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Antibody against Interleukin-6 Reduces Inflammation and Numbers of Cysts in Brains of Mice with Toxoplasmic Encephalitis

YASUHIRO SUZUKI,[1,2] QING YANG,[1] FRANCES K. CONLEY,[3] JOHN S. ABRAMS,[4] AND JACK S. REMINGTON[1,2]*

Department of Immunology and Infectious Diseases, Research Institute, Palo Alto Medical Foundation, Palo Alto, California 94301[1]; Division of Infectious Diseases and Geographic Medicine, Department of Medicine, Stanford University School of Medicine, Stanford, California 94305[2]; and Section of Neurosurgery, Veterans Administration Medical Center,[3] and DNAX Research Institute,[4] Palo Alto, California 94304

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Treatment of toxoplasmic encephalitis in C57BL/6 mice with monoclonal antibody (MAb) against interleukin-6 (IL-6) resulted in a remarkable decrease in the number of foci of acute inflammation in their brains caused by proliferation of tachyzoites. In brains of mice treated with isotype control MAb and those treated with anti-IL-6 MAb, tachyzoites were observed only in foci of acute inflammation. Immunoperoxidase staining revealed a greatly diminished frequency of tachyzoites in brains of mice treated with anti-IL-6 MAb. Of interest, treatment with MAb against IL-6 was also associated with reduced numbers of *Toxoplasma gondii* cysts in the brains and with higher serum levels of gamma interferon than in control mice. Paradoxically, the mice treated with anti-IL-6 MAb had higher serum levels of IL-6 as measured by an enzyme-linked immunosorbent assay than controls. These results revealed the importance of IL-6 in the immunopathogenesis of murine toxoplasmic encephalitis.

Toxoplasmic encephalitis (TE) is a major cause of morbidity and mortality in patients with AIDS (7, 10). In these patients, the encephalitis occurs almost solely in individuals with preexisting antibodies to toxoplasma (9, 10, 17). Thus, TE is due primarily to recrudescence of a previous, latent toxoplasma infection in individuals with defective T-cell immunity. These facts demonstrate that immunity during chronic (latent) *Toxoplasma gondii* infection is critically important for prevention of TE. Critical to this immune status are gamma interferon (IFN-γ) (13, 15) and tumor necrosis factor alpha (5). Recently, our group found that treatment with monoclonal antibody (MAb) against interleukin-6 (IL-6) prolonged the time to death in mice with acute toxoplasmosis (1). This fact led us to examine the effect of administration of MAb against IL-6 on TE in a murine model. The studies described below reveal that administration of anti-IL-6 MAb remarkably reduced the frequency of tachyzoites and the inflammatory changes caused by the tachyzoites as well as the number of *T. gondii* cysts in the brains of mice with TE.

MATERIALS AND METHODS

Mice. Male C57BL/6 mice obtained from Simonsen Laboratories (Gilroy, Calif.) were 7 to 8 weeks of age when used. There were four mice in each experimental group in the first experiment and three mice per experimental group in one additional experiment. The results in the two experiments were comparable.

Toxoplasma infection. Cysts of the ME49 strain of *T. gondii* were obtained from chronically infected C57BL/6 mice. Briefly, mice were sacrificed by asphyxiation with CO_2, and their brains were removed and triturated in phosphate-buffered saline (pH 7.2) (16). An aliquot of the brain suspension was examined microscopically for the numbers of cysts, and after appropriate dilution of the aliquot in phosphate-buffered saline, each mouse was injected with 10 cysts intraperitoneally (i.p.) (13).

MAbs. The MAbs used for neutralization of cytokine activity in vivo were rat anti-mouse IL-6 (MP5-20F3) (12) and anti-mouse IFN-γ (XMG1.2) (2). Rat anti-*Escherichia coli* β-galactosidase (GL113) was used as the control MAb.

