

Astrocytic Alterations in Interleukin-6/Soluble Interleukin-6 Receptor α Double-Transgenic Mice

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Interleukin-6 (IL-6), a major cytokine with diverse effects on cells mainly of the immune and hematopoietic systems, has been linked to several neurological disorders such as acquired immune deficiency syndrome dementia, multiple sclerosis, and Alzheimer's disease. Central nervous system (CNS)-specific expression of IL-6 caused neurodegeneration, massive gliosis, and vascular proliferation in transgenic mice. However, the effects of systemically circulating IL-6 and its receptor IL-6R α on the CNS are unknown. IL-6R α is the specific component of the IL-6 receptor system and hence an important co-factor of IL-6. IL-6R α is bioactive in a membrane-bound and in a soluble (s) form. We investigated the effects of systemically elevated levels of either human IL-6 or human sIL-6R α or both on the CNS of transgenic mice. Although IL-6 and sIL-6R α single transgenic mice were free of neurological disease, IL-6/sIL-6R α double-transgenic mice showed neurological signs, such as tremor, gait abnormalities, and paresis. However, these mice also frequently showed prominent general weakness probably because of the systemic effects of IL-6/IL-6R α such as liver damage and plasmacytomas. IL-6/sIL-6R α transgenic mice exhibited massive reactive gliosis. Lack of signs of neuronal breakdown versus ample astrogliosis suggested that astrocytes were selectively affected in these mice. There was neither vascular proliferation nor inflammatory infiltration. Ultrastructural analysis revealed blood-brain barrier (BBB) changes manifested by hydropic astrocytic end-feet. However, albumin immunohistochemistry did not reveal major BBB leakage. Our results indicate that increased and constitutive systemic expression of IL-6 together with its soluble receptor sIL-6R α is less harmful to the brain than to other organs. The BBB remains primarily intact. IL-6/IL-6R α , however, might be di-

rectly responsible for the selective activation of astrocytes. (Am J Pathol 2000, 157:1485-1493)

Interleukin-6 (IL-6) was initially described as a central mediator of the immune system, hemopoiesis, and acute phase reaction.¹ IL-6 is a member of the neurocytokine family, which also includes ciliary neurotrophic factor, leukemia inhibitory factor, oncostatin M, and cardiotrophin-1. These molecules have overlapping biological activities; they possess a similar secondary structure and exert their activities through related multisubunit receptors.² The binding of IL-6 to its receptor IL-6R α triggers the association of the IL-6R α subunit with a second membrane glycoprotein, gp¹³⁰, which transduces the IL-6 signal.^{2,3} Neurons and glial cells can produce IL-6.⁴⁻⁸ IL-6 has pleiotropic effects within the nervous system^{9,10} including neurotrophic^{7,8} and neuronal differentiation promoting activities.¹¹ In addition, IL-6 promotes peripheral nerve fiber outgrowth *in vivo*.¹²

Elevated IL-6 blood levels are found in diseases such as plasmacytoma/myeloma, osteoporosis, autoimmune diseases, and acquired immune deficiency syndrome (AIDS).¹ IL-6 has also been implicated as an important mediator of pathophysiological processes in the central nervous system (CNS), such as demyelination,^{13,14} neurodegeneration,¹⁵ and neoplastic transformation of glial cells.¹⁶⁻¹⁸ In two human disorders involving the nervous system, Castleman's disease and polyneuropathy, organomegaly, endocrinopathy, M protein, skin changes (POEMS) syndrome, highly elevated systemic IL-6 levels have been detected, suggesting that systemically increased IL-6 might be harmful to the nervous system.¹⁹⁻²³ Mice overexpressing IL-6 in bone marrow cells develop a syndrome resembling Castleman's disease.²⁴ In both conditions, polyneuropathy can be a prominent symptom; additionally, chordoid meningeal tumors with prominent lymphocytic infiltration have been reported in Castleman's disease.^{19,22,25}

Transgenic mouse models have advanced the understanding of the CNS pathobiology of IL-6 *in vivo*.²⁶ These

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studies highlighted the central role of astrocytes. For example, expression of murine IL-6 from the prenatally active astrocyte-specific glial fibrillar acidic protein (GFAP) promoter²⁷ induced a strong and lifelong reactive gliosis as well as microglia activation, vascular proliferation, and neuronal damage.^{27,28} The central role of IL-6 in regulating astrocyte responses is emphasized by the reciprocal mouse model deficient in IL-6 showing impairment of neuroglial activation after injury.²⁹ In addition, a ciliary neurotrophic factor transgene under the control of GFAP promoter sequences also induced prominent reactive gliosis in mice.³⁰ Wild-type mice that had been injected with ciliary neurotrophic factor into uninjured brain showed the same glial response.³⁰ When IL-6 was targeted to neurons by the rat neuron-specific enolase promoter, neuron-specific enolase-IL-6 transgenic mice also developed reactive astrocytosis and an increase in ramified microglial cells, but no apparent neuronal damage.³¹ In GFAP-IL-6 mice, the blood-brain barrier (BBB) never developed completely.³²

