INFECTION AND IMMUNITY, June 1997, p. 2339–2345 Vol. 65, No. 6
0019-9567/97/$04.00+0

Impaired Resistance to the Development of Toxoplasmic Encephalitis in Interleukin-6-Deficient Mice

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Received 8 November 1996/Returned for modification 7 January 1997/Accepted 10 March 1997

The role of interleukin-6 (IL-6) in the pathogenesis of toxoplasmic encephalitis (TE) was examined by using IL-6-targeted mutant (IL-6$^{-/-}$) mice. At 4 and 8 weeks after infection with the ME49 strain of *Toxoplasma gondii*, significantly greater numbers of *T. gondii* cysts and areas of inflammation associated with tachyzoites were observed in brains of IL-6$^{-/-}$ mice than in those of control mice. Large areas of necrosis were observed only in brains of IL-6$^{-/-}$ mice. Tachyzoites were frequently detected in the areas of necrosis, suggesting that necrosis was caused by proliferation of the parasite. These results indicate that IL-6 is protective against development of TE by preventing formation of *T. gondii* cysts and proliferation of tachyzoites in brains of infected mice. Whereas in brains of control mice, large numbers of inflammatory cells were always observed in areas where tachyzoites were detected, in brains of IL-6$^{-/-}$ mice, only small numbers of inflammatory cells were observed in many areas with tachyzoites. Lymphocyte preparations isolated from brains of infected control mice had significantly higher ratios of γ/δ T cells and CD4$^+$ α/β T cells but lower ratios of CD8$^+$ α/β T cells compared to those of infected IL-6$^{-/-}$ mice. There were no differences in the ratios of these T-cell subsets in spleens between these mice. The amounts of mRNA for gamma interferon (IFN-γ) detected by reverse transcriptase PCR were significantly smaller in brains of IL-6$^{-/-}$ mice than in those of control mice, whereas amounts of IL-10 mRNA were greater in the former than in the latter. IL-6 mRNA was detected only in infected control mice. The protective activity of IL-6 against development of TE appears to be through its ability to stimulate IFN-γ production and induce infiltration and accumulation of different T-cell subsets in brains of infected mice.

Toxoplasmic encephalitis (TE) has been the major opportunistic infectious disease in the central nervous system in patients with AIDS. TE in AIDS patients is almost solely caused by recrudescence of a latent chronic infection with *Toxoplasma gondii* (22, 24). However, the pathogenesis of the encephalitis remains to be defined. Since immunocompetent individuals do not usually suffer apparent untoward effects from infection with *T. gondii* (26), it is clear that the immune response is critical for prevention of TE. We and others have found that gamma interferon (IFN-γ) (13, 30) and tumor necrosis factor alpha (TNF-α) (13, 32) are important in prevention of development and/or progression of TE in murine models. In addition to these two cytokines, interleukin-6 (IL-6) has been reported to be involved in the immunopathogenesis of TE (33). IL-6 is a multifunctional cytokine that regulates various aspects of the immune response, acute-phase reaction, and hematopoiesis (1) and acts in the nervous system (15). IL-6 mRNA is expressed in brains of mice infected with *T. gondii* (9, 13, 18). We previously reported that treatment of mice with a monoclonal antibody (MAb) against IL-6 reduces the inflammatory response as well as numbers of *T. gondii* tachyzoites and cysts in the brains of mice with TE (33). However, it is not clear from these results whether IL-6 is protective or whether it contributes to development of TE, since mice treated with an anti-IL-6 MAb had paradoxically higher levels of IL-6 in serum than did untreated mice (33). Deckert-Schlüter et al. (9) recently reported that mice with a strong increase in intracerebral mRNA for IFN-γ, TNF-α, and IL-6 were significantly better protected against *T. gondii*. In the present study, to determine the role of IL-6 in the pathogenesis of TE, we examined the development of TE following infection in IL-6-deficient (IL-6$^{-/-}$) mice which had been generated by gene targeting. IL-6 was found to play a critical role in prevention of development of TE.

MATERIALS AND METHODS

Mice. Male IL-6$^{-/-}$ and wild-type control mice (129/SV × C57BL/6 background) (8) were 8 to 12 weeks old when used. They were bred in our animal facilities. Female C57BL/6 mice obtained from Bantin and Kingman (Fremont, Calif.) were 7 to 8 weeks old when used. There were four or five mice in each experimental group. Control mice were generated from the same breeding program as IL-6$^{-/-}$ mice, where littermate animals were initially classified as +/+ or −/− by Southern blot analysis as described before (8). Mice of each of these two genotypes were selected at random for additional breeding to generate experimental groups. Mice used had not been backcrossed to either the C57BL/6 or 129/SV strain.