Mice were injected i.p. with 2 mg of anti-IL-6 MAb weekly for 4 weeks beginning 4 weeks after infection. Control mice were injected with 2 mg of MAb GL113 with the same

TABLE 1. Effect of treatment with MAbs against IL-6 and IFN-γ on acute focal inflammation and numbers of *T. gondii* cysts in the brain of mice with TE

Treatment[a]	No. of areas of acute focal inflammation[b]		No. of cysts[c,d]
	Total no. of areas[e]	No. of areas with *T. gondii* antigens[c]	
None	19.4 ± 9.0	ND[f]	19.4 ± 10.7
GL113 (control)	13.6 ± 7.2	6.0 ± 4.0	15.6 ± 7.4
Anti-IL-6	2.8 ± 1.4[g]	0.2 ± 0.5[h]	4.3 ± 3.3[i]
Anti-IFN-γ	32.8 ± 12.9[h]	ND	169.8 ± 12.3[i]

[a] Mice were injected i.p. with 2 mg of either GL113 or anti-IL-6 MAb once weekly for 4 weeks beginning 4 weeks after infection. For treatment with anti-IFN-γ MAb, mice were injected with 2 mg of antibody once weekly for 2 weeks beginning 6 weeks after infection.

[b] Number of areas of acute focal inflammation per coronal section of brain. Two sections (distance between sections, 150 μm) from each mouse were evaluated for the counting. A total of eight sections were examined for each group. Results of two separate experiments were comparable (see Materials and Methods).

[c] Sections were stained with immunoperoxidase by using anti-toxoplasma immunoglobulin G.

[d] Three sections (distance between sections, 150 μm) from each mouse were examined. A total of 12 sections were examined for each group.

[e] Sections were stained with hematoxylin and eosin.

[f] ND, not done.

[g] $P < 0.001$ when compared with control mice treated with GL113.

[h] $P < 0.005$ when compared with control mice treated with GL113.

[i] $P < 0.0001$ when compared with control mice treated with GL113.

* Corresponding author. Mailing address: Department of Immunology and Infectious Diseases, Research Institute, Palo Alto Medical Foundation, 860 Bryant St., Palo Alto, CA 94301. Phone: (415) 326-8120. Fax: (415) 329-9114.

schedule. Another group was injected with 2 mg of anti-IFN-γ MAb once weekly beginning 6 weeks after infection and continuing for 2 weeks; mice did not survive for 4 weeks since this MAb results rapidly in development of severe lethal encephalitis. One week following the final injection of MAb (8 weeks after infection), the mice were sacrificed and their brains were fixed in a solution that contained 10% formalin, 70% ethanol, and 5% acetic acid.

Histologic evaluation. Two to three coronal sections (5-μm thick; distance between sections, 150 μm) of each brain were stained with hematoxylin and eosin. The presence of toxoplasma antigens was examined by immunoperoxidase staining as described previously (3).

Serum levels of IL-6 and IFN-γ. One week after the final injection of MAb, mice were sacrificed for bleeding and the concentrations of IL-6 and IFN-γ in their sera were measured by an enzyme-linked immunosorbent assay (ELISA) using MAbs against IL-6 (MP5-20F3 as capture and MP5-32C11 as secondary) or IFN-γ (R4-6A2 as capture and XMG1.2 as secondary; all obtained from PharMingen, San Diego, Calif.).

Statistical analysis. Levels of significance for comparisons between groups of mice were determined by using the Student *t* test distribution.