While the role of IL-6 expressed in astrocytes and neurons has been extensively investigated, the effects of systemically circulating IL-6 and sIL-6R α in the CNS have not been addressed so far. Because IL-6R α is bioactive in a membrane-bound and in a soluble form, sIL-6R α is an important cofactor of IL-6. In fact, the IL-6/sIL-6R α complex rather than IL-6 is believed to be the active form *in vivo*.³³ Interestingly, mice transgenic for IL-6 and a membrane-anchored IL-6R α developed hypertrophy of ventricular myocardium.³⁴ Involvement of the CNS has not been described in this model, even though mice overexpressing IL-6 locally in the brain display marked CNS alterations and at least two human conditions associated with high IL-6 levels, Castleman's disease and polyneuropathy, organomegaly, endocrinopathy, M protein, skin changes syndrome, show nervous system involvement (see above). Therefore, we investigated the effects of high levels of circulating IL-6 and its soluble α receptor on the CNS in a transgenic mouse model, focusing on astroglial and BBB alterations.

Materials and Methods

Generation of IL-6/IL-6R α Double-Transgenic Mice

Heterozygous double-transgenic mice were obtained by cross-breeding metallothionein-I/IL-6 mice³⁵ with phosphoenolpyruvate carboxy kinase/hslIL-6R α mice.³⁶ These double-transgenic mice expressed the transgenic proteins systemically under the control of the metallothionein-I promoter³⁷ and the neonatal active phosphoenolpyruvate carboxy kinase promoter,³⁸ respectively.

Detection of IL-6 and IL-6R α Gene Products

For transgenic RNA analysis, total RNA from different organs was isolated by the guanidine isothiocyanate method.³⁹ Expression of IL-6 and sIL-6R α mRNAs was analyzed previously by Northern blots probed with either

a ³²P-labeled cDNA of IL-6³⁵ or with a *Pst*I-*Xho*I fragment of the IL-6R α cDNA.³⁶ Transgenic protein expression was investigated by enzyme-linked immunosorbent assay and immunoprecipitation as reported earlier.³⁶ Biological activity of IL-6 was determined by either using the 7TD1 proliferation assay⁴⁰ or the IL-6-dependent cell line B9.⁴¹

Histology and Immunohistochemistry

Most major organs (brain, spinal cord, heart, lung, thymus, liver, kidney, spleen, ovary/testis, intestine, eye, skeletal muscle) were fixed in formaldehyde and embedded in paraffin. Hematoxylin and eosin staining was performed on 4- μ m sections. For GFAP immunohistochemistry of brain sections, the indirect peroxidase-staining method was applied using a rabbit anti-cow antiserum (DAKO, Glostrup, Denmark) and a peroxidase-conjugated goat anti-rabbit IgG (Dianova, Hamburg, Germany) secondary antibody. For the detection of BBB leakage, albumin immunohistochemistry was performed by use of a rabbit anti-human antiserum (DAKO) and a biotinylated swine anti-rabbit secondary antibody. The reaction was visualized using an avidin biotin alkaline phosphatase system and the chromogen new-fuchsin (DAKO).

The degree of gliosis was scored as follows: 0, no GFAP immunoreactive astrocytes; +, less than three GFAP immunoreactive astrocytes/high-power field (with $\times 40$ lens); ++, less than 10 GFAP immunoreactive astrocytes/high-power field; +++, more than 10 GFAP immunoreactive astrocytes/high-power field. Liver infiltration by plasmacytoma cells and hematopoietic cells was scored as follows: 0, no infiltration; +, minor increase in mononuclear cell content; ++, moderate mononuclear cell infiltration and moderate widening of few portal canals, minor liver cell necrosis; +++, massive infiltration of numerous portal canals with major widening as well as lobular infiltration and major liver cell necrosis. The infiltration of lungs and kidneys by plasmacytoma cells was scored as follows: 0, no infiltration; +, one to three small infiltrates on cross sections of the whole organs; ++, one large or more than three small infiltrates; +++, large, confluent infiltrates. The scoring was performed by two independent observers (AGB, JW) blinded to the identity (wild type, single, or double transgenic) of the mice.

