimental tuberculosis of mice has provided important clues into the complexity of the protective host response against tuberculosis (30). Convincing evidence suggests that different T-cell populations are required for acquisition of protection to mycobacterial infections. This includes CD4, CD8, and γ/δ T lymphocytes (25, 30, 36, 38, 39, 41, 43). Although the specific roles of these different T-cell subsets remain incompletely understood, the crucial role of the Th1 immune response is beyond question (14, 30, 31). In contrast, Th2 cells

TABLE 1. Cytofluorometric analyses of spleen cells from infected IL-6$^{-/-}$ and IL-6$^{+/+}$ mice[a]

Infection type and mice	Day p.i.	Spleen cells						CD4/CD8 ratio	B220/CD3 ratio
		Total (10^8)	% CD4	% CD8	% CD3	% NK1.1	% B220		
None									
IL-6$^{+/+}$		1.1	19.8	12.9	32.5	5.91	59.1	1.54	1.82
IL-6$^{-/-}$		1.0	22.1	13.6	34.4	5.60	57.4	1.62	1.67
M. bovis BCG									
IL-6$^{+/+}$	15	1.2	23.2	13.1	43.8	4.15	47.3	1.77	1.08
	30	1.5	16.5	10.4	29.8	6.89	50.9	1.59	1.71
	60	2.6	22.2	17.1	35.1	8.23	52.6	1.30	1.50
	90	1.7	27.7	20.1	54.1	3.56	36.8	1.38	0.68
IL-6$^{-/-}$	15	2.2	17.7	13.5	32.6	8.05	49.5	1.31	1.52
	30	3.5	13.3	7.5	27.0	3.18	60.7	1.77	2.25
	60	1.8	18.3	10.8	38.6	2.65	55.2	1.69	1.43
	90	1.6	22.7	17.7	44.5	5.73	40.9	1.28	0.92
M. tuberculosis									
IL-6$^{+/+}$	15	2.3	19.2	15.0	33.6	10.1	14.1	1.28	0.42
	30	2.0	19.7	18.6	36.2	5.6	40.9	1.06	1.13
	60	8.8	16.8	8.8	24.7	4.7	42.9	1.91	1.74
	120	9.0	12.8	6.4	20.2	4.6	50.2	2.00	2.48
IL-6$^{-/-}$	15	2.0	12.9	5.3	18.3	10.6	33.8	2.45	1.85
	30	1.5	18.6	4.6	18.6	12.9	51.7	4.01	2.78
	60	1.3	15.7	4.8	21.7	14.8	50.7	3.30	2.34

[a] Gated on lymphoid cells by morphological characteristics (forward versus sideward scatter). Note that IL-6$^{-/-}$ mice died after day 51 p.i. (Fig. 2). Results are from the experiments depicted in Fig. 4. Similar results were obtained in two experiments.

are ineffective or even harmful in tuberculosis, and antibodies play a minor role, if any, in protection against tuberculosis.

Consistent with the central contribution of Th1 cells to protection against tuberculosis, IFN-γ-mutant mice succumb to infection with *M. tuberculosis* (11, 23). These IFN-γ-deficient mutants are also more susceptible to BCG vaccination (13, 29). Further, a critical role of TNF alpha (TNF-α) in protection against tuberculosis has been demonstrated (24). This cytokine is produced by activated macrophages and synergizes with IFN-γ in macrophage activation (20). Moreover, a role of TNF in necrotic tissue reactions has been established (24, 33). Finally, treatment with rIL-12 has been found to increase resistance against tuberculosis (10). Although the great complexity of the immune response which controls tuberculosis strongly suggests that multiple other cytokines are required, thus far virtually no information on this issue has been available.

Our data add IL-6 to the list of cytokines which are critical to resistance against tuberculosis. To our knowledge, this is the first report describing an essential role of IL-6 in protective immunity to *M. tuberculosis*. In vitro, CD4 T cells from *M. tuberculosis*-infected mice produce high levels of IL-6 during the early immune response, and IL-6 induces mycobacterial growth inhibition in macrophages (19, 46). Moreover, a previous study found that treatment of mice with anti-IL-6 MAb impaired protective immunity against *Mycobacterium avium* (3). However, compelling evidence showing that treatment with anti-IL-6 MAb increases serum IL-6 levels, probably through chaperoning effects, has been presented (27). Hence, phenotypic characterization of IL-6 functions remained inconclusive. Consistent with the functional effects of IL-6 in murine tuberculosis, analyses of human pulmonary tuberculosis and of levels of IL-6 in human plasma point to a role of IL-6 in infection with *M. tuberculosis* (17, 40).

