Animal Model

Role of Interleukin-1 in Prion Disease-Associated Astrocyte Activation

Julia Schultz,* Anja Schwarz,* Sabine Neidhold,*
Michael Burwinkel,* Constanze Riemer,*
Dietrich Simon,* Manfred Kopf,[†] Markus Otto,[‡]
and Michael Baier*

From the Robert-Koch-Institute,* Berlin, Germany; the
Department of Neurology,[‡] Georg-August-University, Göttingen,
Germany; and the Department of Environmental Sciences,[†]
Eidgenössische Technische Hochschule Zürich, Swiss Federal
Institute of Technology, Zürich, Switzerland

Prion-induced chronic neurodegeneration has a substantial inflammatory component, and the activation of glia cells may play an important role in disease development and progression. However, the functional contribution of cytokines to the development of the gliosis *in vivo* was never systematically studied. We report here that the expression of interleukin-1 β (IL-1 β), IL-1 β -converting enzyme, and IL-1 receptor type 1 (IL-1RI) is up-regulated in a murine scrapie model. The scrapie-induced gliosis in IL-1RI^{-/-} mice was characterized by an attenuated activation of astrocytes in the asymptomatic stage of the disease and a reduced expression of CXCR3 ligands. Furthermore, the accumulation of the misfolded isoform of the prion protein PrP^{Sc} was significantly delayed in the IL-1RI^{-/-} mice. These observations indicate that IL-1 is a driver of the scrapie-associated astrocytosis and possibly the accompanying amyloid deposition. In addition, scrapie-infected IL-1RI-deficient (IL-1RI^{-/-}) mice showed a delayed disease onset and significantly prolonged survival times suggesting that an anti-inflammatory therapeutical approach to suppress astrocyte activation and/or glial IL-1 expression may help to delay disease onset in established prion infections of the central nervous system. (*Am J Pathol* 2004, 165:671–678)

Prion infections of the central nervous system cause a progressive and ultimately lethal degeneration of neuronal tissue but the underlying pathomechanisms are still elusive.^{1–3} The activation of glial cells precedes neuronal death and is a general hallmark of neurodegenerative

amyloidoses.^{4–7} In prion diseases, the reactive gliosis is most likely a consequence of the accumulation of disease-associated isoform of the prion protein (PrP^{Sc}). In agreement with this hypothesis, microglia are activated by the aggregated, amyloid-like, form of a PrP-derived peptide (PrP_{106–126}) *in vitro*. Supernatants from PrP_{106–126}-treated microglia cultures activate astrocytes and are neurotoxic.^{8–11} Thus, cytokines and chemokines released by PrP^{Sc}-activated glia cells may contribute indirectly to the pathogenesis of prion infections as mediators of the gliosis and/or directly via cytotoxicity to neurons. However, the actual role of prion-induced glia activation and subsequent cytokine secretion in disease development is still far from clear. So far, cytokines reportedly overexpressed in scrapie-infected brain tissue such as tumor necrosis factor- α and interleukin (IL)-6 were shown to have no effect on scrapie pathogenesis and length of incubation periods on intracerebral infections.^{5,12,13}

IL-1, often regarded as prototypic inflammatory cytokine, is known to play a detrimental role in acute neurodegeneration after brain injury and stroke^{14,15} and was suggested to be involved in Alzheimer's disease pathogenesis.¹⁶ In addition, certain polymorphisms in the genes encoding IL1-1 α and IL1-1 β are considered to increase the risk for early disease onset in Alzheimer's disease.¹⁷

Previous studies demonstrated that IL-1 is produced by microglia on exposure to PrP_{106–126} and β -amyloid *in vitro*.^{18,19} Glial overexpression of IL-1 was previously observed in murine scrapie models during preclinical and clinical stages of the disease,^{5,13,20,21} in brain tissue of mice transgenic for the human amyloid precursor protein (huAPP) and in Alzheimer's disease.^{16,22,23} However, despite this accumulated evidence for an involvement of IL-1 in chronic neurodegeneration, the functional biology of IL-1 overexpression in neurodegenerative amyloidoses

Supported in part by the Federal Ministry for Health and Social Security (grant 325-4471-02/45) and the Federal Ministry for Education and Research, Germany (grant 01KO0111).

Accepted for publication April 12, 2004.

Address reprint requests to Dr. Michael Baier, Project "Neurodegenerative Diseases," Robert-Koch-Institute, Nordufer 20, 13353 Berlin, Germany. E-mail: baierm@rki.de.

was never experimentally addressed. Here we investigated the role of IL-1 in the scrapie-associated glia activation.