Ultrastructural Examination

For electron microscopy, brain samples of wild-type, IL-6, sIL-6R α , and IL-6/sIL-6R α transgenic mice were fixed in 3.9% phosphate-buffered glutaraldehyde and embedded in epoxy resin. Semithin sections were stained with *p*-phenyldiamine and toluidine blue. Ultrathin sections were contrast enhanced with lead citrate and uranyl acetate, and examined using a Philips EM 300 as described elsewhere.⁴²

Table 1. Histological Findings in IL-6 and IL-6/sIL-6R α Animals

Animal no.	Age (mo)	Liver	Lung	Kidney	Gliosis
IL-6-transgenics					
JR4	5.5	+	+	+	+
JR5	7	+	+	++	++
JR12	4	-	++	++	-
JR13	2.5	-	+	-	-
JR17	3	-	-	-	-
JR18	3	-	-	-	-
JR23	11	-	++	++	-
JR24	11	-	-	+	-
JR25	11	+	++	++	-
JR28	3	-	-	-	-
IL-6/sIL-6R α transgenics					
JR2	5.5	-	++	++	+++
JR6	7	+	+	+	+++
JR8	2.5	+	+	+	+++
JR9	2.5	+	++	-	++
JR11	4	++	++	++	+++
JR19	3.5	-	++	-	++
JR20	3.5	-	++	-	++
JR21	8	++	+++	+	++
JR22	8	+++	+++	-	+
JR26	2	+	++	-	++
JR27	2	-	+	-	++
JR29	6	++	+++	+++	+++
JR30	6	+	+++	+	+
JR31	6	+++	+++	++	+++

The scores were given as described in Material and Methods.

Statistical Analysis

To determine whether gliosis is interrelated with age and/or mononuclear cell infiltration in lung, liver, and kidney we used Pearson correlation coefficients.⁴³ The Kruskal-Wallis test was used to assess statistical differences in the distribution of various parameters from the wild-type and the two transgenic mouse lines. Where differences were found a pairwise comparison with Bonferroni correction was applied to compare the different lines.⁴⁴

Results

Control Animals

Nontransgenic littermates of various ages stayed healthy and symptom-free throughout the whole duration of the study (11 months). Autopsy of all three wild-type control animals and subsequent histological examination revealed no pathological changes in any organ examined except for minor gliosis of unknown cause in one wild-type animal. The sIL-6R α single-transgenic mice were also considered to be negative controls, because mouse IL-6 does not bind to human sIL-6 receptor α .⁴⁵ Accordingly, the three human sIL-6R α transgenic mice were completely inconspicuous and also served as controls.

Analysis of Transgene Expression

Hemizygous IL-6/sIL-6R α double-transgenic mice co-expressing both transgenes were used to determine the effect of the IL-6/sIL-6R α complex on the CNS *in vivo*. In

IL-6 mice transgene expression is driven by the metallothionein-I promoter.³⁵ Even though it has been described that this promoter is preferentially active in fibrous and protoplasmatic astrocytes,⁴⁶ Northern blot analysis of total RNA from several organs, including brain, had revealed that the liver is the major organ for hIL-6 mRNA expression.³⁵ Metallothionein-I/IL-6 transgenic mice express IL-6 constitutively in the liver and secrete the cytokine into the blood.³⁵ Serum concentrations ranged between 10 and 20 ng/ml.⁴⁷

Expression of the sIL-6R α transgene was controlled by the neonatally active promoter of the phosphoenolpyruvate carboxy kinase gene that drives expression in the liver, kidney, and adipose tissue.^{38,48} As shown previously, Northern blot analysis from different tissues showed transcripts of the hIL-6R α in the liver and kidney, but not in the heart, skeletal muscle, or brain.⁴⁵ In the brain, we could detect mRNAs of both transgenes only by use of the reverse transcriptase-polymerase chain reaction (not shown). Serum levels of sIL-6R α were quantified by Western blot analysis and ranged between 4 and 8 μ g/ml.⁴⁵ The IL-6/hIL-6R α complex could be immunoprecipitated from the serum.⁴⁵ IL-6/IL-6R α double-transgenic mice had elevated IL-6 plasma levels, and the plasma half-life of IL-6 was significantly prolonged.⁴⁵

Clinical Phenotype of Double-Transgenic Animals

IL-6/sIL-6R α double-transgenic mice have already been shown previously to differ from sIL-6R α and IL-6 single transgenic mice mainly in three aspects: 1) plasmacytoma development was significantly accelerated and ag-

gravated; 2) extramedullary hematopoiesis was strongly activated first in the spleen and later in the liver, and subsequently all peripheral blood cell counts were highly increased; and 3) significant hepatocellular hyperplasia and secondary liver pathology, including peliosis and reactive necroses were induced.^{45,49,50}

Here, we investigated the neuropathological consequences of either circulating IL-6 and sIL-6R α or both in transgenic mouse brains. In single transgenic mouse lines neurological symptoms were absent. In contrast, all IL-6/sIL-6R α double-transgenic mice developed neurological symptoms, such as tremor, gait abnormalities, and paresis after 2 to 6 months of life. However, these symptoms were part of a larger complex of effects of IL-6/IL-6R α including weight loss; fur abnormalities; liver, kidney, and lung damage; and plasmacytomas and can therefore at least in part be attributed to the general weakness.