IL-6 has a molecular weight of 22,000 to 29,000 and is encoded by a gene located on chromosome 5 (34, 35). It is a pleiotropic cytokine which plays a major role in hematopoiesis, T- and B-cell differentiation, and inflammation (1, 34, 54). Furthermore, IL-6 synergizes with other cytokines in macrophage activation (19). Therefore, it is possible that different mechanisms are responsible for the high degree of susceptibility of IL-$6^{-/-}$ mutants to tuberculosis. IL-6, together with IL-1 and TNF-α, is a potent proinflammatory cytokine, and it is the major inducer of the acute-phase response and of neutrophil stimulation in microbial infections (1, 34, 48, 54). Accordingly, the increased susceptibility of IL-$6^{-/-}$ mutants to *L. monocytogenes* infection is correlated with impaired blood neutrophilia (12, 35). In other models, IL-6 has been shown to promote differentiation of T cells (51, 53, 54). IL-6 is generally grouped as a Th2 cytokine, and recently it was found to promote Th2 responses (14, 47, 54). However, it has also been shown to stimulate cytotoxic T lymphocyte and NK functions and to favor Th1 cell development (48, 51, 53). In a recent report, impaired Th1 cell development in IL-$6^{-/-}$ mutant mice infected with *Candida albicans* has been described, although the precise mechanisms through which IL-6 contributes to Th1 cell differentiation remain to be identified (48). The data in Fig. 4 are consistent with an impact of IL-6 in Th1 cell responses in tuberculosis. Taking the published data on biological activities of IL-6 into account, we consider the possibility that IL-6 participates in defense against tuberculosis in the immunocompetent host by inducing inflammatory responses and by promoting IFN-γ production.

In our experiments, we inoculated mice with an *M. tuberculosis* strain of low mouse virulence by the i.v. route (16, 36). Differential virulence of wild-type *M. tuberculosis* is also considered an important factor in the epidemiology of human tuberculosis and is thought to influence the efficacy of BCG vaccination (18). It is therefore noteworthy that the IL-$6^{-/-}$ mutants were highly susceptible to the weakly virulent *M. tuberculosis* substrain used in our studies but still were capable of controlling the vaccine strain *M. bovis* BCG efficiently. However, in IL-$6^{-/-}$ mutants, *M. bovis* BCG apparently also favored IL-4 production and impaired IFN-γ secretion. We assume that default cytokine secretion in IL-$6^{-/-}$ mutants had more-dramatic consequences for control of *M. tuberculosis* than for the vaccine strain *M. bovis* BCG. Similarly, gene disruption mutants with a deficiency in CD8 T cells, γ/δ T cells, IFN-γ, or TNF receptor type I are more susceptible to *M. tuberculosis* than to *M. bovis* BCG (11, 13, 23–25, 29, 36, 38, 39). It is possible that such differences at least in part contribute to the failure of the *M. bovis* BCG vaccine to stimulate satisfactory protection against tuberculosis (6, 9). Although the IL-$6^{+/+}$ and IL-$6^{-/-}$ mice were from the same breedings, the genetic background between individual mice was heterogeneous. It is therefore of note that the IL-$6^{-/-}$ mice succumbed to tuberculosis under conditions which were still tolerated by the IL-$6^{+/+}$ mice.

With the emergence of tuberculosis in human immunodeficiency virus-positive individuals and of multidrug-resistant strains of *M. tuberculosis*, immunotherapy with recombinant cytokines in adjunct to chemotherapy is currently being considered for treatment of certain cases of tuberculosis (4, 44). Studies using gene disruption mutant mice with defined immunodeficiencies may help to define the cytokines which are critical to protective immunity against tuberculosis and therefore may facilitate the development of new immunologic means for prevention and therapy of tuberculosis.

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