RESULTS

Effect of injection of anti-IL-6 MAb or anti-IFN-γ MAb on TE. Four weeks after infection, at which time treatment with MAb was begun, remarkable inflammatory changes were present in the brains of the mice (Fig. 1A). Eight weeks after infection, at which time the mice were sacrificed after treatment with MAb, the brains of untreated mice also had remarkable infiltration of mononuclear cells in their meninges and around blood vessels (Fig. 1B) and numerous sites of acute focal inflammation (Table 1). The presence of both tachyzoites and toxoplasma antigens was demonstrable in the areas of acute focal inflammation by immunoperoxidase staining (Fig. 1C). Injection of the control MAb (GL113) did not affect the inflammatory changes in the brains (Fig. 1D and Table 1). Tachyzoites and toxoplasma antigens were demonstrable in the brains of these mice by immunoperoxidase staining in areas of acute focal inflammation. Injection of anti-IFN-γ MAb markedly increased the inflammatory changes (Fig. 1F) and the numbers of foci of acute inflammation caused by proliferation of tachyzoites ($P < 0.005$) (Table 1). This is consistent with our previous findings on the effect of anti-IFN-γ MAb on TE (13) and indicates that systemic administration of MAb against a cytokine can have a profound effect on the pathology in the central nervous system of mice. In contrast, mice treated with MAb against IL-6 had significantly less inflammation in the brain than did controls (Fig. 1E). The numbers of inflammatory mononuclear cells infiltrated into the meninges and around blood vessels were markedly lower than those in controls, and the number of foci of acute inflammation in the brain parenchyma was significantly reduced when compared with that of control mice ($P < 0.001$) (Table 1). Tachyzoites were observed only in areas of acute focal inflammation in the control and experimental mice treated with anti-IL-6 MAb. The frequency of the presence of tachyzoites was remarkably and significantly less in the experimental than in the control mice ($P < 0.005$) (Table 1).

Effect of anti-IL-6 or anti-IFN-γ MAb on numbers of *T. gondii* cysts in the brains. Table 1 shows the numbers of *T. gondii* cysts in coronal sections of the brains of mice after treatment with MAb. Mice treated with the control MAb had the same numbers of cysts in their brains as did mice that did not receive the MAb. In contrast, mice treated with MAb against IL-6 had remarkably fewer cysts in their brains than mice treated with the control MAb ($P < 0.0001$). As we have observed previously (13), the mice treated with MAb against IFN-γ had greatly increased numbers of cysts ($P < 0.0001$).

IFN-γ levels in sera of mice treated with anti-IL-6 MAb. Since IFN-γ is known to provide protection against TE (13, 14), concentrations of this cytokine in sera of infected mice treated with control MAb or anti-IL-6 MAb were compared. IFN-γ concentrations were twice as high in sera of mice treated with anti-IL-6 MAb as in sera from control mice (320.0 ± 97.9 pg/ml [mean ± standard deviation] versus 150.3 ± 85.6 pg/ml; $P < 0.05$). IFN-γ was not detectable (i.e., <80 pg/ml) in sera of infected mice treated with anti-IFN-γ MAb. When mice received no treatment, the IFN-γ concentration in their sera was 153.5 ± 105.4 pg/ml.

