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IL-6-deficient mice are resistant to the induction of experimental autoimmune encephalomyelitis provoked by myelin oligodendrocyte glycoprotein

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Abstract

The role of IL-6 in experimental autoimmune encephalomyelitis (EAE) provoked by myelin oligodendrocyte glycoprotein (MOG) was investigated using IL-6-deficient mice. We show here that IL-6-deficient mice were resistant to the MOG-induced EAE as compared to wild-type mice (one out of 18 versus 17 out of 20). The delayed-type hypersensitivity response, lymphocyte proliferation response and antibody reactivity to MOG in IL-6-deficient mice were significantly lower than those in wild-type mice. Furthermore, the histological examination revealed that no infiltration of inflammatory cells was observed in the central nervous system of IL-6-deficient mice. These results indicate that IL-6 may play a crucial role in the induction phase of EAE. Given the potential relevance of this animal model for multiple sclerosis (MS), it is possible that anti-IL-6 therapy may be useful in the prevention of relapses of MS.

IL-6 is a multifunctional cytokine that regulates immune response. IL-6 provides multiple signals such as B cell differentiation, induction of acute phase proteins, neural cell differentiation, cytotoxic T cell differentiation and T cell growth, and it is considered to contribute to proliferative and autoimmune diseases (1,2). Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) and is believed to be an autoimmune disease associated with abnormalities in immunoregulation (3). Several studies have suggested that IL-6 plays a role in MS. Augmented levels of IL-6 in cerebrospinal fluid (CSF) and in serum of MS patients have been reported (4–6). IL-6 has been detected in MS lesions (7,8), and mononuclear cells in CSF and in blood of MS patients have expressed high levels of IL-6 mRNA (9). However, the role of IL-6 in MS has not clearly been understood, particularly since IL-6 is a pleiotropic cytokine involved in the regulation of immune responses (1,2).

To clarify the uncertainty, we investigated the role of IL-6 on experimental autoimmune encephalomyelitis (EAE), an inflammatory demyelinating disease that serves as a model for MS. The up-regulation of IL-6 gene expression in the CNS of animals with EAE correlated well with the severity (10–12) and the elevation of IL-6 gene expression in peripheral blood mononuclear cells was observed at the pre-stage of EAE (13). These results suggested that IL-6 might be a pathogenetic factor of EAE. However, there have been two conflicting results with regard to anti-IL-6 therapy in EAE. Willenborg *et al.* have shown that anti-IL-6 has no significant effect on EAE, but exogenous IL-6 inhibits EAE when delivered by the recombinant vaccinia virus system (14). By contrast, Gijbels *et al.* have reported that the administration of anti-IL-6 antibody prevented actively induced and adoptively transferred EAE in mice (15). In the latter study, however, it is uncertain whether neutralization of the IL-6 activity or enhancement of the IL-6 activity by anti-IL-6 antibody caused suppression of EAE.

To further define the role of IL-6 in autoimmune demyelinating disease, such as MS, we have investigated the influence

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of IL-6 on the course of EAE using IL-6-deficient mice with the C57BL/6 background. Myelin oligodendrocyte glycoprotein (MOG) was chosen as an encephalitogen since EAE induced with MOG may closely resemble MS (16) and C57BL/6 mice (H-2^b) are highly susceptible to MOG-induced EAE (17,18).

Human recombinant MOG (rMOG) was prepared with the previously described method (19). The non-glycosylated glutathione sulfotransferase fusion protein representing the Ig extracellular domain (amino acids 1–125) of rMOG from *Escherichia coli* was solubilized with urea and then purified by CM-Sephadex 50 chromatography after being cleaved with thrombin. SDS–PAGE and Western blot analysis revealed that highly purified rMOG was obtained (19). Highly specific T cell and antibody responses to rMOG in C57BL/6 mice immunized with rMOG have also been confirmed (20). 129/Sv×C57BL/6 mice with a disrupted IL-6 gene have been previously described (21) and were bred under specific pathogen-free conditions. These mice were backcrossed onto the C57BL/6 background for seven generations. The presence of the defective IL-6 allele in mice was determined by PCR on genomic DNA as previously described (21). Female IL-6 knockout [IL-6(–/–)] mice, wild-type [IL-6(+/+)] mice and heterozygous [IL-6(+/–)] mice of H-2^b haplotype (C57BL/6) were used between the ages of 8 and 16 weeks for EAE experiments.