Infection with *T. gondii*. Cysts of the ME49 strain of *T. gondii* were obtained from brains of chronically infected C57BL/6 mice as previously described (33). IL-6$^{-/-}$ and control mice were infected intraperitoneally with 10 cysts. The intraperitoneal route was chosen for infection because this route was used in our previous study to examine the effect of an anti-IL-6 MAb on development of TE (33).

Histopathology. At 4 and 8 weeks after infection with the ME49 strain, mice were euthanized by asphyxiation with CO_2. Their brains were removed and immediately fixed in a solution containing 10% formalin, 70% ethanol, and 5% acetic acid. Two to four 5-μm-thick sagittal sections (50- or 100-μm distance

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between sections) of the brain from each mouse were stained with hematoxylin and eosin or by the immunoperoxidase method with rabbit anti-*T. gondii* immunoglobulin G antibody (7, 33). Sections stained with hematoxylin and eosin were evaluated for inflammatory changes, and sections stained by the immunoperoxidase method were evaluated for the number of *T. gondii* cysts and areas of inflammation associated with tachyzoites.

Flow cytometry. MAbs used for flow cytometry were obtained from PharMingen (San Diego, Calif.). Single-cell suspensions of spleen cells from each of the infected IL-$6^{-/-}$ and control mice were prepared individually by mincing the spleens on frosted glass slides. The lymphocyte population infiltrated into the brain of each mouse was isolated individually by a modification of the method described by Lindsley and Rodriguez (23). In brief, perfused brains were disrupted by using frosted glass slides. The cells were washed in minimum essential medium (Sigma BioScience, St. Louis, Mo.) by centrifugation at 500 × *g* for 10 min. The pellets were suspended in 30% Percoll (Pharmacia, Uppsala, Sweden) in minimum essential medium containing heparin (100 U/ml) and centrifuged at 500 × *g* for 15 min. The pellets were used for flow cytometry after lysing erythrocytes. Then 10^6 spleen cells and 10^5 lymphocytes isolated from brains were pretreated on ice for 10 min with 10 μl of a predetermined optimal concentration of anti-FcγII/III receptors (2.4G2) to block non-antigen-specific binding of antibodies to the FcγII/III receptors. Thereafter, the cells were incubated for 30 min with 10 μl of optimal concentrations of phycoerythrin-conjugated anti-T-cell receptor α/β MAb (H57-597) and fluorescein isothiocyanate-conjugated anti-T-cell receptor γ/δ MAb (GL3) or phycoerythrin-conjugated anti-CD4 MAb (RM4-5) and fluorescein isothiocyanate-conjugated anti-CD8 MAb (53-6.7). Analysis of stained cells was performed with a FACScan (Becton Dickinson, Mountain View, Calif.). Dead cells were gated out on the basis of propidium iodide staining.

Detection of cytokine mRNAs. RNA was isolated from brains of infected IL-$6^{-/-}$ and control mice by an acid guanidinium thiocyanate-phenol-chloroform single-step method (6). The optical density at 260 nm was used to estimate the concentration of total RNA. cDNA was synthesized by using the RNA as described previously (19, 32). PCR for cytokines was performed with 1 μl of the original cDNA reaction mixture with a Geneamp 9600 thermocycler (Perkin-Elmer, Emeryville, Calif.), using 30 cycles to produce amounts of DNA within a linear range as described previously (34). This number of cycles was determined in preliminary studies using different amounts of cDNA of the same sample. Specific primers for β-actin, IFN-γ, TNF-α, IL-4, IL-6, and IL-10 (Clontech, Palo Alto, Calif.) designed to span at least one intron allowed differentiation of amplified target DNA derived from either cDNA or genomic DNA in the PCR.

Detection of PCR products. Ten-microliter aliquots of the final PCR mixtures were electrophoresed at 90 V for 3 h on a 1.5% agarose gel and denatured (19, 32). The DNA was then transferred to a Duralon-UV membrane (Stratagene, La Jolla, Calif.) by standard blotting procedures (27) and UV cross-linked. Oligonucleotide probes for β-actin and cytokines (Clontech) which hybridize to the PCR products wholly within the region amplified by the primers were end labeled as described for the 3′-end labeling and signal amplification system for a FluorImager (Amersham, Little Chalfont, England), and hybridization was detected by scanning of the membranes with a FluorImager Storm 860 (Molecular Dynamics, Sunnyvale, Calif.). The quantification of mRNA was performed by densitometry analysis with the FluorImager and normalized to the β-actin level.