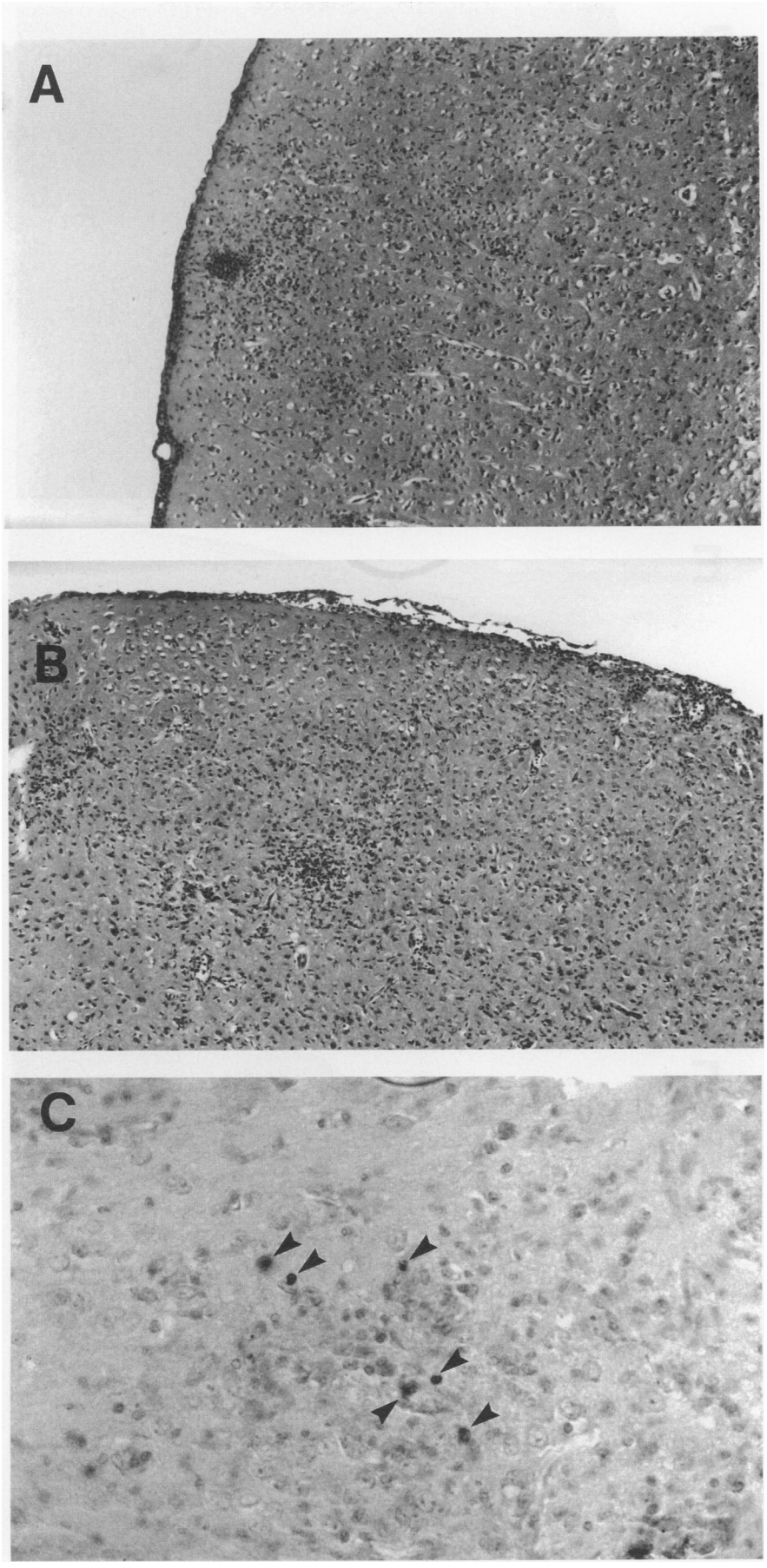
IL-6 levels in sera of mice treated with anti-IL-6 MAb. IL-6 levels in sera of infected mice treated with neutralizing MAb against IL-6 were measured to examine whether the MAb affected the levels of IL-6. In contrast to the effect of treatment with the MAb against IFN-γ, which markedly reduced the blood level of this cytokine, mice treated with anti-IL-6 MAb had concentrations of IL-6 in their sera (1,207 ± 743 pg/ml [mean ± standard deviation]) more than three times higher than those of the control mice treated with control MAb (373 ± 87 pg/ml), although this difference did not achieve statistical significance because one mouse in the experimental group had a low IL-6 level which was similar to the levels in the control mice (Table 2).

DISCUSSION

The results described above reveal that injection of neutralizing MAb against IL-6 remarkably reduced inflammatory changes in the brains of chronically infected mice with TE. The fact that tachyzoites were observed only in the areas of acute focal inflammation in both control and experimental mice treated with anti-IL-6 MAb and that the numbers of areas of acute focal inflammation with tachyzoites were significantly lower in the experimental than in the control mice suggest that treatment of mice with anti-IL-6 MAb prevented disruption of cysts or proliferation of tachyzoites released from disrupted cysts in the brain and/or at extraneural sites.

The mechanism of protective effect of MAb against IL-6 on TE is not clear. It has been reported that both $CD4^+$ and $CD8^+$ T cells are responsible for protective immunity against this disease in mice (4) and that IFN-γ (13) and tumor necrosis factor alpha (5) are critical mediators of this immunity. In the