The course of the MOG-induced disease in IL-6(–/–), IL-6(+/–) and IL-6(+/+) mice is shown in Fig. 1(A and B) and summarized in Table 1. While IL-6(–/–) mice immunized with rMOG did not lose body weight, IL-6(+/–) and IL-6(+/+) mice showed weight loss after immunization (Fig. 1A). While only one out of 18 IL-6(–/–) mice developed mild EAE, IL-6(+/–) and IL-6(+/+) mice developed severe EAE at high frequency (Table 1 and Fig. 1B). No significant difference was observed between IL-6(+/–) mice and IL-6(+/+) mice. This result indicates that IL-6 is an important component in the pathogenesis of EAE. In an attempt to elucidate the mechanism for resistance of IL-6-deficient mice to EAE, we examined the immune response to MOG in the periphery and the histopathology in the CNS.

Delayed-type hypersensitivity (DTH) responses to rMOG were measured 15 days after the primary immunization with rMOG and CFA in IL-6(+/+) and IL-6(–/–) mice. As illustrated in Fig. 2, both IL-6(+/+) and IL-6(–/–) mice displayed a significant increase in footpad swelling in response to MOG; however, IL-6(–/–) mice had a significantly lower DTH response as compared to IL-6(+/+) mice. In addition, lymphocyte proliferation in response to rMOG was tested. As shown in Fig. 3, lymph node (LN) cells from IL-6(+/+) and IL-6(–/–) mice proliferated in response to rMOG, but a significantly lower response to rMOG was observed in IL-6(–/–) mice as compared to IL-6(+/+) mice. These results indicate that T cell responses to MOG are lower in IL-6-deficient mice as compared to wild-type mice. Impairment in generation and/or activation of encephalitogenic T cells could well explain the resistance to induction of EAE in IL-6-deficient mice, since EAE is mediated by $CD4^+$ T cells of the T_h1 phenotype in response to CNS myelin proteins (18,22),

To further elucidate the mechanisms for impairment of T cell responses to MOG in IL-6-deficient mice, phenotypic analysis of LN cells in IL-6(+/+) and IL-6(–/–) mice immunized with or without rMOG were performed. As shown in Table 2, a significantly lower population of T cells in IL-6(–/–) mice was observed, although the ratio of CD4/CD8 was not significantly

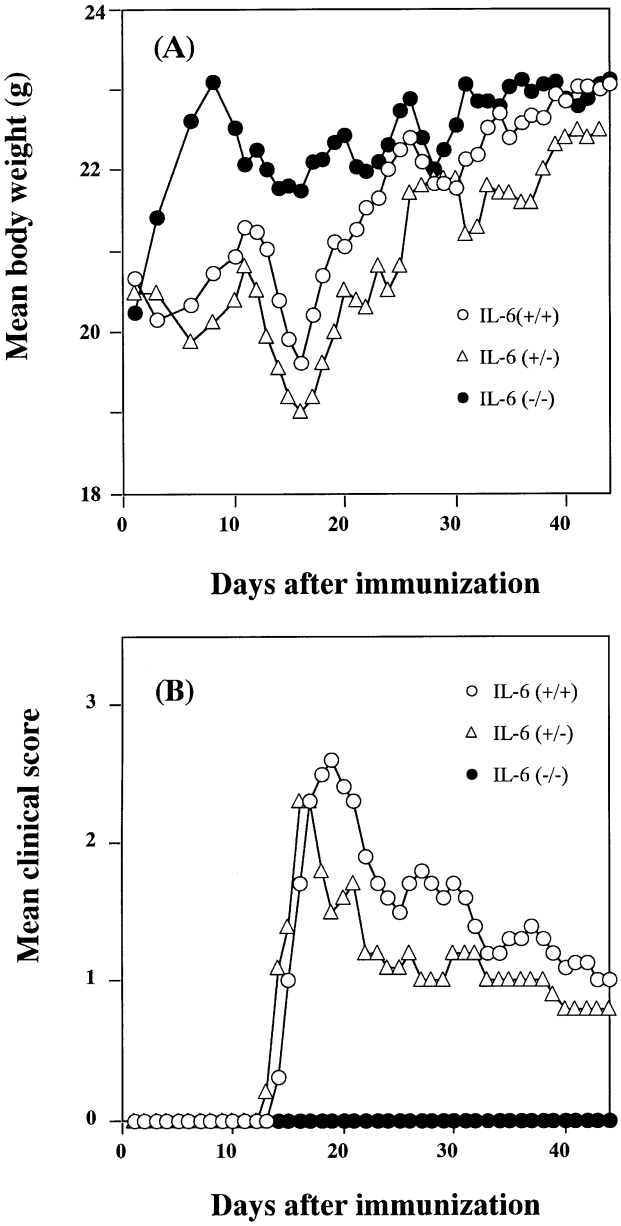

Fig. 1. Clinical course of MOG-induced EAE in IL-6(+/+) mice (open circle), IL-6(+/–) mice (open triangle) and IL-6(–/–) mice (closed circle). Mice were immunized s.c. in the femoral region on both sides with 100 μg of rMOG emulsified in complete Freund's adjuvant (CFA) containing 400 μg of H37Ra *Mycobacterium tuberculosis*, and 400 ng of *Bordetella pertussis* toxin was given i.p. at the time of immunization and 2 days later. Mice were weighed and examined for clinical score daily for 60 days after immunization. Clinical score was graded as follows: 0, no clinical sign; l, limp tail; 2, limp tail and impaired righting reflex; 3, apparent hind limb paresis; 4, complete hind limb paralysis; 5, moribund or death. Results are expressed as the mean body weight (A) and the mean clinical score (B). The data were collected from one of four independent experiments (n = 5 for each group).