Histopathological Findings

At the light microscopic level, wild-type as well as sIL-6R α transgenics were virtually free of reactive gliosis (not shown). In addition, these mice did not show impairments or defects in other organs, such as liver, lung, kidney, or muscle (not shown). A small number of reactive astrocytes could be detected in the brains of two IL-6 single transgenic animals by GFAP immunohistochemistry (Figure 1a). In kinetical terms, gliosis in IL-6 transgenic mice did not increase during a follow-up time of 11 months. The low level of gliosis in these mice was not significantly different from the levels found in the sIL-6R α and wild-type control mice ($P = 0.074$). Neurons of these mice appeared unaltered in shape and number when compared to normal control animals (Figure 1). Compared to the brain, which showed minor to moderate gliosis in only two out of 10 animals, the lungs and kidneys of the IL-6 transgenic mice were considerably more affected; they showed infiltration by lymphoid cells in six out of 10 animals each.

In general, the brains of the IL-6/sIL-6R α double-transgenic mice were also considerably less affected compared to the extent of lesions in lungs, livers, and kidneys, which showed prominent lymphoid infiltration in most cases (Table 1). However, numerous astrocytes in the gray and white matter of the IL-6/IL-6R α mice displayed the typical hallmarks of reactive astrogliosis, ie, hypertrophy and strong GFAP expression (Figure 1, b and c). Pairwise Kruskal-Wallis analysis with Bonferroni correction revealed that the levels of gliosis in the IL-6/IL-6R α double-transgenic animals differed significantly from the degree of gliosis found in the IL-6 and IL-6R α single transgenic as well as in the wild-type mice ($P < 0.05$). Histological examination of brain sections at different time points throughout a period of 2.5 to 8 months demonstrated that the degree of gliosis in IL-6/sIL-6R α mice was stable and not time-dependent. The reactively transformed astrocytes were distributed all throughout the brain and not at preferred sites. The number of neurons in IL-6/sIL-6R α mice appeared neither reduced nor in-

creased when compared to the wild-type, sIL-6R α , and IL-6 mice. There were no histopathological signs of neuronal damage such as chromatolysis or shrinkage/eosinophilia. Cerebral vascular proliferation, necrosis, inflammatory cellular infiltrates, or accumulations of macrophages or activated microglial cells were absent in the histological sections of any group examined. Importantly, no leptomeningeal infiltrates or tumors were found.

Notably, the prominent systemic effects such as liver damage and expansion of lymphoid or hematogenic progenitor cells typical for IL-6/IL-6R α double-transgenic mice^{45,47,49} did not parallel the development of gliosis in the brains of the IL-6/sIL-6R α double-transgenic mice. For example, the 6-month-old mouse that suffered from splenomegaly and major intestinal infiltration of hematogenic precursor cells (JR22) had only minor astrogliosis. On the other hand, another IL-6/sIL-6R α mouse 7 months of age (JR6) diagnosed with only minor liver damage was assigned strongly gliotic (Table 1). Statistical analysis showed that there was no correlation between the degrees of infiltration of liver, lungs, and kidneys on the one hand and gliosis on the other hand in the group of IL-6/sIL-6R α double-transgenic mice. Pearson's coefficient analysis, Kruskal-Wallis one-way analysis of variance, and pairwise Kruskal-Wallis analysis did not show any significant correlation for all comparisons (gliosis versus liver, kidney, and lung damage).

Albumin immunohistochemistry of the brains of all mice examined in the present study did not reveal any leakage of albumin into the brain parenchyma, indicating that the BBB was primarily intact even in the IL-6/sIL-6R α double-transgenic mice (Figure 1, e and f).