FIG. 1. Effect of MAb against IL-6 and IFN-γ on inflammatory changes in the brains of mice with TE. Beginning 4 weeks after infection, mice were injected i.p. with 2 mg of either GL113 or anti-IL-6 once weekly for 4 weeks. For treatment with MAb against IFN-γ, mice were injected with 2 mg of antibody once weekly for 2 weeks beginning 6 weeks after infection. One week after the final injection of MAb (8 weeks after infection), histologic studies were performed on their brains. (A) Mice at 4 weeks after infection at which time treatment was begun; (B and C) mice which did not receive MAb; (D) control mice injected with GL113 MAb; (E) mice injected with anti-IL-6 MAb; (F) mice injected with anti-IFN-γ MAb. Panels A, B, D, E, and F were stained with hematoxylin and eosin; panel C was stained with immunoperoxidase by using rabbit anti-toxoplasma antibodies. Arrowheads indicate collections of toxoplasma antigen in areas of acute focal inflammation.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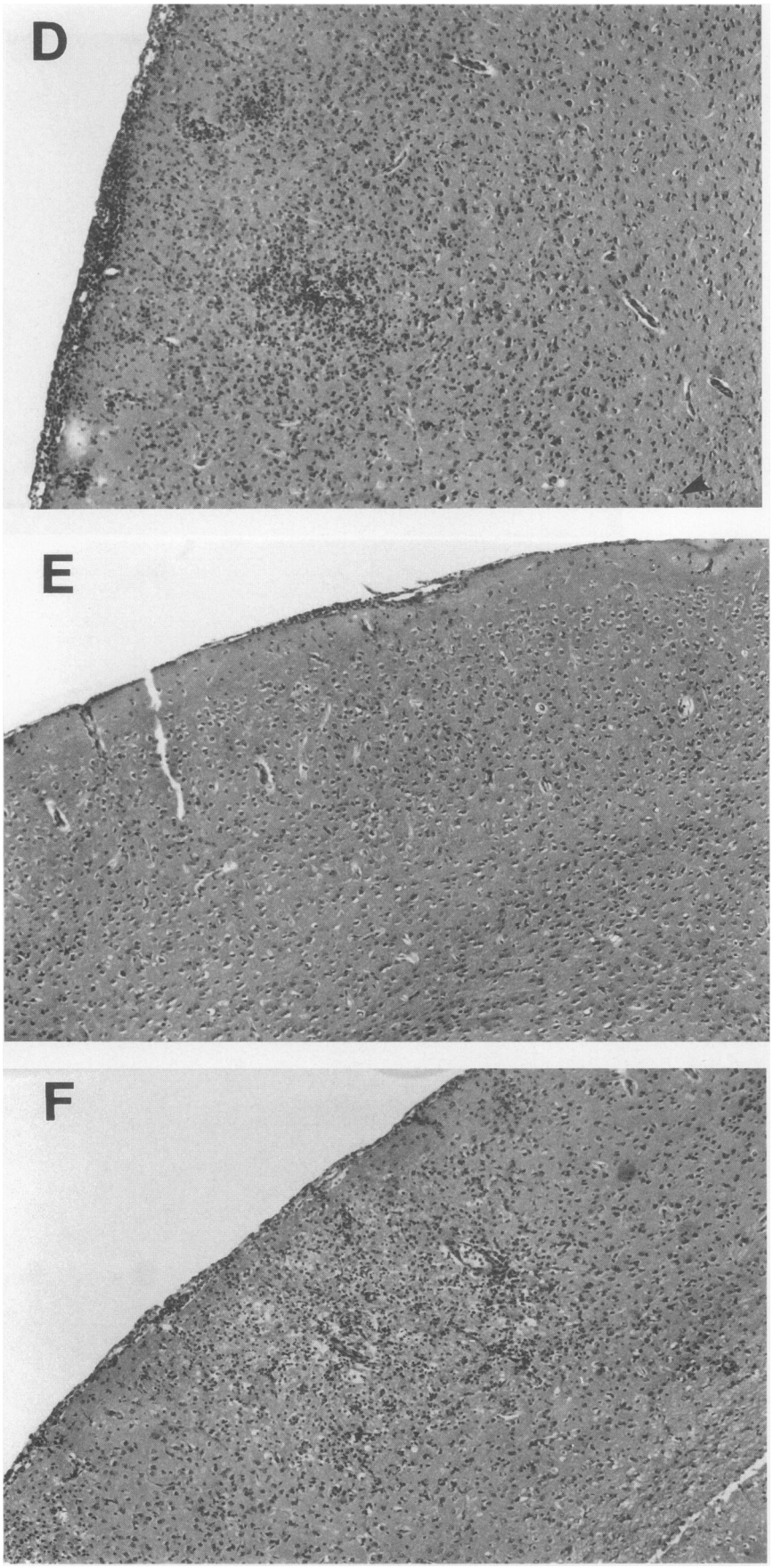