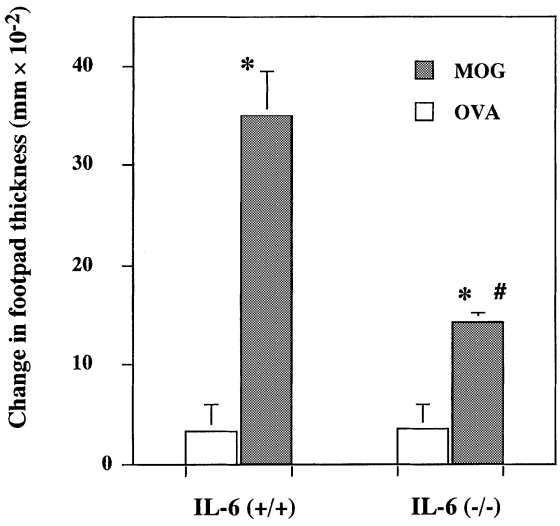

Fig. 2. DTH reactions to rMOG in IL-6(+/+) and IL-6(–/–) mice. Twenty-five micrograms of rMOG or ovalbumin (OVA) in PBS were injected into each footpad 15 days after primary immunization with rMOG (100 μg/mouse) and CFA. Footpad swelling was measured at 0 and 24 h after the secondary antigenic challenge by using micrometer callipers. The difference in the footpad thickness before and after challenge for individual mice was recorded. Histograms represent the mean increase in footpad thickness ($mm \times 10^{-2}$) for each group of mice (n = 4). Significant DTH responses were observed in both IL-6(+/+) and IL-6(–/–) mice ($^{*}P<0.05$ with Student's t-test as compared to OVA-treated controls), while a significantly lower DTH response was observed in IL-6(–/–) mice as compared to IL-6(+/+) mice ($^{\#}P< 0.05$ with Student's t-test).

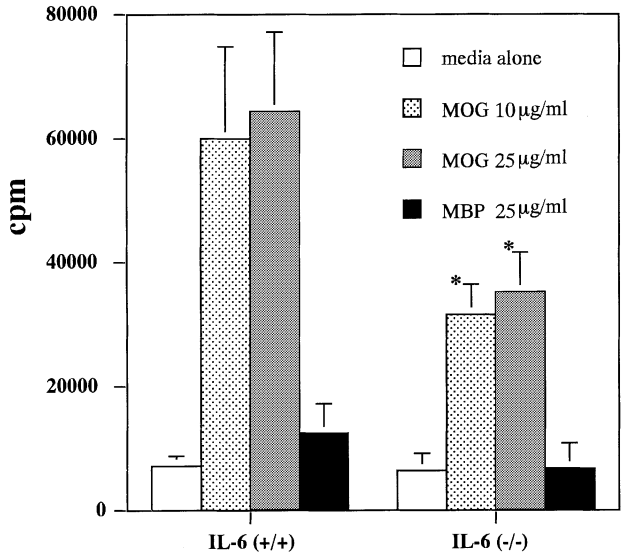

Fig. 3. Lymphocyte proliferation in response to rMOG in IL-6(+/+) and IL-6(–/–) mice. At 10–12 days after immunization, mice were killed and a single-cell suspension was prepared from the inguinal lymph nodes (LN). After two washes, the LN cells were cultured with or without 10 μg/ml or 25 μg/ml of rMOG or 25 μg/ml of myelin basic protein (MBP) (Sigma, St Louis, MO) at a concentration of 2×10^6/ml in RPMI 1640 medium supplemented with 5% FCS serum, HEPES (20 mM), 2-mercaptoethanol (3×10^5 M), penicillin (100 U/ml) and streptomycin (100 μg/ml). Cultures were incubated for 72 h in 96-well, round-bottomed microtiter plates (0.2 ml/well) at 37°C in humidified 5% CO_2. Wells were pulsed for the final 18 h of culture with 2 μCi [^{3}H]thymidine. [^{3}H]Thymidine uptake was measured by a Betaplate Counter (Wallac, Gaithersburg, MD). Results are expressed as the mean c.p.m. ± SD based on three independent experiments. A significantly lower response to rMOG was observed in IL-6(–/–) mice as compared with IL-6(+/+) mice ($^{*}P<0.05$ with Student's t-test).