Statistical analysis. Levels of significance for differences between groups of mice were determined by the Student *t* or Mann-Whitney U test. The latter was applied when standard deviations were significantly different between groups tested. Differences which provided *P* values of less than 0.05 were considered significant. For comparison of amounts of mRNA for cytokines in brains of infected mice, we calculated corrected *P* (*Pc*) values to correct for numbers of independent variables by multiplying each *P* value by the number of cytokines studied (3). In this case, differences which provided *Pc* values of less than 0.05 were considered significant.

RESULTS

Development of TE in IL-$6^{-/-}$ mice. At both 4 and 8 weeks after infection, IL-$6^{-/-}$ mice had more severe histological changes and significantly greater numbers of *T. gondii* cysts in their brains than did control mice (Fig. 1A) ($P = 0.014$ and $P = 0.012$, respectively). Tachyzoites were always detected in association with inflammatory cells in brains of both strains of mice. The numbers of areas in which tachyzoites were detected in association with inflammatory cells were significantly greater in brains of IL-$6^{-/-}$ mice than in those of control mice at these two time periods (Fig. 1B) ($P = 0.003$ and $P = 0.006$, respectively). In addition, the numbers of parasitophorous vacuoles containing tachyzoites were significantly greater in brains of IL-$6^{-/-}$ mice than in those of control mice at 8 weeks after infection (the median [with the maximum and minimum values per sagittal section] was 13.6 [5, 33] versus 3.3 [0, 11]; $P = 0.003$, 9 mice for each group). These results indicate that IL-6 is protective against formation of *T. gondii* cysts and the appearance and/or proliferation of tachyzoites in brains of mice. Whereas in control mice, tachyzoites were always observed in association with large numbers of inflammatory cells (Fig. 2A and B), in IL-$6^{-/-}$ mice, tachyzoites were frequently (but not always) associated with only small numbers of inflammatory cells (Fig. 2C and D). These results suggest that IL-6 plays an important role in induction of accumulation of inflammatory cells in the areas where tachyzoites appear. Large areas of necrosis in brains were observed only in IL-$6^{-/-}$ mice (Fig. 2E), and tachyzoites were frequently detected in these areas (Fig. 2F), but with only a few inflammatory cells (Fig. 2E). These results indicate that IL-6 is important in prevention of development of necrosis caused by proliferation of tachyzoites in brains of mice. There was no mortality in either the IL-$6^{-/-}$ or control mice during the observation period.

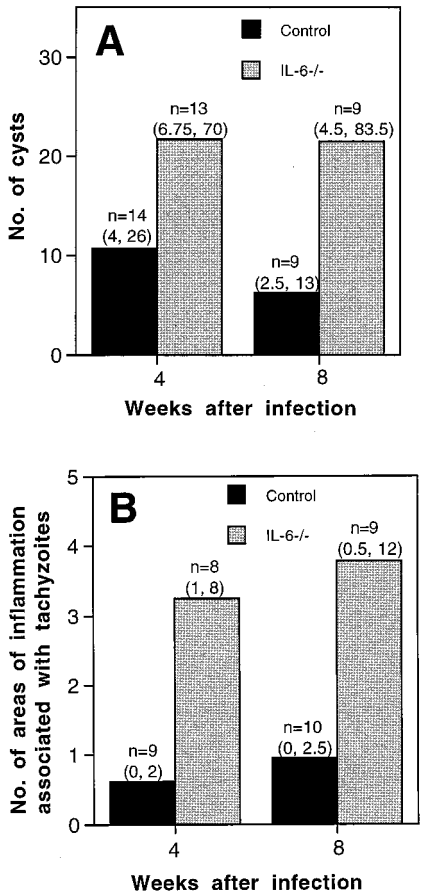

FIG. 1. Numbers of *T. gondii* cysts (A) and areas of inflammation associated with *T. gondii* tachyzoites (B) in brains of IL-$6^{-/-}$ and control mice infected with *T. gondii*. Mice were infected intraperitoneally with 10 cysts of the ME49 strain. Four and eight weeks later, histological study was performed. Two to four sagittal sections (distance between sections of 50 μm) from each mouse were stained with an immunoperoxidase stain by using anti-*T. gondii* antibodies and evaluated for counting. The mean value from these sections for each mouse was calculated as the number per section. These values are shown in each panel and were used for statistical analysis to compare differences between groups. *P* values shown were obtained by the Mann-Whitney *U* test. The numbers in parentheses represent minimum and maximum values for each group. The number above the parentheses indicates the number of mice used in each group. The data reflect results of two or three separate experiments. Each experimental group in each experiment contained four or five mice.