Materials and Methods

Mouse Strains and Scrapie Infections

IL-1RI^{-/-} mice, which originated from 129Sv × C57/B6 mouse strain crosses, were maintained at the ETH Zurich, Zurich, Switzerland, and were backcrossed for seven generations into the C57/B6 strain. All infections were performed intracerebrally using 20 μl of diluted 10% brain homogenates prepared from scrapie strain 139A (kindly provided by R.H. Kimberlin, Edinburgh, UK)-infected mice as previously described.²⁴ Further infection experiments using first generation wild-type 129Sv × C57/B6 crossbreeds showed no significant survival time differences to similarly infected wild-type C57/B6 mice, indicating that the genetic background has no influence on the disease development in the used combination of scrapie and mouse strain. Mock infections were similarly performed with 10% brain homogenates prepared from uninfected, healthy C57/B6 mice. All infections with a given dilution were performed at the same day from the same brain homogenate aliquot to minimize variations of the infectious doses. The statistical analysis of the survival of the IL-1RI^{-/-} mice and the wild-type controls infected with a 10⁻⁴ diluted 10% brain homogenate was performed by the Kaplan-Meier method.²⁵ Kaplan-Meier survival curves were plotted for the IL-1RI^{-/-} and wild-type mice (data not shown). Differences between the survival curves were statistically evaluated using the log rank test.²⁶ In addition, the survival in all groups was statistically analyzed using the unpaired *t*-test.

Northern Blot Analysis

RNA, isolated from scrapie-infected and mock-infected mice brains by acid guanidinium thiocyanate-phenol-chloroform extraction,²⁷ was fractionated by agarose gel electrophoresis, transferred to nylon membranes, and hybridized with probes specific for IL-1β, IL-1RI, IL-1β-converting enzyme (ICE), glial fibrillary acidic protein (GFAP), and actin. The GFAP-specific probe was obtained from a cDNA library enriched for up-regulated genes in scrapie-infected brain tissue as previously described,⁶ all other DNAs used as hybridization probes were amplified by polymerase chain reaction (PCR) starting from murine spleen cDNA. Applied oligonucleotides for the PCR amplifications were: IL-1β(+) CCTGTGTCTTTCCCGTG; IL-1β(-) GAGTGCTGCCTAATGTCC; IL-1RI(+) GGGACTC-CAGGATTCATCAG; IL-1RI(-) GCTCTTCAGCCACATTCC; ICE(+) CGTGGAGAGAAACAAGGAG; ICE(-) CAGCAGT-GGGCATCTGTAG; actin(+) AAGGCCAACCGTGAAAA-GAT; actin(-) CGCTCGTTGCCAATAGTGAT.

Quantitation of Chemokine mRNA Levels

RNAs were reverse-transcribed using the First-Strand cDNA synthesis kit (Amersham, Freiberg, Germany). Gene expression levels from three mice per group were subsequently determined by TaqMan real-time PCR using an GeneAmp 5700 sequence detection system (Perkin Elmer, Boston, MA) and the SYBR Green PCR kit (Qiagen, Hilden, Germany). Amplifications were performed with the primer sets mig-1 TTTTGGGCATCATCTTCCTG and mig-2 TTCCCCCTCTTTTGCTTTTT for CXCL9; and IP10-1 GCAACTGCATCCATATCGATGAC combined with IP10-2 TGTGCGTGGCTTCACTCCA for CXCL10, respectively. To ensure equivalence of the samples results were normalized for glyceraldehyde-3-phosphate dehydrogenase mRNA levels, which were detected using the primers GAPDH-1 GACCTCACCATCCCGCATCT and GAPDH-2 GCGG-GAGTCGGCCAGTTACC.

Immunohistochemistry and PET Blot Analysis

All immunohistochemistry was performed according to previously published procedures.²⁸ Examined areas were the cortex, hippocampus, brain stem, cerebellum, and the olfactory bulb from three mice per group and time point. Differences between IL-1RI^{-/-} mice and the C57/B6 controls were evaluated by independent scoring of all tissue samples by three investigators without previous knowledge of the group to which the mice belonged. Antibodies used were a polyclonal anti-GFAP antiserum (DAKO, Glostrup, Denmark) and a monoclonal antibody against the CD 11b (Mac-1) antigen (kindly provided by B. Engelhardt, Munster, Germany). The prion protein was detected using the anti-PrP monoclonal antibody 6H4 (Prionics, Schlieren, Switzerland).

The PET blot analysis was performed as previously described.²⁹ Briefly, 6-μm paraffin-sections of formalin-fixed brain tissues were transferred onto a nitrocellulose membrane and, after dewaxing, treated with proteinase K for digestion of normal PrP^C. Remaining membrane-bound protein was subsequently denatured with 3 mol/L guanidine isothiocyanate. The actual immunodetection was performed with the anti-PrP 6H4 antibody followed by incubation with an alkaline phosphatase-linked anti-mouse immunoglobulin antiserum. The final staining was performed with nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate.