Table 1. MOG induced EAE in IL-6(+/+), IL-6(+/–) and IL-6(–/–) mice[a]

	No. sick/ total[b]	Maximal clinical score (mean ± SD)[c]	Day of onset (mean ± SD)
IL-6(+/+)	17/20	3.0 ± 1.0	15.1 ± 3.1
IL-6(+/–)	14/20	2.9 ± 0.9	15.6 ± 3.9
IL-6(–/–)	1/18 [d]	1	28

[a]The method for EAE induction was described in Fig. 1.
[b]Number of mice with clinical signs per total number of mice from four experiments.
[c]Data were based on mice with clinical disease.
[d]$P<0.001$ versus IL-6(+/+) mice with Fisher's exact probability test

different between IL-6(+/+) and IL-6(–/–) mice. These results are in agreement with the previous report showing that T cells expressed TCR, CD4 and CD8, and B cells expressed B220, IgM and IgD within normal ranges, and that the number of peripheral T cells was reduced by 20–40% in IL-6-deficient mice as compared to wild-type mice (21). These findings suggest that a lower population of T cells in IL-6-deficient mice may be associated with impairment of T cell responses *in vivo* and contribute to the resistance to EAE.

Figure 4 shows that, while the levels of anti-MOG IgG antibody in serum from IL-6(+/+) and IL-6(–/–) mice were elevated at day 18 and 60 after immunization, significantly lower anti-MOG IgG levels were observed in IL-6(–/–) mice as compared with IL-6(+/+) mice. A recent study using B cell-deficient mice has demonstrated that B cells were not necessary for induction of EAE provoked by MBP peptide (23). Given the fact that MOG-specific mAb enhanced EAE (24,25) and that high levels of anti-MOG antibodies appeared to be necessary for development of MOG-induced EAE (26), however, these results suggested that antibodies against myelin antigens might not be necessary for induction of EAE but might have a harmful influence in the course of the disease. Thus, low anti-MOG IgG antibody levels may partially account for the resistance of IL-6-deficient mice in developing neurological deficit.

The histological examination of the spinal cord from IL-6(+/+) mice with EAE revealed that the histological score based on inflammation correlated well with the clinical score (Table 2). The presence of myelin breakdown as determined by pale klüver–Barrera (KB) staining was confined to the area where mononuclear cells infiltrated. No inflammation nor myelin breakdown was observed in IL-6(–/–) mice immunized with rMOG. This result may be explained by the lower immune response to MOG in the periphery of IL-6(–/–) mice and may suggest that IL-6 participates in the breakdown of the blood–brain barrier in EAE as previously pointed out by Lassmann (27).

The results in the present study suggesting that the immune

Table 2. Phenotype of LN cells from IL-6(+/+) and IL-6(–/–) mice immunized with or without rMOG[a]

	CD3 (%)[b]	CD4 (%)	CD8 (%)	B220 (%)
LN from non-treated mice				
IL-6(+/+)	66.3±2.1	37.3±2.1	27.3±1.2	28.0±2.7
IL-6(–/–)	61.0±1.7[c]	32.0±1.7[c]	30.0±1.4	33.5±2.7[c]
LN from MOG-immunized mice				
IL-6(+/+)	43.0±6.6	25.7±5.5	17.3±2.9	47.7±4.7
IL-6(–/–)	34.7±3.8 [c]	19.3±1.5 [c]	14.3±1.5 [c]	58.3±3.8[c]

[a]Freshly isolated inguinal LN cells (5×10^5 cells) from IL-6(+/+) and IL-6(–/–) mice 15 days after immunization with rMOG or from non-treated IL-6(+/+) and IL-6(–/–) mice were used to determine the phenotypes of these cells. After two washes, LN cells were incubated with rat anti-mouse mAb specific for FITC-conjugated CD3 (Serotec, Oxford, UK), phycoerythrin (PE)-conjugated CD4 (PharMingen, San Diego, CA), PE-conjugated CD8 (PharMingen) or FITC-conjugated B220 (Caltag, La Jolla, CA) for 45 min on ice and analyzed on FACScan flow cytometer with CellQuest software (Becton Dickinson, Mountain View CA). Data for $>1\times10^4$ cells were collected.

[b]The data are expressed as percentage (mean ± SD from three independent experiments) of total lymphocytes, characterized by their scatter properties.

[c]$P<0.05$ versus IL-6(+/+) mice with Student's *t*-test.