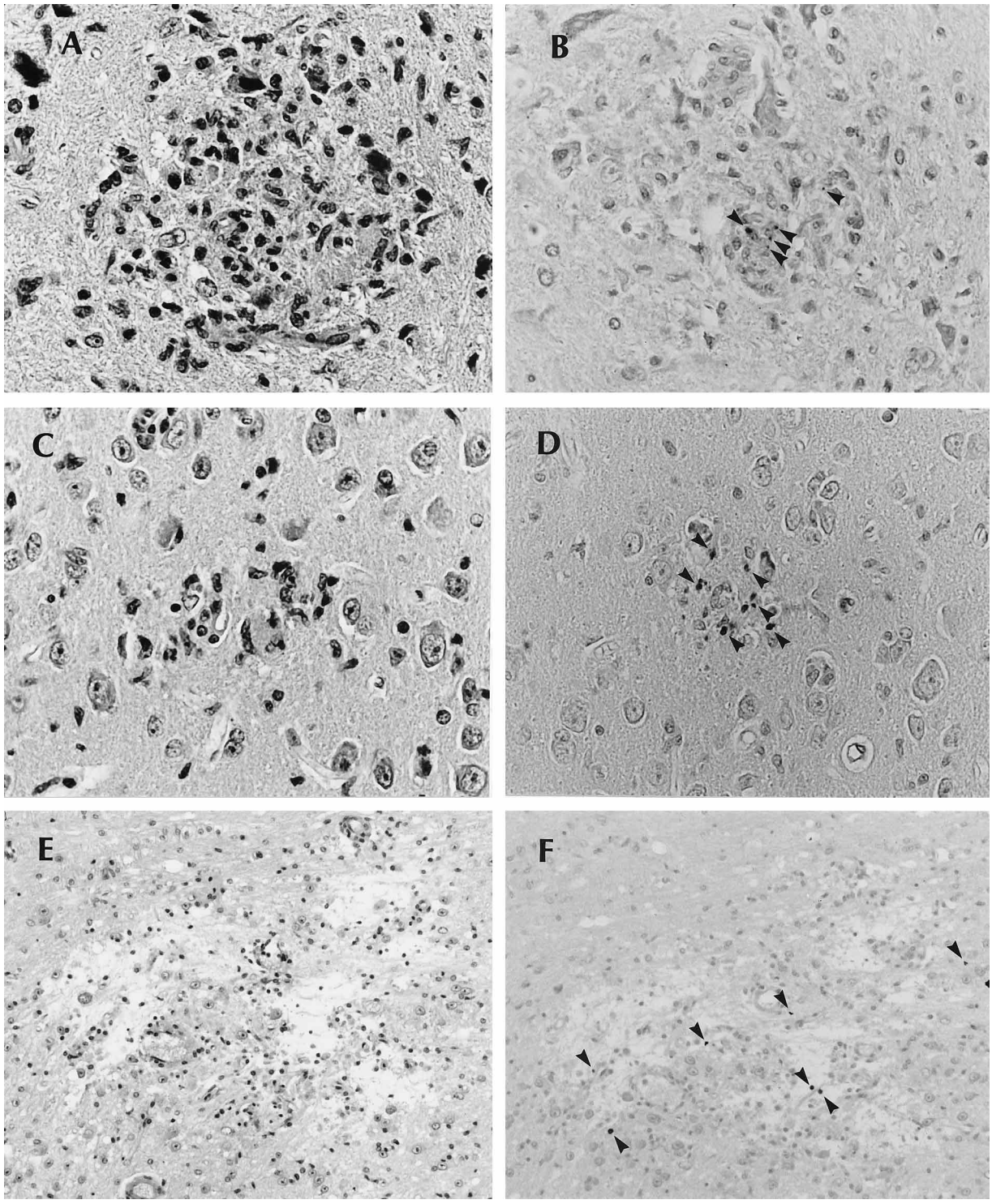

FIG. 2. Histological changes in brains of IL-$6^{-/-}$ and control mice infected with *T. gondii*. Mice were infected intraperitoneally with 10 cysts of the ME49 strain, and histological study was performed 8 weeks later. (A and B) Area of acute inflammation associated with tachyzoites in control mice; (C and D) area of inflammation associated with tachyzoites in IL-$6^{-/-}$ mice (note only a few inflammatory cells); (E and F) large area of necrosis in IL-$6^{-/-}$ mice. (A, C, and E) Hematoxylin and eosin stain; (B, D, and F) immunoperoxidase stain using rabbit anti-*T. gondii* immunoglobulin G antibodies (arrowheads indicate collections of *T. gondii* tachyzoites and antigens). Each photograph is representative of the histological changes observed in each experimental group. The experiment was performed twice, and there were four or five mice in each experimental group in each experiment.