Ultrastructural Alterations of the CNS

In the present study we focused on glial and BBB abnormalities in the CNS of IL-6 and IL-6/sIL-6R α transgenic mice. Wild-type and sIL-6R α transgenic mice served as controls. The capillary walls of these normal control mice (Figure 2a), as well as of IL-6 (Figure 2b) and IL-6/sIL-6R α (Figure 2, c and d) mice displayed no signs of endothelial damage such as fenestration or nuclear or cytoplasmic irregularities. There were no detectable alterations of endothelial tight junctions and no thickening or any other alteration of the vascular basement membranes in any mouse line. The glial sheath in the control mice looked normal with only minor focal hydropic changes (Figure 2a). In IL-6 mice, swelling of astrocyte end-feet was moderate, if present at all. In contrast, perivascular astrocytes of IL-6/sIL-6R α transgenics were swollen considerably (Figure 2, c and d). These hydropic astrocytic processes were a very frequent finding. The degree of vascular luminal narrowing increased with the degree of vacuolization (Figure 2, c and d).

Electron microscopically, no signs of neuronal damage such as chromatolysis, shrinkage, or apoptotic bodies in the gray matter were found in any group. There was no apparent activation of perivascular monocytic cells or microglial cells detectable on the electron microscopic level.

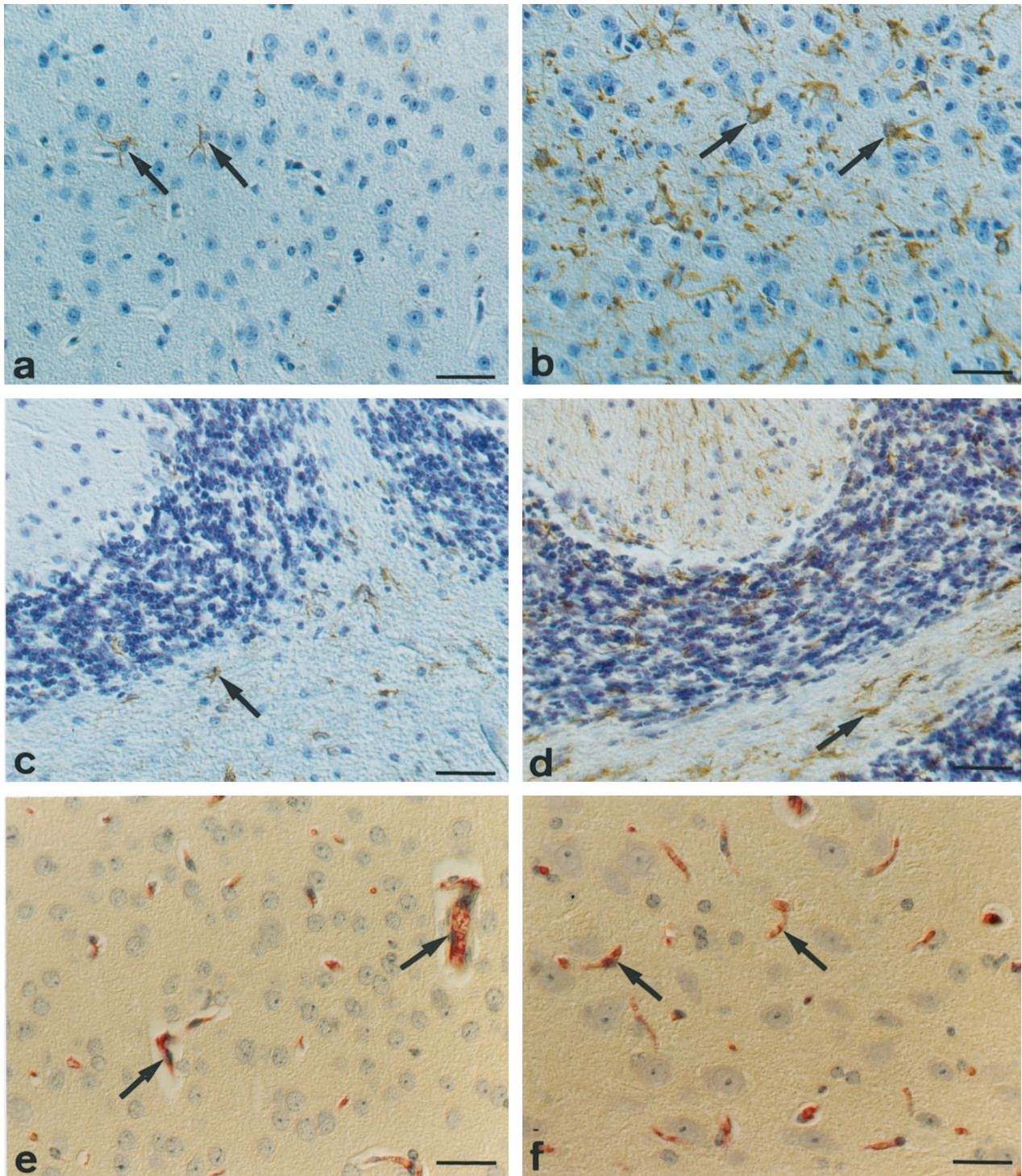


Figure 1. a, c: IL-6 transgenic mice with high levels of circulating human IL-6 showed minor astrogliosis reflected by a low number of GFAP immunoreactive astrocytes (arrows). In contrast, IL-6/sIL-6R α transgenics exhibited massive reactive gliosis with numerous GFAP immunoreactive astrocytes in all parts of the CNS including the cerebral cortex (b) and the cerebellum (d). Scale bars, 30 μ m (a-d). However, no impairment of BBB function was detected by albumin immunohistochemistry even in the IL-6/sIL-6R α mice. There was a prominent intravascular staining in all brain sections analyzed (arrows); extravascular immunoreactivity was absent (e, normal control mouse; f, double-transgenic mouse). Scale bars, 30 μ m (c), 20 μ m (f).

Discussion

Many neurological disorders are paralleled by changes in cytokine expression.⁹ Mounting evidence implies that elevated IL-6 expression is rather a cause than a result of

glial as well as neuronal responses.⁵¹ We used single-transgenic mice overexpressing human IL-6 and sIL-6R α and double-transgenic mice overexpressing IL-6/sIL-6R α systemically to clarify the contribution of the different components of this ligand/receptor system. In IL-6 and