Table 3. Analysis of spinal cords of IL-6(+/+) and IL-6(–/–) mice immunized with rMOG[a]

	Mean clinical score[b]	No. of histological abnormalities[c]	Histological score (mean ± SD)[d]
IL-6(+/+)			
day 18	2.0	3/3	2.0 ± 1.3
day 35	1.3	3/3	1.7 ± 0.7
day 60	1.0	2/3	0.9 ± 0.7
IL-6(–/–)			
day 18	0	0/3	0
day 35	0	0/3	0
day 60	0	0/3	0

[a]Spinal cords were obtained from three IL-6(+/+) mice and three IL-6(–/–) mice at day 18, 35 and 60 respectively. Each spinal cord was removed, fixed in 4% paraformaldehyde in 0.1 M phosphate buffer and then embedded in paraffin. Sections (5 μm) were prepared and stained with H & E and KB for assessment of inflammation and demyelination respectively. The sections at cervical, thoracic and lumbar levels of the spinal cord from each mouse were examined.

[b]Mean clinical score of mice at each day used for histological examination.

[c]Number of mice with histological abnormalities (inflammation and/or demyelination) per total number of mice ($n = 3$ at each day).

[d]Semiquantitative histological evaluation based on the severity of inflammation was performed by two observers in a blind fashion using H & E-stained sections with the following score: 0, no inflammation; 1, cellular infiltrate only in the perivascular areas and meninges; 2, mild cellular infiltrate in parenchyma (1–20/section); 3, moderate cellular infiltrate in parenchyma (21–100/section); 4, severe cellular infiltrate in parenchyma (>100/section). Data were based on nine sections, three different levels of spinal cords from three mice at each day.

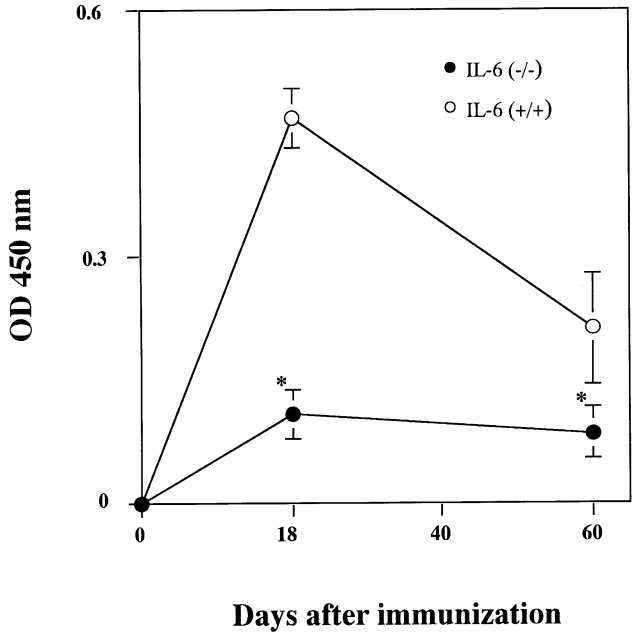

Fig. 4. Anti-rMOG antibody responses in IL-6(+/+) and IL-6(–/–) mice. Serum samples were prepared from peripheral blood of rMOG injected or control IL-6(+/+) and IL-6(–/–) mice. Blood was collected by cardiac puncture from two non-treated IL-6(+/+) mice and two non-treated IL-6(–/–) mice at the age of 8 and 12 weeks, from three IL-6(+/+) mice and three IL-6(–/–) mice at day 18, and from three IL-6(+/+) mice and three IL-6(–/–) mice 60 days after immunization. Reactivity to rMOG was determined by ELISA as previously reported (16). Briefly, rMOG was diluted to a concentration of 1 μg/ml in 0.1 M carbonate buffer, pH 9.6, and coated to 96-well microtiter plates that had been preincubated with 0.2% glutaraldehyde. After coating overnight at 4°C, the plates were blocked with 3% BSA/PBS and were incubated with 1:10,000 dilution of mouse serum for 1 h at 37°C and the specific antibody binding was determined using an anti-mouse IgG biotin conjugate (Nitirei, Tokyo, Japan) and streptavidin–horseradish peroxidase. Tetramethylbenzidine was used as a substrate and the OD was measured at 450 nm. The results were expressed as OD values. Significantly lower anti-rMOG IgG levels in IL-6(–/–) mice were observed as compared with IL-6(+/+) mice at day 18 and 60 ($^*P<0.05$ with Student's *t*-test).

response in IL-6-deficient mice might be generally suppressed are consistent with the previous reports concerning the function of IL-6 (1,2,21). However, as for DTH response, there have been conflicting results. Jayaraman *et al.* showed that IL-6 enhanced DTH (28), while Mihara *et al.* reported that IL-6 inhibited it (29). The mechanism for the inhibitory effect of IL-6 on DTH remains unclear but it may be mediated by indirect ways such as the induction of glucocorticoid or the inhibition of IL-1 and/or tumor necrosis factor (TNF)-α production (30,31) as Mihara *et al.* suggested (29). Based

on the results of the present study, we believe that the relative reduction in the number of T cells and the impaired T cell proliferation and/or activation, which are possibly caused by the loss of IL-6, may explain the lower DTH response to MOG in IL-6-deficient mice. Therefore, it is likely that IL-6 itself plays an important role in DTH response.