Results

As a first step we determined whether IL-1β, ICE, which is required to generate active IL-1β,³⁰ and IL-1 receptor type 1 (IL-1RI) are expressed in a well-characterized murine scrapie model system; the scrapie-strain 139A-infected C57/B6 mouse.³¹ Northern blot analysis of total brain RNA showed the up-regulation of the respective mRNA levels in the scrapie-infected brain in comparison to brain tissue from age- and sex-matched mock-infected controls (Figure 1). The analyzed components of the IL-1 system were hardly expressed in the normal brain,

Figure 1. Northern blot analysis of the expression of IL-1 β , IL-1RI, and ICE in scrapie-infected and mock-infected (normal) mice. The total brain RNAs analyzed were isolated from two animals sacrificed at the terminal stage of the disease (176 dpi) and two mock-infected controls of the same age. The terminal stage is defined as the state when the symptoms, specifically the general reduction in mobility, the hind leg paralysis, and the hunched posture, indicate that the animals would die, if not sacrificed, within the next 72 hours. The actin controls confirm the presence of comparable amounts and qualities of the RNA samples.

whereas in the course of the infection the expression levels increased fourfold to sixfold.

The biological effects of IL-1 α and IL-1 β are mediated via binding to IL-1RI. The only other known IL-1 receptor, IL-1R type 2, lacks an intracellular domain necessary for signal transduction and is believed to act as a decoy receptor.³² To assess the role of IL-1 α and IL-1 β during a prion disease of the central nervous system, the intracerebral scrapie infection of mice deficient for the IL-1RI³³ was compared to similarly infected wild-type C57/B6 mice. The mean survival time for the scrapie-infected IL-1RI^{-/-} mice was 197 (± 7) days in comparison to 172 (± 4) days for C57/B6 mice receiving the same infectious dose using a 10⁻² diluted brain homogenate prepared from a terminally ill animal as inoculum (Table 1). Moreover, C57/B6 mice intracerebrally infected on the same day with a 10⁻³ dilution of the same brain homogenate survived for 181 (± 13) days on average (Table 1). Hence, the significantly ($P < 0.001$) increased resistance of the IL-1RI^{-/-} mice is equivalent to the survival of wild-type mice, which received a more than 10 times

lower challenge dose. In separate experiments IL-1RI^{-/-} and C57/B6 mice were intracerebrally infected with a 10⁻⁴-diluted brain homogenate (Table 1). Again, the incubation periods of the IL-1RI^{-/-} [226 $\pm$ 14 days after infection (dpi)] were significantly longer ($P < 0.001$) than those of the wild-type controls (204 $\pm$ 7 dpi). Furthermore, the survival time spread increased from 26 days for the controls to 43 days for the IL-1RI^{-/-} mice, which additionally indicates the higher resistance of the IL-1RI-deficient mice against scrapie pathogenesis.¹²

The animals were kept under close veterinary supervision throughout the entire experiment to determine whether the disease developed differently in the IL-1RI^{-/-} versus the control mice. Monitored clinical symptoms of the disease and the duration of the symptomatic phase were primarily similar for the IL-1RI^{-/-} mice in comparison to the controls. However, the onset of the characteristic reduction in mobility and other typical manifestations of the symptomatic phase such as poor coat condition, hunched posture, weight loss, and behavioral changes (hyperresponsiveness to touch, nervousness) was delayed in the IL-1RI^{-/-} mice (onset at 172 dpi) in comparison to the wild-type controls (onset at 150 dpi).

The next parameter investigated was the scrapie-associated astrocytosis in IL-1RI^{-/-} compared to C57/B6 mice, which was examined by immunohistochemistry in the asymptomatic stage of the disease (125 dpi) and in terminally ill animals. At the terminal stage of the disease no significant difference concerning activated, GFAP-expressing, astrocytes was seen between wild-type controls and the IL-1RI^{-/-} mice. However, at 125 dpi, a pronounced astrocytosis in the cerebellum, olfactory bulb, and the brain stem was readily detectable in C57/B6 mice, whereas in IL-1RI^{-/-} mice GFAP-positive astrocytes were nearly absent at this asymptomatic stage of the infection (Figure 2). A further comparison of terminally diseased wild-type controls (169 dpi) with still hardly symptomatic IL-1RI^{-/-} mice sacrificed at the same day confirmed these observations, but the difference in GFAP-positive astrocytes between the two groups was at this time point only twofold to fourfold in the areas examined (data not shown). Additional Northern blot analysis of total brain RNA demonstrated the presence of fourfold to sixfold elevated GFAP mRNA levels at 125 dpi in the scrapie-infected C57/B6 mice. In contrast, GFAP expression in brain tissue of IL-1RI^{-/-} mice at the same stage of the infection was still comparable to the mock-infected controls (Figure 2). The astrocytosis was further investigated by quantitative reverse transcriptase-PCR expression analysis of the CXC chemokines CXCL9 and CXCL10, which are in the central nervous system predominantly produced by reactive astrocytes.^{34,35} CXCL9 and CXCL10 mRNA levels were found to be 4.2 and 7.4 times higher at 125 dpi in the wild-type control brains compared to the IL-1RI^{-/-} mice, respectively. At the terminal stage of the disease the expression of CXCL9 and CXCL10 was 8.3- and 4.6-fold increased in the scrapie-infected C57/B6 controls (Figure 3).