Relative percentages of T-cell subsets in lymphocyte preparations isolated from brains of infected IL-$6^{-/-}$ and control mice. Since fewer inflammatory cells were observed in areas of brains associated with tachyzoites in IL-$6^{-/-}$ mice than in control mice, we examined relative percentages of T-cell subsets in lymphocyte preparations isolated from brains of IL-$6^{-/-}$ and control mice by flow cytometry. At 8 weeks after infection, lymphocyte populations were isolated from the brain

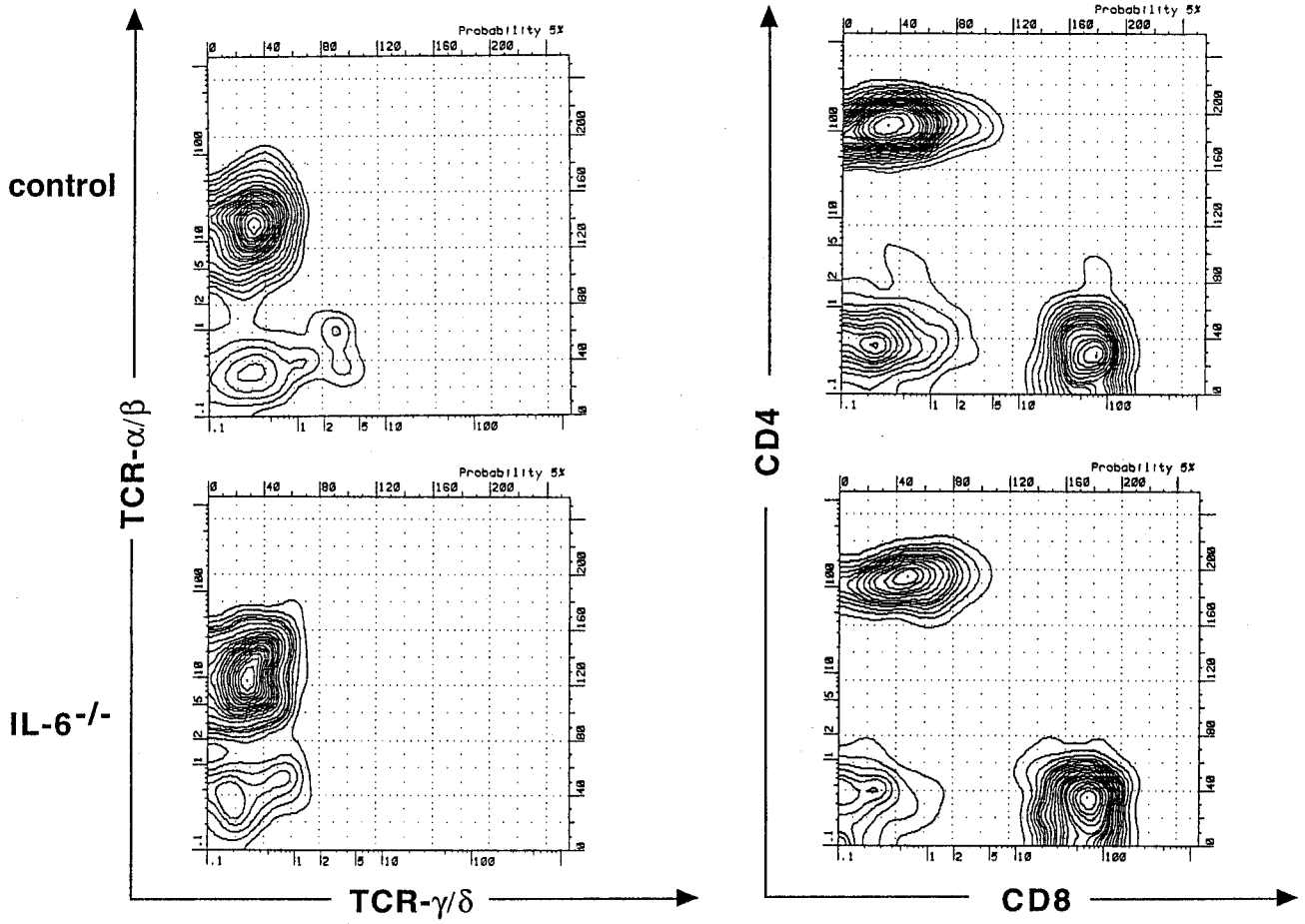

FIG. 3. Relative percentages of γ/δ and α/β T cells and of $CD4^+$ and $CD8^+$ T cells in the lymphocyte preparations isolated from brains of IL-$6^{-/-}$ and control mice infected with *T. gondii*. Mice were infected intraperitoneally with 10 cysts of the ME49 strain; 8 weeks later, lymphocyte preparations were isolated from their brains (see Materials and Methods). Essentially identical results were obtained in each mouse of the same experimental group (four mice for each group). TCR, T-cell receptor.