Considering the report that the T_h1 response was impaired in IL-6-deficient mice (32) and the reduced immune response to MOG in IL-6-deficient mice as we observed in this study, either a shift from T_h1 to T_h2 and/or a diminished levels of their corresponding cytokines might well be responsible for the resistance of IL-6-deficient mice to EAE, since EAE is believed to be mediated by T_h1 cytokines (22). Taken together, we believe that IL-6 plays an important role in the induction phase of EAE, perhaps by enhancing the myelin antigen-specific T and B cell responses and/or by modulating production of cytokines in the periphery.

It is also possible that IL-6 may have an effect on the effector phase of EAE. The up-regulation of IL-6 has been detected within the CNS of animals with EAE and correlated with the severity and/or the course of EAE (10–12). In addition, overexpression of IL-6 in the CNS of mice has been reported to cause neurological symptoms (33), implying that IL-6 participated in CNS inflammation. However, since no inflammatory response was observed in the CNS of IL-6-deficient mice, we could not elucidate the role of IL-6 in the effector phase of EAE. To clarify the role of IL-6 in the induction and the effector phase of EAE, experiments on passively transferred EAE using IL-6(–/–) mice are necessary and currently being attempted.

Although inflammatory cytokines such as IL-1, IL-2, IL-6, IFN-γ, TNF-α and TNF-β have been considered to participate in the pathogenesis of EAE, recent studies using cytokine knockout mice have demonstrated that some of them are not essential for the induction of EAE. IFN-γ-deficient mice and IFN-γ receptor-deficient mice have been shown to be susceptible to MBP- and MOG-induced EAE respectively (34,35). TNF-α-deficient mice have also been shown to be susceptible to MOG-induced EAE (20,36). TNF-β-deficient mice immunized with MOG have developed EAE (67%), but had significantly milder EAE (37). On the contrary, TNF-α and TNF-β double knockout mice immunized with the mouse spinal cord homogenate or with proteolipid protein developed a very severe form of EAE (38). Although there are some differences among those reports, which may be due to differences in materials such as encephalitogenic antigens and mouse strains, those results indicate that other cytokines can compensate for those three proinflammatory cytokines in induction of EAE. To the best of our knowledge, IL-6 is the only cytokine so far reported that may have a crucial role in induction of EAE.

In conclusion, the frequency of EAE was significantly low and the infiltration of inflammatory cells in the CNS was significantly reduced in IL-6 knockout mice immunized with MOG. Based on our findings, we believe that IL-6 may be an important factor for development of MS and anti-IL-6 therapy may be promising in MS, especially in the prevention of relapse.

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Abbreviations

CFA	complete Freund's adjuvant
CNS	central nervous system
CSF	cerebrospinal fluid
DTH	delayed-type hypersensitivity
EAE	experimental autoimmune encephalomyelitis
KB	klüver–Barrera
LN	lymph node
MBP	myelin basic protein
MOG	myelin oligodendrocyte glycoprotein
MS	multiple sclerosis
OVA	ovalbumin
rMOG	recombinant MOG
TNF	tumor necrosis factor