The scrapie-induced microgliosis was examined by immunohistochemistry in coronal sections using a monoclonal antibody against the CD11b (Mac-1) antigen,

Table 1. Survival Times (dpi) of Scrapie-Infected IL-1RI^{-/-} and C57/B6 Control Mice*

Dilution of the 10% brain homogenates used as inoculum	IL-1RI ^{-/-}	C57/B6
10 ⁻²	197 $\pm$ 7, <i>n</i> = 4	172 $\pm$ 4, <i>n</i> = 8
10 ⁻³	203 $\pm$ 3, <i>n</i> = 3	181 $\pm$ 13, <i>n</i> = 13
10 ⁻⁴	226 $\pm$ 14, <i>n</i> = 9	204 $\pm$ 7, <i>n</i> = 14

*All infected animals succumbed to the disease.

Figure 2. Astrocytosis in scrapie-infected IL-1RI^{-/-} and C57/B6 mice. **A:** Immunohistochemical detection of GFAP. At 125 dpi activated astrocytes in scrapie-infected IL-1RI^{-/-} mice were hardly detectable (**a, b**), whereas a prominent astrocytosis was readily detectable in similarly infected C57/B6 mice (**c, d**). No difference concerning GFAP-expressing astrocytes was seen between terminally diseased wild-type controls (**f**) and IL-1RI^{-/-} mice (**e**). Mock-infected animals showed no indications of an astrocytosis (**g, h**) in the areas examined. Some age-related astrocytosis occurred in 5-month-old and older mock-infected and uninfected animals (not shown). **B:** Northern blot analysis of GFAP mRNA levels in total brain RNA. **Lane 1:** RNA from two mock-infected C57/B6 mice; **lane 2:** IL-1RI^{-/-} mice (125 dpi); **lane 3:** C57/B6 mice (125 dpi); **lane 4:** C57/B6 mice (185 dpi, terminal stage). Original magnifications: $\times 50$ (**a, c, g**); $\times 400$ (**b, d-f, h**).

a widely used microglia activation marker.³⁶ For both time points analyzed, at 125 dpi and in the terminal stage of the infection, the activation of microglia in all areas

Figure 3. Quantitative reverse transcriptase-PCR analysis of relative CXCL9 and CXCL10 expression levels. Chemokine expression in scrapie-infected wild-type C57/B6 controls was compared to IL-1RI^{-/-} mice at 125 dpi and in terminally diseased animals.

examined was slightly (approximately twofold) more pronounced in the IL-1RI^{-/-} mice (Figure 4).

Having shown that the IL-1RI deficiency modifies the scrapie-induced gliosis, we then investigated whether this would influence the accumulation of PrP^{Sc} during the infection. The accumulation of the disease-associated isoform of the prion protein PrP^{Sc} in IL-1RI^{-/-} and C57/B6 mice was examined by immunohistochemistry and the recently developed paraffin-embedded tissue blot (PET blot) technique.²⁹ Using conventional immunohistochemistry less stainable PrP^{Sc} was detectable in the IL-1RI^{-/-} mice than in the C57/B6 controls at 125 dpi (Figure 5), but both groups appeared primarily similar at the terminal stage of the disease (data not shown). The PET blot technique facilitates the depiction of larger brain areas and allows for a stringent discrimination between normal PrP and proteinase K-resistant PrP^{Sc}. In agreement with the immunohistochemistry, PET blots from animals of the IL-1RI^{-/-} mice at 125 dpi showed significantly less PrP^{Sc} accumulation in the cortex and thalamus region than the wild-type controls. In the terminal stage, the PrP^{Sc} accumulations of the disease were primarily indistinguishable between the two groups and were particularly intense in the cortex and the hippocampus (Figure 5).

Discussion

The data presented here show that essential components of the IL-1 system are activated in the brains of scrapie-infected mice (Figure 1). The overexpression of IL-1 β (Figure 1) is in good agreement with previous observations in various murine scrapie models,^{5,13,20,21} but contrasts the apparent lack of IL-1 β production in scrapie

Figure 4. Microgliosis in scrapie-infected IL-1RI^{-/-} and C57/B6 mice. The activation of microglia in the cerebellum was increased in the IL-1RI^{-/-} mice (**a, f**: 125 dpi; **c, h**: terminal stage) in comparison to the scrapie-infected C57/B6 mice (**b, g**: 125 dpi; **d, i**: terminal stage). No activated microglia were found in mock-infected mice (**e, j**). Original magnifications: $\times 50$ (**a–e**); $\times 400$ (**f–j**).

strain ME7-infected C57/B6 mice.³⁷ Interestingly, Campbell and colleagues²⁰ described a predominant induction of IL-1 α expression in the Chandler/SWRj scrapie model, whereas an increase of IL-1 β levels was evident but comparatively low, which may require very sensitive methods to be detectable.²¹ Thus, besides possible sensitivity issues, to look for expression levels of a single component of the IL-1 system does not necessarily inform about its functional activation.

The additional induction of ICE and IL-1RI is likely to contribute significantly to the biological activity of IL-1 as more proIL-1 β is converted into its active form and increased receptor expression renders cells more responsive to IL-1 binding. The combined effects of this IL-1 system activation may well lead to a considerably higher

degree of available IL-1 bioactivity than the Northern blot data in Figure 1 indicate. To identify the functions of IL-1 in a model system for chronic neurodegeneration we investigated the scrapie infection of IL-1RI^{-/-} mice in comparison to C57/B6 controls.