of each of the IL-$6^{-/-}$ and control mice. The total numbers of cells in the lymphocyte preparations did not differ between IL-$6^{-/-}$ and control mice ([13.4 ± 4.21] × 10^6 versus [9.20 ± 2.69] × 10^6; $P = 0.142$). However, relative percentages of γ/δ T cells and of $CD4^+$ and $CD8^+$ α/β T cells differed significantly between these mice. Figure 3 shows the results of flow cytometric analysis from one representative mouse of each group. Relative percentages of γ/δ T cells were 2.6-fold higher in lymphocyte preparations from brains of infected control mice than in those of infected IL-$6^{-/-}$ mice ($P = 0.004$) (Table 1; Fig. 3). In addition, relative percentages of $CD4^+$ T cells were significantly higher in lymphocyte preparations from brains of infected control mice than in those of infected IL-$6^{-/-}$ mice ($P = 0.048$), whereas relative percentages of $CD8^+$ T cells were significantly lower in the former than in the latter ($P = 0.002$) (Table 1; Fig. 3). The majority of those $CD4^+$ and $CD8^+$ T cells were α/β T cells in both IL-$6^{-/-}$ and control mice (data not shown).

Relative percentages of T-cell subsets in spleens of infected IL-$6^{-/-}$ and control mice. Since relative percentages of T-cell subsets in lymphocyte preparations isolated from brains of IL-$6^{-/-}$ mice differed significantly from those in infected control mice, we examined relative percentages of T-cell subsets in spleens of IL-$6^{-/-}$ and control mice individually 8 weeks after infection. Relative percentages of γ/δ T cells and of $CD4^+$ and $CD8^+$ α/β T cells did not differ between infected IL-$6^{-/-}$ and control mice (Table 1).

Levels of cytokine mRNAs in brains of infected IL-$6^{-/-}$ and control mice. Amounts of mRNAs for IFN-γ, TNF-α, IL-4, IL-6, and IL-10 in the total RNA fractions obtained from

TABLE 1. Relative percentages of T-cell subsets in lymphocyte preparations isolated from brains and spleens of IL-$6^{-/-}$ and control mice infected with *T. gondii*

Mice[a]	Source of lymphocytes[b]	Relative % of T-cell subsets[c] (mean ± SD)		
		γ/δ	$CD4^+$	$CD8^+$
Control	Brain	5.32 ± 0.69	41.9 ± 2.96	34.7 ± 3.89
IL-$6^{-/-}$	Brain	2.05 ± 0.62[d]	32.4 ± 7.10[e]	53.7 ± 6.30[d]
Control	Spleen	1.53 ± 0.56	13.8 ± 2.54	11.2 ± 3.46
IL-$6^{-/-}$	Spleen	1.29 ± 0.43	15.2 ± 4.53	15.4 ± 3.62

[a] Mice were infected intraperitoneally with 10 cysts of the ME49 strain of *T. gondii*.
[b] Spleen cells and lymphocyte preparations from brains were obtained from mice 8 weeks after infection.
[c] Determined by flow cytometric analysis.
[d] $P < 0.005$, Student's *t* test.
[e] $P < 0.05$, Student's *t* test.

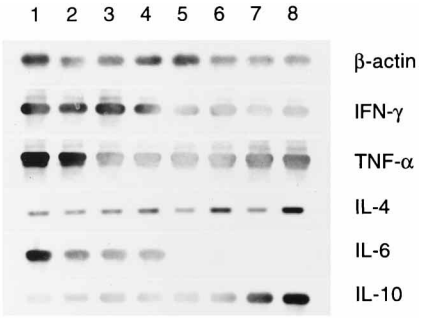

FIG. 4. Detection of cytokine mRNAs by using PCR-assisted amplification of RNA in brains of IL-$6^{-/-}$ and control mice infected with *T. gondii*. Groups of four mice were killed 8 weeks after infection with the ME49 strain, and their brains were analyzed for the presence of cytokine mRNAs (see Materials and Methods). Lanes 1 to 4, infected control mice; lanes 5 to 8, infected IL-$6^{-/-}$ mice.

brains of mice were measured by reverse transcriptase PCR 8 weeks after infection. As expected, IL-6 mRNA was detected in the total RNA of infected control mice but not in that of IL-$6^{-/-}$ mice (Fig. 4). mRNA for each of the other cytokines was detected in both infected IL-$6^{-/-}$ and control mice. The amounts of IFN-γ mRNA were significantly small in IL-$6^{-/-}$ than in control mice ($P = 0.002$, $Pc = 0.010$). In contrast, although relatively few mice were used in the experiment, there was a tendency for greater amounts of IL-10 mRNA in the former than the latter mice (Fig. 4). This difference did not, however, reach statistical significance when corrected for the number of variables tested ($P = 0.029$, $Pc = 0.143$). There were no differences in the amounts of mRNAs for IL-4 and TNF-α between infected IL-$6^{-/-}$ and control mice (Fig. 4).

