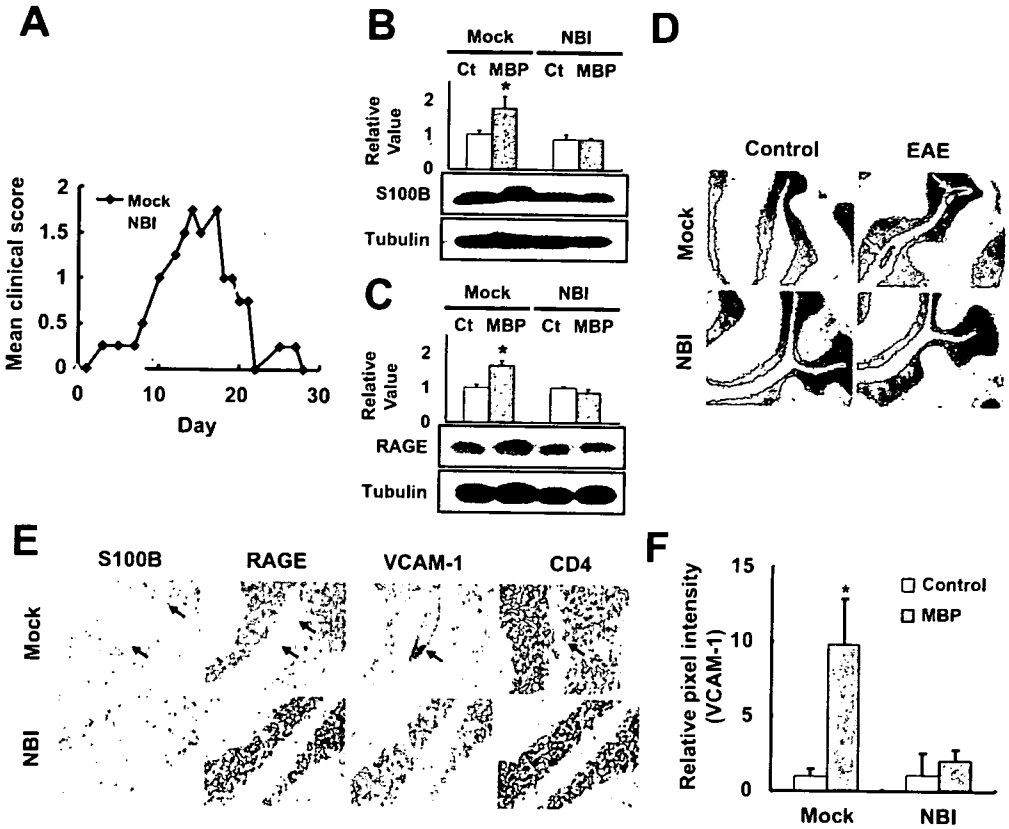


FIG. 4. Vascular inflammatory responses in EAE-induced rat brains. (A) Clinical symptoms of EAE. The means of daily clinical scores are shown (see Materials and Methods). Expressions of S100B (B) and RAGE (C) in the cerebellum of the BDV-infected rats at 14 days after the MBP injection are shown. Quantitative analyses of the expressions are also shown. The band intensities were determined by NIH image. Values were normalized to the tubulin level. (*, $P < 0.05$ with PBS-injected age-matched control [Ct] rats). Mock, age-matched mock-infected rats. (D) Neuropathological analysis of MBP-injected rats. Brain sections were stained with H&E. Arrows indicate the regions of vasodilatation in the cerebellum of a mock-infected, EAE-induced rat. (E) IHC analysis of EAE-induced rat brains. Serial brain sections from the cerebellar regions were stained with anti-S100B, RAGE, VCAM-1, and CD4 antibody. Magnification, ×200. Arrows indicate positive signals in the perivascular regions. (F) Quantification of VCAM-1 expression in rat brains. For a quantitative analysis of VCAM-1 levels in the brain, the optical density of positive signals was measured as described in Materials and Methods. All optical density measurements were performed under the same optical and lighting conditions. The relative pixel intensities to PBS-injected control animals are shown. (*, $P < 0.05$).

positive and -negative cells in the cerebellum (Fig. 3B). These results indicated that S100B signaling may be disturbed in the persistently infected NBI rat brains.