The differences between the IL-1RI^{-/-}-deficient mice and the controls concerning the scrapie-associated astrocyte activation were striking (Figure 2). At the early stage of the infection the astrogliosis in the C57/B6 group greatly exceeded the astroglia activation in the IL-1RI^{-/-} mice. The data indicate that IL-1 is a driver of the early scrapie-associated astrogliosis *in vivo*. Given that in the late stage of the infection the staining of GFAP-positive reactive astrocytes was similar for wild-type controls and IL-1RI^{-/-} mice, other factors, among them possibly IL-6, which is up-regulated during scrapie infections^{5,13} and activates astrocytes *in vitro*,¹¹ appear to compensate for the absence of IL-1 bioactivity in astrocyte activation. In agreement with this hypothesis, similar effects were observed for the activation of astrocytes throughout time in IL-1RI^{-/-} mice after brain injury.¹⁵ Furthermore, it was recently shown that astrocytes interact directly with amyloid fibrils via scavenger receptors yet to be identified.³⁸ Thus, the activation of astrocytes may also be promoted by scavenger receptor-mediated signaling as a consequence of the ongoing deposition of misfolded PrP^{Sc}. This activating stimulus could represent a further mechanism to compensate for the absence of IL-1 bioactivity in the IL-1RI^{-/-} mice.

In addition to using GFAP detection to determine the extent of astrogliosis in the scrapie-infected mice we assessed the expression of the chemokines CXCL9 and CXCL10, known to be produced by activated astrocytes. As expected, the expression of CXCL9 and CXCL10 was significantly elevated in the wild-type controls compared to the IL-1RI^{-/-} mice (Figure 3). However, even in the terminal stage of the disease, which showed no changes in GFAP expression, CXCL9 and CXCL10 mRNA levels were 8.3 and 4.6 times higher in the C57/B6 control group than in the IL-1RI^{-/-} mice (Figure 3). This suggests that, in addition to the quantitative differences observed, the activation status of astrocytes (and hence the chemokine/cytokine profile) differs in the presence or absence of IL-1 bioactivity. It was previously shown that IL-1 is a strong inducer of CXCR3 ligand expression in astrocytes.^{39,40} The sustained increase of astrocytic CXCL9 and CXCL10 mRNA levels in the wild-type mice compared to the IL-1RI^{-/-} group is therefore most likely because of the direct induction of chemokine expression by IL-1. Given that the chemokine receptor CXCR3 is expressed on microglia, astrocytes, and neurons it is well possible that CXCR3 ligands are involved in glial and glial/neuronal interactions.⁴¹ CXCR3 ligands may trigger the migration of microglia toward regions of neuronal damage,⁴² and could thereby promote the activation of glial cells by degenerating nervous tissue. Moreover, CXCL9 and CXCL10 activate the ERK1/2 pathway in murine neurons and sustained ERK1/2 activation was suggested to play a detrimental role in acute and chronic neurodegeneration.⁴¹ The infiltration of T cells, which are most likely attracted by CXCR3 ligands of astrocytic origin, into the

Figure 5. PrP^{Sc} accumulation in scrapie-infected IL-1RI^{-/-} and C57/B6 mice. **a:** Immunohistochemical quantitation of the PrP^{Sc} accumulation in various brain areas at 125 dpi. 1, cortex; 2, hippocampus; 3, brain stem; 4, cerebellum; 5, olfactory bulb. Note that no PrP^{Sc} depositions were detectable in the cortex, the hippocampus, and the olfactory bulb of the IL-1RI^{-/-} mice at this stage. **b:** PET blot analysis of PrP^{Sc} deposition. Scrapie-infected IL-1RI^{-/-} mice at 125 dpi (**a–c**), scrapie-infected C57/B6 controls at 125 dpi (**d–f**), scrapie-infected IL-1RI^{-/-} mouse at the terminal stage of the disease (**g**), scrapie-infected C57/B6 control at the terminal stage of the disease (**h**), and mock-infected C57/B6 control (**i**). **Arrows** point at some of the areas with beginning PrP^{Sc} deposition at 125 dpi.

brain in murine and human prion diseases is a further consequence of chemokine overexpression with a possible influence on the disease development.⁴³

Several other aspects link the astrocytosis to scrapie pathogenesis. First of all, *in vitro* experiments demonstrated that astrocyte activation is associated with the secretion of neurotoxic factors.¹⁰ Thus, the scrapie-associated, in part IL-1-driven, astrocytosis could promote disease development. Furthermore, the observation that PrP-expression specifically targeted to astrocytes is sufficient to render resistant PrP knockout mice susceptible to scrapie suggests that PrP-expressing astrocytes are a relevant reservoir for prion replication *in vivo*.⁴⁴ In addition, the accumulation of PrP^{Sc} was previously described to occur in astrocytes in scrapie-infected mice and hamsters.^{45,46} Therefore, the delayed PrP^{Sc} deposition in the

IL-1RI^{-/-} mice may be linked to the reduced astrocytosis in these animals (Figures 2, 3, and 5).