DISCUSSION

This study reveals the importance of IL-6 in resistance against development of TE. Greater numbers of *T. gondii* tachyzoites and cysts were found in brains of IL-$6^{-/-}$ mice than in those of control mice. These results indicate that endogenous IL-6 plays a significant role in prevention of the formation of *T. gondii* cysts and of the appearance and/or proliferation of *T. gondii* tachyzoites in brains of mice.

Histological changes in brains of infected IL-$6^{-/-}$ mice were remarkable for large areas of necrosis. Such histological changes were not noted in control mice. Tachyzoites were frequently detected in those areas of necrosis, suggesting that the necrosis was caused by proliferation of tachyzoites. These results indicate that IL-6 plays a critical role in prevention of proliferation of tachyzoites and thereby of necrosis. In this study, large areas of necrosis of the brain were observed in infected IL-$6^{-/-}$ mice of the 129/SV × C57BL/6 background. In addition, IL-$6^{-/-}$ mice of the 129/SV × C57BL/6 × MF1 (an outbred strain) background (kindly supplied by Valeria Poli, Instituto di Richerche di Biologia Molecolare [IRBM], Pomezia, Italy) also developed large areas of necrosis in their brains following infection with *T. gondii* (data not shown). Thus, IL-6 appears to be important for prevention of necrosis of brains (caused by proliferation of tachyzoites) of mice with different genetic backgrounds.

We previously reported that treatment of mice with an anti-IL-6 MAb reduced the numbers of tachyzoites and cysts in brains of mice with TE (33). In those studies, mice treated with an anti-IL-6 MAb had paradoxically higher levels of IL-6 in serum than did untreated controls (33). Induction of high levels of IL-6 in serum by an anti-IL-6 MAb has also been observed in endotoxin shock (14) and infection with *Staphylococcus aureus* (25). Since the present study reveals that IL-6 is protective against the formation of *T. gondii* cysts and the appearance and/or proliferation of tachyzoites in brains of mice, it appears likely that the protective effect of the anti-IL-6 MAb observed in our previous study was mediated by higher levels of IL-6 in serum induced by the anti-IL-6 MAb. Deckert-Schlüter et al. (9) reported that genetically regulated resistance against *T. gondii* correlated with the amount of intracerebrally produced cytokine mRNAs for IFN-γ, TNF-α, and IL-6. Thus, IL-6 may be involved in the mechanism(s) which determines genetic resistance of mice against development of TE previously reported (4, 31, 32).

Beaman et al. (2) previously reported that treatment of murine peritoneal macrophages with human recombinant IL-6 enhanced intracellular replication of tachyzoites in vitro. In contrast, the present study reveals that IL-6 is protective against development of TE (cyst formation and proliferation of tachyzoites). In the in vitro study by Beaman et al. (2), human recombinant IL-6 was used with murine macrophages, and the concentrations that they used may not reflect those in the microenvironment of the brain. In addition, the effect of IL-6 on glial cells in the brain may be different from its effect on peritoneal macrophages. In this regard, Chao et al. (5) reported that treatment of human fetal microglia with IL-6 inhibited intracellular replication of tachyzoites in vitro.

IL-$6^{-/-}$ mice have been reported to be significantly more susceptible to infection with *Listeria monocytogenes*, a facultative intracellular bacterium (8, 21), and Dalrymple et al. (8) recently reported that IL-6 is important for induction of neutrophilia in resistance against *L. monocytogenes*. Although it is not known whether murine neutrophils are able to kill *T. gondii* tachyzoites and although neutrophils were usually not a major population of inflammatory cells observed in areas of brains where tachyzoites were detected in control mice, it is possible that neutrophils are involved in killing of tachyzoites in brains of infected mice. Human neutrophils have been shown to phagocytose and kill tachyzoites (11, 35).