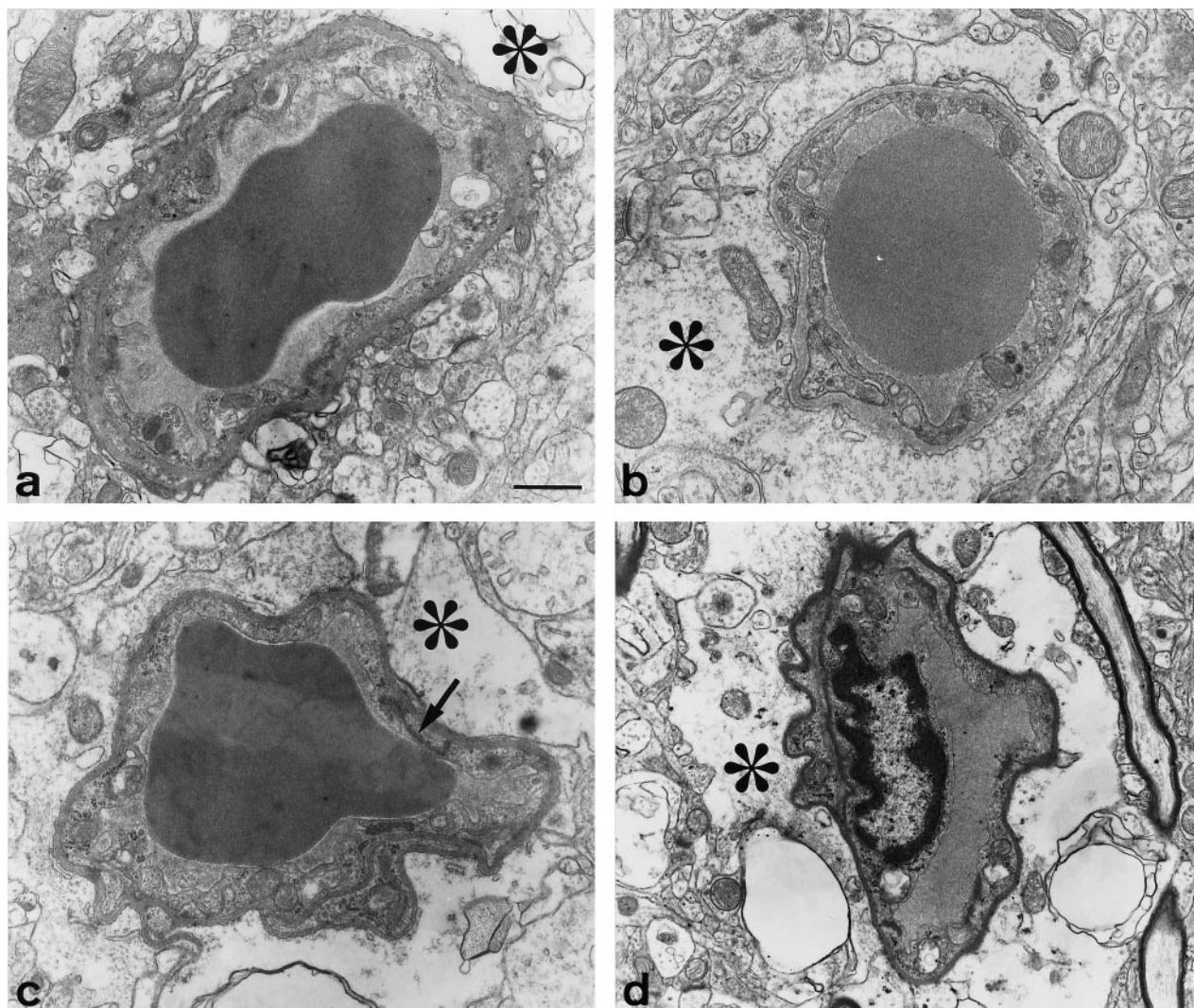


Figure 2. Electron micrographs of brain sections from different transgenic mouse lines. **a:** Brain capillary of wild-type control animal with normal endothelial cell, tight junctions, and basal lamina and only minor focal swelling of astroglial ensheathment (asterisk). **b:** Moderately hydropic astrocyte end-feet covering an endothelial cell in an IL-6 mouse. The astrocytic end-feet are moderately swollen (asterisk). **c** and **d:** Swollen astrocytic end-feet (asterisks) covering indented brain capillaries in IL-6/sIL-6R α double-transgenic mice. There are no major endothelial cell or basal lamina abnormalities. Scale bar, 0.5 μ m (**a-d**).

sIL-6R α single-transgenic mice, neurological symptoms were absent. As predicted, sIL-6R α mice were normal, because mouse IL-6 does not crosstalk with human sIL-6R α . We found only few GFAP-positive reactive astrocytes in IL-6 mice even though systemic effects of IL-6 overexpression including plasmacytoma development were prominent in these mice. In contrast, IL-6/sIL-6R α double-transgenic mice had marked neurological symptoms and showed massive reactive gliosis in the context of severe general weakness. Because neuronal density was apparently not reduced on the histological level, a selective effect on astrocytes seems to be operative in these mice.

Typically, reactive gliosis was manifested by hypertrophy and hyperplasia of astrocytes similar to the GFAP-IL-6 mice reported previously.²⁷ GFAP-IL-6 mice suffered from tremor, ataxia, and seizures. Life expectancy of GFAP-IL-6 mice was approximately 6 months for high expressors and 12 months for low expressors, respec-

tively.²⁷ Our IL-6/sIL-6R α mice lived for 2 to 7 months until the onset of severe symptoms which were, however, caused predominantly by extracerebral alterations such as plasmacytomas and liver cell necrosis.