As outlined in the introduction, microglia are likely to be initially activated by the PrP^{Sc} deposition. Accordingly, both the IL-1RI^{-/-} mice and the wild-type controls developed a vigorous microgliosis in response to the ongoing PrP^{Sc} accumulation (Figures 4 and 5). Thus, IL-1 is not essential for microglia activation during infections with the scrapie agent. In contrast to this model of an amyloid-driven microgliosis the microglia activation in acute neurodegeneration after brain injury is primarily IL-1-dependent.¹⁵

It is however less clear why the microglia activation is moderately more pronounced in the IL-1RI^{-/-} mice than in the wild-type controls. Interestingly, microglia activation and function can be negatively affected by soluble

factors produced by astrocytes as well as by direct astrocytic contact to microglia.^{47–50} Hence, the more pronounced microgliosis in the IL-1RI^{−/−} mice could be because of the reduced astrocytosis in these animals and possibly affects the deposition of PrP^{Sc} by enhanced microglial phagocytosis of amyloid.^{51–54}

Our observations indicate that IL-1 acts as a driver of the scrapie-associated activation of astrocytes during early stages of the disease. Although IL-1 is clearly not essential for the development of the ultimately lethal neurodegeneration (Table 1), the experimental model suggests that therapeutic agents that suppress astrocyte activation and/or glial IL-1 expression may help to delay disease onset in established prion infections of the central nervous system. In addition, our study encourages further research efforts to identify other host factors, which participate in disease development and in particular in the deleterious inflammatory aspects of the disease-associated gliosis.

Acknowledgments

We thank K. Krohn, S. Lichy, and H. Wohler for expert technical assistance; U. Erikli for critical reading of the manuscript; and U. Mönning and N. Holtkamp for helpful discussions.