References

1 Kishimoto, T. 1989. The biology of interleukin-6. *Blood* 74:1.
2 Hirano, T., Akira, S., Taga, T. and Kishimoto, T. 1990. Biological and clinical aspect of interleukin 6. *Immunol. Today* 11:443.
3 Bansil, S., Cook, S. D. and Rohowsky-Kochan, C. 1995. Multiple sclerosis: immune mechanism and update on current therapies. *Ann. Neurol.* 37:87.
4 Nishimoto, N., Yoshizaki, K., Eiraku, N., Machigashira, K., Tagoh, H., Ogata, A., Kuritani, T., Osame, M. and Kishimoto, T. 1990. Elevated levels of interleukin-6 in serum and cerebrospinal fluid of HTLV-1-associated myelopathy/tropical spastic paraparesis. *J. Neurol. Sci.* 97:183.
5 Maimone, D., Gregory, S., Arnason, B. G. W. and Reder, A. T. 1991. Cytokine levels in the cerebrospinal fluid and serum of patients with multiple sclerosis. *J. Neuroimmunol.* 32:67.
6 Frei, K., Fredrikson, S., Fontana, A. and Link, H. 1991. Interleukin-6 is elevated in plasma in multiple sclerosis. *J. Neuroimmunol.* 31:147.
7 Woodroofe, M. N. and Cuzner, M. L. 1993. Cytokine mRNA expression in inflammatory multiple sclerosis lesions: detection by non-radioactive *in situ* hybridization. *Cytokine* 5:583.
8 Maimone, D., Guazzi, G. C. and Annunziata, P. 1997. IL-6 detection in multiple sclerosis brain. *J. Neurol. Sci.* 146:59.
9 Navikas, V., Matusevicius, D., Söderström, M., Fredrikson, S., Kivisäkk, P., Ljungdahl, Å., Höjeberg, B. and Link, H. 1996. Increased interleukin-6 mRNA expression in blood and cerebrospinal fluid mononuclear cells in multiple sclerosis. *J. Neuroimmunol.* 64:63.
10 Kennedy, M. K., Torrance, D. S., Picha, K. S. and Mohler, K. M. 1992. Analysis of cytokine mRNA expression in the central nervous system of mice with experimental autoimmune encephalomyelitis reveals that IL-10 mRNA expression correlates with recovery. *J. Immunol.* 149:2496.
11 Okuda, Y., Nakatsuji, Y., Fujimura, H., Esumi, H., Ogura, T., Yanagihara, T. and Sakoda, S. 1995. Expression of the inducible isoform of nitric oxide synthase in the central nervous system of mice correlates with the severity of actively induced experimental allergic encephalomyelitis. *J. Neuroimmunol.* 62:103.
12 Diab, A., Zhu, J., Xiao, B. G., Mustafa, M. and Link, H. 1997. High IL-6 and low IL-10 in the central nervous system are associated with protracted relapsing EAE in DA rats. *J. Neuropathol. Exp. Neurol.* 56:641.
13 Okuda, Y., Sakoda, S., Fujimura, H. and Yanagihara, T. 1996. Pentoxifylline delays the onset of experimental allergic encephalomyelitis in mice by modulating cytokine production in peripheral blood mononuclear cells. *Immunopharmacology* 35:141.
14 Willenborg, D. O., Fordham, S. A., Cowden, W. B. and Ramshaw, I. A. 1995. Cytokines and murine autoimmune encephalomyelitis:

Inhibition or enhancement of disease with antibodies to select cytokines, or by delivery of exogenous cytokines using a recombinant vaccinia virus system. *Scand. J. Immunol.* 41:31.
15 Gijbels, K., Brocke, S., Abrams, J. S. and Steinman, L. 1995. Administration of neutralizing antibodies to interleukin-6(IL-6) reduces experimental autoimmune encephalomyelitis and is associated with elevated levels of IL-6 bioactivity in central nervous system and circulation. *Mol. Med.* 1:795.
16 Johns, T. G., Kerlero de Rosbo, N., Menson, K. K., Abo, S., Gonzales, M. F. and Bernard, C. C. A. 1995. Myelin oligodendrocyte glycoprotein induces a demyelinating encephalomyelitis resembling multiple sclerosis. *J. Immunol.* 154:5536.
17 Mendel. I., Kerlero de Rosbo, N. and Ben-Nun, A. 1995. A myelin oligodendrocyte glycoprotein peptide induces typical chronic experimental autoimmune encephalomyelitis in H-2^b mice: fine specificity and T cell receptor V_β expression of encephalitogenic T cells. *Eur. J. Immunol.* 25:1951.
18 Bernard, C. C. A., Johns, T. G., Slavin, A., Ichikawa, M., Ewing, C., Liu, J. and Bettadapura, J. 1997. Myelin oligodendrocyte glycoprotein: a novel candidate autoantigen in multiple sclerosis. *J. Mol. Med.* 75:77.
19 Bettadapura, J., Menon, K. K., Moritz, S., Liu, J. and Bernard, C. C. A. 1998. Expression, purification and encephalitogenicity of recombinant human myelin oligodendrocyte glycoprotein. *J. Neurochem.*, in press.
20 Liu, J., Marino, M. W., Wong, G., Grail, D., Dunn, A., Bettadapura, J., Slavin, A. J., Old, L. and Bernard, C. C. A. 1998. TNF is a potent anti-inflammatory cytokine in autoimmune-mediated demyelination. *Nat. Med.* 4:78.
21 Kopf, M., Baumann, H., Freer, G., Freudenberg, M., Lamers, M., Kishimoto, T., Zinkernagel, R., Bluethmann, H. and Köhler, G. 1994. Impaired immune and acute-phase responses in interleukin-6-deficient mice. *Nature* 368:339.
22 Tsukada, I. and Fujinami, R. S. 1996. Two models for multiple sclerosis: experimental allergic encephalomyelitis and Theiler's murine encephalomyelitis virus. *J. Neuropathol. Exp. Neurol.* 55:673.
23 Wolf, S. D., Dittel, B. N., Hardardottir, F. and Janeway, C. A., Jr. 1996. Experimental autoimmune encephalomyelitis induction in genetically B cell-deficient mice. *J. Exp. Med.* 184:2271.
24 Schluesener, H. J., Sobel, R. A., Linington, C. and Weiner, H. L. 1987. A monoclonal antibody against a myelin oligodendrocyte glycoprotein induces relapses and demyelination in central nervous system autoimmune disease. *J. Immunol.* 139:4016.
25 Linington, C., Berger, T., Perry, L., Weerth, S., Hinze-Selch, D., Zhang, Y., Lu, H., Lassmann, H. and Wekerle, H. 1993. T cells specific for the myelin oligodendrocyte glycoprotein mediate an unusual autoimmune inflammatory response in the central nervous system. *Eur. J. Immunol.* 23:1364.
26 Ichikawa, M., Johns, T. G., Liu, J. and Bernard, C. C. A. 1996. Analysis of the fine B cell specificity during the chronic/relapsing course of a multiple sclerosis-like disease in Lewis rats injected with the encephalitogenic myelin oligodendrocyte glycoprotein peptide 35–55. *J. Immunol.* 157:919.
27 Lassmann, H., Zimprich, F., Rössler, K. and Vass, K. 1991. Inflammation in the nervous system. Basic mechanisms and immunological concepts. *Rev. Neurol.* 147:763.
28 Jayaraman, S., Martin, C. A. and Dorf, M. E. 1990. Enhancement of *in vivo* cell-mediated immune responses by three distinct cytokines. *J. Immunol.* 144:942.
29 Mihara, M., Ikuta, M., Koishihara, Y. and Ohsugi, Y. 1991. Interleukin 6 inhibits delayed-type hypersensitivity and the development of adjuvant arthritis. *Eur. J. Immunol.* 21:2327.
30 Aderka, D., Le, J. and Vilcek, J. 1989. IL-6 inhibits lipopolysaccharide-induced tumor necrosis factor production in cultured human monocytes, U937 cells, and in mice. *J. Immunol.* 143:3517.
31 Schindler, R., Mancilla, J., Endres, S., Ghorbani, R., Clark, S. C. and Dinarello, C. A. 1990. Correlations and interactions in the production of interleukin-6(IL-6), IL-1, and tumor necrosis factor (TNF) in human blood mononuclear cells: IL-6 suppresses IL-1 and TNF. *Blood* 75:40.
32 Romani, L., Mencacci, A., Cenci, E., Spaccapelo, R., Toniatti, C., Puccetti, P., Bistoni, F. and Poli, V. 1996. Impaired neutrophil response and $CD4^+$ T helper cell 1 development in interleukin 6-deficient mice infected with *Candida albicans*. *J. Exp. Med.* 183:1345.
33 Campbell, I. L., Abraham, C. R., Masliah, E., Kemper, P., Inglis, J. D., Oldstone, M. B. A. and Mucke, L. 1993. Neurologic disease induced in transgenic mice by cerebral overexpression of interleukin 6. *Proc. Natl Acad. Sci. USA* 90:10061.
34 Ferber, I. A., Brocke, S., Edwards, C. T., Ridgway, W., Dinisco, C., Steinman, L., Dalton, D. and Fathman, C. G. 1996. Mice with a disrupted IFN-γ gene are susceptible to the induction of experimental autoimmune encephalomyelitis (EAE). *J. Immunol.* 156:5.
35 Willenborg, D. O., Fordham, S., Bernard, C. C. A., Cowden, W. B. and Ramshaw, I. A. 1996. IFN-γ plays a critical down-regulatory role in the induction and effector phase of myelin oligodendrocyte glycoprotein-induced autoimmune encephalomyelitis. *J. Immunol.* 157:3223.
36 Körner, H., Riminton, D. S., Strickland, D. H., Lemckert, F. A., Pollard, J. D. and Sedgwick, J. D. 1997. Critical points of tumor necrosis factor action in central nervous system autoimmune inflammation defined by gene targeting. *J. Exp. Med.* 186:1585.
37 Suen, W. E., Bergman, C. M., Hjelmström, P. and Ruddle, N. H. 1997. A critical role for lymphotoxin in experimental allergic encephalomyelitis. *J. Exp. Med.* 186:1233.
38 Frei, K., Eugster, H., Bopst, M., Constantinescu, C. S., Lavi, E. and Fontana, A. 1997. Tumor necrosis factor α and lymphotoxin α are not required for induction of acute experimental autoimmune encephalomyelitis. *J. Exp. Med.* 185:2177.