In control mice, large numbers of inflammatory mononuclear cells were always observed in areas of the brain where tachyzoites were detected. In contrast, in IL-$6^{-/-}$ mice, tachyzoites were frequently observed in association with only small numbers of inflammatory cells. The areas of necrosis and tachyzoites in IL-$6^{-/-}$ mice were also observed to have only small numbers of inflammatory cells. These results suggest that IL-6 plays an important role in inducing an accumulation of inflammatory cells into the areas of the brain where tachyzoites appear and that such accumulation of inflammatory cells is critical for prevention of proliferation of tachyzoites and prevention of necrosis of the brain which occurs following proliferation of tachyzoites.

Flow cytometric study of lymphocyte populations that infiltrated the brains of infected mice revealed significant differences in the relative percentages of T-cell subsets between IL-$6^{-/-}$ and control mice. Control mice had significantly higher ratios of γ/δ T cells and $CD4^+$ α/β T cells but lower ratios of $CD8^+$ α/β T cells in their brains at 8 weeks after infection compared to IL-$6^{-/-}$ mice (at that time, IL-$6^{-/-}$ mice had more severe TE than control mice). Of interest is that there were no differences in relative percentages of these T-cell subsets in spleens of the mice. These results indicate that IL-6 plays an important role in regulating infiltration by different T-cell subsets of brains of mice infected with *T. gondii*. IL-6 may play an important role in activating different T-cell subsets, which thereby confers activity to these cells to infiltrate

the brains and/or induce accumulation of different T-cell subsets which have already been activated during infection with *T. gondii*. In this regard, Kopf et al. (21) reported that generation and activity of cytotoxic T cells against vaccinia virus was 3- to 10-fold reduced in IL-$6^{-/-}$ mice compared to control mice.

Gazzinelli et al. (12) previously reported that $CD4^+$ and $CD8^+$ T cells collaborate to prevent development of TE in C57BL/6 mice which have the H-2^b haplotype, as do the IL-$6^{-/-}$ and control mice used in the present study. Since brains of infected IL-$6^{-/-}$ mice were found to have significantly different ratios of $CD4^+$ and $CD8^+$ α/β T cells and γ/δ T cells compared to infected control mice, infiltration of appropriate numbers of different T-cell subsets in the areas of the brain where tachyzoites appear may be critical in prevention of proliferation of tachyzoites and prevention of TE, although the role of γ/δ T cells in the pathogenesis of TE is unknown. Infiltration of γ/δ T cells has also been found in brains of athymic nude rats with TE (28). Recently, Hisaeda et al. (16) and Kasper et al. (20) reported that γ/δ T cells play a protective role against death in mice during the early stage of the infection. In studies of human peripheral blood γ/δ T cells, Subauste et al. (29) reported the cytotoxicity of these cells for *T. gondii*-infected target cells and production of IFN-γ, IL-2, and TNF-α.

IFN-γ has been shown to be critical for the activity of T cells against development of TE (13, 30). In the present study, infected IL-$6^{-/-}$ mice were found to have significantly less IFN-γ mRNA in their brains than infected control mice, indicating that IL-6 plays an important role in activating production of IFN-γ in the brain during infection. The major source of IFN-γ in brains of infected IL-$6^{-/-}$ and control mice appeared to be T cells, since natural killer cells ($NK1.1^+$ cells) were not detected by flow cytometry (data not shown). The upregulating effect of IL-6 on IFN-γ production in *T. gondii*-infected mice is supported by our previous finding that infected mice treated with an anti-IL-6 MAb had higher levels of both IL-6 and IFN-γ in serum than did infected, untreated controls (33).

It has been reported that IFN-γ may play a critical role in homing of lymphocytes to the brain (10, 17). IFN-γ can upregulate expression of adhesion molecules involved in lymphocyte traffic on endothelial cells in vitro, and lymphocytes show much greater adhesion to cerebrovascular endothelial cells in vitro when monolayers are pretreated with IFN-γ, TNF-α, or IL-1 (10, 17). Therefore, impaired production of IFN-γ by T cells in brains of infected IL-$6^{-/-}$ mice may have contributed to their failure to accumulate lymphocytes into areas of the brain where tachyzoites were demonstrable.

In conclusion, IL-6 plays a critical role in prevention of development of TE. This protective activity in brains of infected mice appears to be, at least in part, through its effect to stimulate IFN-γ production and induce infiltration of different T-cell subsets and their accumulation into areas where tachyzoites appear.

ACKNOWLEDGMENTS

We thank Pauline Chu for excellent technical assistance with histological studies.

This work was supported by grant R94-PAM-100 from the University of California Universitywide AIDS Research Program, Public Health Service grants AI04717, AI30230, and AI35956 from the National Institutes of Health, and a grant from Japan Immunoresearch Laboratories, Co., Ltd. Oliver Liesenfeld is a recipient of an Infectious Disease Research Fellowship from the German Ministry of Research and Technology.

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Editor: S. H. E. Kaufmann