References

1. Prusiner SB: Prions. *Proc Natl Acad Sci USA* 1998, 95:13363–13383
2. Chesebro B: Prion protein and the transmissible spongiform encephalopathy diseases. *Neuron* 1999, 24:503–506
3. Rezaie P, Lantos PL: Microglia and the pathogenesis of spongiform encephalopathies. *Brain Res Brain Res Rev* 2001, 35:55–72
4. Williams AE, Lawson LJ, Perry VH, Fraser H: Characterization of the microglial response in murine scrapie. *Neuropathol Appl Neurobiol* 1994, 20:47–55
5. Williams A, Van Dam AM, Ritchie D, Eikelenboom P, Fraser H: Immunocytochemical appearance of cytokines, prostaglandin E2 and lipocortin-1 in the CNS during the incubation period of murine scrapie correlates with progressive PrP accumulations. *Brain Res* 1997, 754:171–180
6. Riemer C, Queck I, Simon D, Kurth R, Baier M: Identification of upregulated genes in scrapie-infected brain tissue. *J Virol* 2000, 74:10245–10248
7. Meda L, Baron P, Scarlato G: Glial activation in Alzheimer's disease: the role of Abeta and its associated proteins. *Neurobiol Aging* 2001, 22:885–893
8. Forloni G, Angeretti N, Chiesa R, Monzani E, Salmona M, Bugiani O, Tagliavini F: Neurotoxicity of a prion protein fragment. *Nature* 1993, 362:543–546
9. Brown DR, Schmidt B, Kretschmar HA: Role of microglia and host prion protein in neurotoxicity of a prion protein fragment. *Nature* 1996, 380:345–347
10. Brown DR: Prion protein peptide neurotoxicity can be mediated by astrocytes. *J Neurochem* 1999, 73:1105–1113
11. Hafiz FB, Brown DR: A model for the mechanism of astrogliosis in prion disease. *Mol Cell Neurosci* 2000, 16:221–232
12. Mabbott NA, Williams A, Farquhar CF, Pasarakis M, Kollias G, Bruce ME: Tumor necrosis factor alpha-deficient, but not interleukin-6-deficient, mice resist peripheral infection with scrapie. *J Virol* 2000, 74:3338–3344
13. Kim JI, Ju WK, Choi JH, Choi E, Carp RI, Wisniewski HM, Kim YS: Expression of cytokine genes and increased nuclear factor-kappa B activity in the brains of scrapie-infected mice. *Brain Res Mol Brain Res* 1999, 73:17–27
14. Allan SM, Rothwell NJ: Cytokines and acute neurodegeneration. *Nat Rev Neurosci* 2001, 2:734–744
15. Basu A, Krady JK, O'Malley M, Styren SD, DeKosky ST, Levison SW: The type 1 interleukin-1 receptor is essential for the efficient activation of microglia and the induction of multiple proinflammatory mediators in response to brain injury. *J Neurosci* 2002, 22:6071–6082
16. Griffin WS, Sheng JG, Roberts GW, Mrak RE: Interleukin-1 expression in different plaque types in Alzheimer's disease: significance in plaque evolution. *J Neuropathol Exp Neurol* 1995, 54:276–281
17. Griffin WS, Mrak RE: Interleukin-1 in the genesis and progression of and risk for development of neuronal degeneration in Alzheimer's disease. *J Leukoc Biol* 2002, 72:233–238
18. Peyrin JM, Lasmezas CI, Haik S, Tagliavini F, Salmona M, Williams A, Richie D, Deslys JP, Dormont D: Microglial cells respond to amyloidogenic PrP peptide by the production of inflammatory cytokines. *Neuroreport* 1999, 10:723–729
19. Meda L, Baron P, Prat E, Scarpini E, Scarlato G, Cassatella MA, Rossi F: Proinflammatory profile of cytokine production by human monocytes and murine microglia stimulated with beta-amyloid[25–35]. *J Neuroimmunol* 1999, 93:45–52
20. Campbell IL, Eddleston M, Kemper P, Oldstone MB, Hobbs MV: Activation of cerebral cytokine gene expression and its correlation with onset of reactive astrocyte and acute-phase response gene expression in scrapie. *J Virol* 1994, 68:2383–2387
21. Brown AR, Webb J, Rebus S, Walker R, Williams A, Fazakerley JK: Inducible cytokine gene expression in the brain in the ME7/CV mouse model of scrapie is highly restricted, is at a strikingly low level relative to the degree of gliosis and occurs only late in disease. *J Gen Virol* 2003, 84:2605–2611
22. Lim GP, Yang F, Chu T, Chen P, Beech W, Teter B, Tran T, Ubeda O, Ashe KH, Frautschy SA, Cole GM: Ibuprofen suppresses plaque pathology and inflammation in a mouse model for Alzheimer's disease. *J Neurosci* 2000, 20:5709–5714
23. Apelt J, Schliebs R: Beta-amyloid-induced glial expression of both pro- and anti-inflammatory cytokines in cerebral cortex of aged transgenic Tg2576 mice with Alzheimer plaque pathology. *Brain Res* 2001, 894:21–30
24. Kimberlin RH, Cole S, Walker CA: Pathogenesis of scrapie is faster when infection is intraspinal instead of intracerebral. *Microbiol Pathog* 1987, 2:405–415
25. Kaplan EL, Meier P: Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958, 53:457–481
26. Peto R, Pike MC, Armitage P, Breslow NE, Cox DR, Howard SV, Mantel N, McPherson K, Peto J, Smith PG: Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. Analysis and examples. *Br J Cancer* 1977, 35:1–39
27. Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987, 162:156–159
28. Jeffrey M, Goodsir CM, Holliman A, Higgins RJ, Bruce ME, McBride PA, Fraser JR: Determination of the frequency and distribution of vascular and parenchymal amyloid with polyclonal and N-terminal-specific PrP antibodies in scrapie-affected sheep and mice. *Vet Rec* 1998, 142:534–537
29. Schulz-Schaeffer WJ, Tschoke S, Kranefuss N, Droese W, Hause-Reitner D, Giese A, Groschup MH, Kretschmar HA: The paraffin-embedded tissue blot detects PrP(Sc) early in the incubation time in prion diseases. *Am J Pathol* 2000, 156:51–56
30. Black RA, Kronheim SR, Cantrell M, Deeley MC, March CJ, Prickett KS, Wignall J, Conlon PJ, Cosman D, Hopp TP, Mochizuki DY: Generation of biologically active interleukin-1 beta by proteolytic cleavage of the inactive precursor. *J Biol Chem* 1988, 263:9437–9442
31. Carp RI, Meeker H, Sersen E: Scrapie strains retain their distinctive characteristics following passages of homogenates from different brain regions and spleen. *J Gen Virol* 1997, 78:283–290
32. O'Neill LA, Dinarello CA: The IL-1 receptor/toll-like receptor superfamily: crucial receptors for inflammation and host defense. *Immunol Today* 2000, 21:206–209
33. Labow M, Shuster D, Zetterstrom M, Nunes P, Terry R, Cullinan EB, Bartfai T, Solorzano C, Moldawer LL, Chizzonite R, McIntyre KW: Absence of IL-1 signaling and reduced inflammatory response in IL-1 type I receptor-deficient mice. *J Immunol* 1997, 159:2452–2461
34. Asensio VC, Maier J, Milner R, Boztug K, Kincaid C, Moulard M, Phillipson C, Lindsley K, Krucker T, Fox HS, Campbell IL: Interferon-