Nevertheless, the GFAP-IL-6 mice are inappropriate for studying the effects of systemically circulating IL-6 on astrocytes. The profound difference between the two models lies in the source of IL-6 production. In GFAP-IL-6 mice, IL-6 expression is tissue-selective, and ensuing damage is confined locally to the CNS. In our model, the distribution of IL-6 and sIL-6R α is systemic. Transgene expression of cytokine and ligand takes place in different organs and outside the CNS. In this way the individual mediators can float freely and interact unbiased with each other. It is interesting to speculate that circulating IL-6 and sIL-6R α have to be complexed first extracerebrally before passing the BBB to induce gliosis. The stronger phenotype in IL-6/sIL-6R α double-transgenic mice might be because of potentiated IL-6 signaling.⁴⁵ In

this way, IL-6 is capable of inducing a vigorous response in primarily less responsive cell populations including neuronal and glial cells.³³ This point is strengthened by the fact that sIL-6R α mice had elevated IL-6 plasma levels and a prolonged plasma half-life of IL-6 when treated with exogenous IL-6.⁴⁵

The central role of astrocytes in inducing BBB properties has been illustrated by several studies *in vivo*⁵² and *in vitro*.⁵³ Astrocytes and their conditioned media can induce BBB properties of the endothelium *in vitro*, and it was suggested that this effect is mediated by astroglia-secreted IL-6.⁵⁴ However, IL-6 is also capable of inducing disruption of the BBB *in vitro*⁵⁵ and *in vivo*.³² In this light, the perturbing effects in GFAP-IL-6 mice on BBB formation as well as the induction of other vascular abnormalities such as neovascularization²⁷ are not surprising. In contrast to normal mice where the BBB develops between day 7 and 14, formation of normal BBB was precluded in the GFAP-IL-6 transgenic mice.³² To obtain an animal model in which BBB formation per se is not precluded, we took advantage of cross-breeding single-transgenic mouse strains to elucidate the individual contributions of the different mediators IL-6, sIL-6R α , and IL-6/IL-6R α on the morphological integrity of the BBB.