FIG. 1—*Continued.*

TABLE 2. Effects of treatment with MAbs against IL-6 on levels of IL-6 in sera of mice with TE

Individual mouse no.	IL-6 concn (pg/ml) in sera of mice treated with[a]:	
	GL113	Anti-IL-6
1	398	1,112
2	262	322
3	360	2,136
4	472	1,260
Mean ± SD	373 ± 87	1,208 ± 743[b]

[a] Mice were injected i.p. with 2 mg of either GL113 or anti-IL-6 MAb once weekly for 4 weeks beginning 4 weeks after infection. Mice were bled 8 weeks after infection (1 week after the final injection of MAb).

[b] $P = 0.0673$ when compared with mice treated with GL113.

present study, the infected mice treated with MAb against IL-6 had significantly higher concentrations of IFN-γ in their sera than did mice treated with control MAb. Thus, it appears that IFN-γ may have been responsible, at least in part, for the improvement in TE conferred by the anti-IL-6 MAb.

It is not clear from our results whether IL-6 was responsible for or protective against pathology associated with TE since injection of neutralizing (see Materials and Methods) MAb against this cytokine resulted in a higher concentration of IL-6 in the sera of the infected mice. This was in contrast to the effect of injection of neutralizing MAb against IFN-γ which resulted in undetectable levels of IFN-γ in the sera of infected mice. This indicates that the effects of neutralizing MAb in vivo may differ among cytokines to which the MAb are directed. The paradoxical effect of anti-IL-6 neutralizing MAb has been reported in other models (6, 8, 11). Injection of anti-IL-6 MAb protected against endotoxin shock in mice (6, 12), but IL-6 levels in the treated mice were higher than those in control mice (6). Our group recently found that treatment of mice with anti-IL-6 MAb prolonged the time to death in mice with acute *T. gondii* infection: higher levels of IL-6 were noted in the sera of the treated mice than in the control mice (1). In the present study, the capture MAb used for the IL-6 ELISA was the same as the neutralizing MAb used for in vivo studies. Therefore, in this ELISA, the increased levels of IL-6 in sera of mice treated with anti-IL-6 MAb suggest that production of IL-6 was increased by injection of the MAb. It is known that a variety of cells, including the murine Th2 subset of T cells as well as macrophages and B cells, produce IL-6. In contrast, production of IFN-γ appears to be confined to T cells and NK cells. These differences in cells of origin might be one of the reasons for the different effects of neutralizing MAb in vivo between these cytokines. May et al. (11) recently reported that neutralizing anti-IL-6 MAb "chaperones" IL-6 into and around the peripheral circulation, leading to a paradoxical sustained elevation of circulating IL-6.

Of interest, treatment with MAb against IL-6 resulted in significantly reduced numbers of *T. gondii* cysts in the brain. Previous reports have not revealed that immunotherapy results in a reduction in the numbers of *T. gondii* cysts in brains of mice with TE. In a previous study, we observed that treatment of TE in mice with recombinant IFN-γ reduced the inflammatory changes but did not reduce the numbers of *T. gondii* cysts in the brains of the mice (14). As mentioned above, TE in AIDS patients is most often due to recrudescence of a previous, latent toxoplasma infection. The effect of anti-IL-6 MAb in reducing the numbers of *T. gondii* cysts in the brains of mice with TE suggests the possibility that adjunctive treatment of TE in AIDS patients with an MAb with similar effects might decrease the risk of recrudescence of the infection. Although the mechanism whereby anti-IL-6 MAb reduces the number of tachyzoites and cysts in TE is unclear, antibodies to IL-6 along with conventional drugs may prove useful in the treatment and prevention of TE in AIDS patients.

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