- independent, human immunodeficiency virus type 1 gp120-mediated induction of CXCL10/IP-10 gene expression by astrocytes in vivo and in vitro. *J Virol* 2001, 75:7067–7077
35. Salmaggi A, Gelati M, Dufour A, Corsini E, Pagano S, Baccalini R, Ferrero E, Scabini S, Silei V, Ciusani E, De Rossi M: Expression and modulation of IFN-gamma-inducible chemokines (IP-10, Mig, and I-TAC) in human brain endothelium and astrocytes: possible relevance for the immune invasion of the central nervous system and the pathogenesis of multiple sclerosis. *J Interferon Cytokine Res* 2002, 22:631–640
36. Ehlers MR: CR3: a general purpose adhesion-recognition receptor essential for innate immunity. *Microbes Infect* 2000, 2:289–294
37. Walsh DT, Betmouni S, Perry VH: Absence of detectable IL-1beta production in murine prion disease: a model of chronic neurodegeneration. *J Neuropathol Exp Neurol* 2001, 60:173–182
38. Wyss-Coray T, Loike JD, Brionne TC, Lu E, Anankov R, Yan F, Silverstein SC, Husemann J: Adult mouse astrocytes degrade amyloid-beta in vitro and in situ. *Nat Med* 2003, 9:453–457
39. Oh JW, Schwiebert LM, Benveniste EN: Cytokine regulation of CC and CXC chemokine expression by human astrocytes. *J Neurovirol* 1999, 5:82–94
40. Cole KE, Strick CA, Paradis TJ, Ogborne KT, Loetscher M, Gladue RP, Lin W, Boyd JG, Moser B, Wood DE, Sahagan BG, Neote K: Interferon-inducible T cell alpha chemoattractant (I-TAC): a novel non-ELR CXC chemokine with potent activity on activated T cells through selective high affinity binding to CXCR3. *J Exp Med* 1998, 187:2009–2021
41. Xia MQ, Bacskaï BJ, Knowles RB, Qin SX, Hyman BT: Expression of the chemokine receptor CXCR3 on neurons and the elevated expression of its ligand IP-10 in reactive astrocytes: in vitro ERK1/2 activation and role in Alzheimer's disease. *J Neuroimmunol* 2000, 108:227–235
42. Rappert A, Biber K, Nolte C, Lipp M, Schubel A, Lu B, Gerard NP, Gerard C, Boddeke HW, Kettenmann H: Secondary lymphoid tissue chemokine (CCL21) activates CXCR3 to trigger a C1– current and chemotaxis in murine microglia. *J Immunol* 2002, 168:3221–3226
43. Lewicki H, Tishon A, Homann D, Mazarguil H, Laval F, Asensio VC, Campbell IL, DeArmond S, Coon B, Teng C, Gairin JE, Oldstone MB: T cells infiltrate the brain in murine and human transmissible spongiform encephalopathies. *J Virol* 2003, 77:3799–3808
44. Raeber AJ, Race RE, Brandner S, Priola SA, Sailer A, Bessen RA, Mucke L, Manson J, Aguzzi A, Oldstone MB, Weissmann C, Chesebro B: Astrocyte-specific expression of hamster prion protein (PrP) renders PrP knockout mice susceptible to hamster scrapie. *EMBO J* 1997, 16:6057–6065
45. Diedrich JF, Bendheim PE, Kim YS, Carp RI, Haase AT: Scrapie-associated prion protein accumulates in astrocytes during scrapie infection. *Proc Natl Acad Sci USA* 1991, 88:375–379
46. Ye X, Scallet AC, Kascsak RJ, Carp RI: Astrocytosis and amyloid deposition in scrapie-infected hamsters. *Brain Res* 1998, 809:277–287
47. DeWitt DA, Perry G, Cohen M, Doller C, Silver J: Astrocytes regulate microglial phagocytosis of senile plaque cores of Alzheimer's disease. *Exp Neurol* 1998, 149:329–340
48. Heyen JR, Ye S, Finck BN, Johnson RW: Interleukin (IL)-10 inhibits IL-6 production in microglia by preventing activation of NF-kappaB. *Brain Res Mol Brain Res* 2000, 77:138–147
49. Hailer NP, Heppner FL, Haas D, Nitsch R: Astrocytic factors deactivate antigen presenting cells that invade the central nervous system. *Brain Pathol* 1998, 8:459–474
50. Hailer NP, Glomsda B, Blaheta RA: Astrocytic factors down-regulate the expression of major histocompatibility complex-class-II and intercellular adhesion molecule-1 on human monocytes. *Neurosci Lett* 2001, 298:33–36
51. Carp RI, Callahan SM: Effect of mouse peritoneal macrophages on scrapie infectivity during extended in vitro incubation. *Intervirology* 1982, 17:201–207
52. Beringue V, Demoy M, Lasmezas CI, Gouritin B, Weingarten C, Deslys JP, Andreux JP, Couvreur P, Dormont D: Role of spleen macrophages in the clearance of scrapie agent early in pathogenesis. *J Pathol* 2000, 190:495–502
53. Bard F, Cannon C, Barbour R, Burke RL, Games D, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Lieberburg I, Motter R, Nguyen M, Soriano F, Vasquez N, Weiss K, Welch B, Seubert P, Schenk D, Yednock T: Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. *Nat Med* 2000, 6:916–919
54. Wyss-Coray T, Lin C, Yan F, Yu GQ, Rohde M, McConlogue L, Masliah E, Mucke L: TGF-beta1 promotes microglial amyloid-beta clearance and reduces plaque burden in transgenic mice. *Nat Med* 2001, 7:612–618