In our model the edematous swelling of perivascular astrocytes in IL-6/sIL-6R α mice presumably represents a transformational step indicating damage, but not necessarily disruption of the BBB. Opening of the BBB can generally be ascribed to three possible mechanisms: 1) separation of the interendothelial tight junctions, 2) increased vesicular transport and the formation of transendothelial channels, and 3) biochemical and structural alteration of the endothelial cell plasma membrane resulting in increased permeability.⁵⁶ We found no endothelial fenestrations, no alterations in brain endothelial tight junctions, and no ultrastructural evidence for increased vesicular transport through cerebral capillaries in the IL-6/sIL-6R α mice. Moreover, albumin immunohistochemistry, an established method to evaluate BBB integrity,^{57,58} gave no indication of a vasogenic edema with altered BBB permeability and leakage of plasma constituents into the brain.⁵⁹ Thus, even high systemic levels of IL-6 and sIL-6R α did not cause BBB breakdown in our mouse model. This result does not support the hypothesis that systemically elevated IL-6 is a major cause of BBB breakdown.^{55,60} In line with our observations, IL-6 injected intravitreally did not cause a measurable increase in blood retinal barrier permeability, whereas tumor necrosis factor- α induced increased permeability.⁶¹ In this context, it is also interesting to note that in mice overexpressing IL-6 in neurons because of an IL-6 transgene regulated by the neuron-specific enolase promoter, no vascular changes were detected in the brain.³¹ Similar to our IL-6/IL-6R α double-transgenic mice, astrogliosis was the major finding in the neuron-specific enolase-IL-6 mice.³¹ These results suggest that systemic and even local cerebral overexpression of IL-6 does not cause BBB disruption. Apparently, IL-6 overexpression has to be directly targeted to astrocytes by use of a GFAP promoter, which leads to early and sustained cerebral IL-6

overexpression from development through adulthood preventing BBB formation.³²

The following hypothesis is proposed to explain the effects of systemically increased IL-6 and IL-6R α on astrocytes. Blood-borne cytokines have been shown to cross the BBB by saturable transport systems.^{62,63} Hence, it is conceivable that IL-6, akin to ciliary neurotrophic factor and tumor necrosis factor,⁶⁴ crossed the BBB by this mechanism. Consistent with our findings, granulocyte macrophage colony-stimulating factor crossed the BBB and blood-spinal cord barrier significantly faster than the control substance, albumin.⁶⁵ Thus, IL-6 and IL-6R α conceivably penetrated the BBB in the double-transgenic mice even though signs of major BBB disruption were absent. In addition, examination of the role of IL-6 and sIL-6R α in IL-6 induction *in vitro* showed that, while treatment of astrocytes with IL-6 and sIL-6R α resulted in modest increases in IL-6 mRNA expression, co-treatment with either tumor necrosis factor- α or IL-1 β plus IL-6/IL-6R α lead to synergistic increases in IL-6 gene expression,⁶⁶ again highlighting the pivotal role of sIL-6R α in determining the effects of IL-6 in astrocytes.

In the present study, systemic overexpression of IL-6 and sIL-6R α induced liver and kidney damage as already reported previously.^{35,45,47,49,50,67} Still, several findings indicate that the vigorous astrocytic response was caused by the systemically expressed and complexed IL-6/IL-6R α transgene product and was not a consequence of the systemic diseases of the double-transgenic animals: the intensity of gliosis was not age-dependent, whereas the systemic consequences of IL-6/IL-6R α overexpression increase with age.⁵⁰ In addition, there was no correlation between the extent of infiltration of liver, lung, and kidneys on the one hand and astrogliosis on the other hand. Moreover, IL-6 single-transgenic mice that also showed considerable plasmacytoma development, liver damage, and other extracerebral changes⁵⁰ displayed only minor, insignificant gliosis. Finally, no histologically or electron microscopically detectable neuronal damage was found in any animal, which should be present in the case of prominent metabolic or other prominent systemic damage affecting the brain. It is, however, important to note that we did not perform detailed stereological measurements to quantify neuronal numbers.

Surprisingly, the brains of the double-transgenic mice also seemed to be protected against plasmacytoma infiltration, because there was no necrosis and no infiltration of the brain by plasmacytoma infiltrates or precursor lesions, even though other organs (ie, liver, kidneys, lung) were heavily infiltrated in many double-transgenic animals. Leptomeningeal lymphocytic infiltrates or meningeal tumors were also absent in both IL-6 and IL-6/IL-6R α overexpressing mice, suggesting that the elevated IL-6 levels found in patients with Castleman's disease are at least not the sole cause of these meningeal alterations.

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