**Reduced vascular inflammatory responses in the brains of persistently infected rats immunized with MBP.** S100B signaling is also known to induce inflammatory reactions through the activation of adhesion molecules on vascular endothelial cells via binding to RAGE (4, 15, 46, 51). The inhibition of RAGE signaling is also known to suppress EAE via a selective blockage of encephalitogenic T-cell infiltration in the mouse CNS (51). These findings give rise to the possibility that brains persistently infected with BDV may have attenuated vascular inflammatory responses to inflammatory stimuli. To understand this, we examined the development of EAE in NBI rats immunized with MBP. We focused on the cerebellum region because this region showed relatively clear vascular inflammatory responses in control rat brains by MBP injection. Following immunization at 7 weeks p.i., NBI rats exhibited a delayed progression of EAE and appeared to induce a significantly less severe EAE than MBP-immunized, mock-infected rats over the observation period (Fig. 4A). We detected the expression of S100B, as well as RAGE, in the cerebellum at 14 days after the sensitization. As shown in Fig. 4B and C, no upregulation of both S100B and RAGE expressions was detected in the MBP-immunized, NBI rat cerebellum, while a significant increase in levels of the proteins was detected in the mock-infected rat brains. The H&E staining revealed that the EAE-developed, mock-infected rats had an apparently increased (in terms of number and diameter) cerebellar vascularity compared with that of the MBP-sensitized NBI rats (Fig. 4D, arrows). The IHC revealed that the immunized, mock-infected rats showed strong immunoreactivity to S100B, RAGE, and VCAM-1 in the perivascular area or vascular endothelium in comparison with that of BDV-positive rats (Fig. 4E, arrows, and F). Furthermore, a large number of CD4-positive cells were found around the neovessels in mock-infected animals (Fig. 4E, arrow). This observation suggested that the persistent infection may prevent the adhesion molecule from being expressed on vascular endothelial cells through the downregulation of S100B expression, resulting in reduced responses during vascular inflammation in the brain.

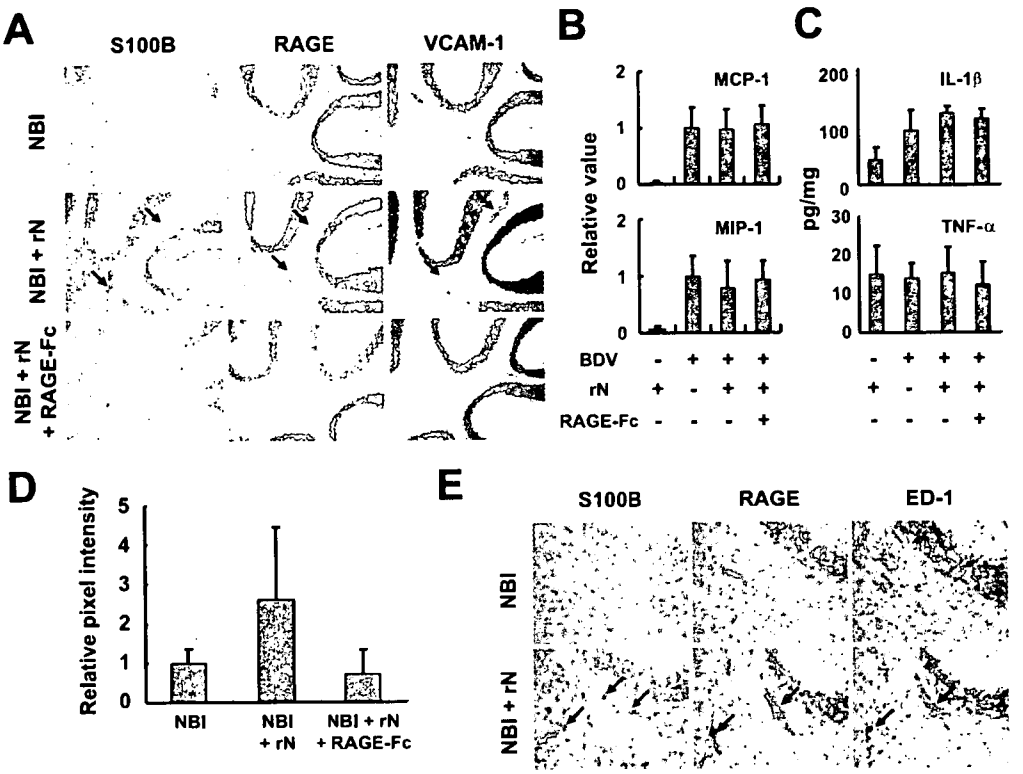


FIG. 5. Expression of S100B is involved in vascular inflammatory responses in BDV-infected neonatal rat brains. (A) IHC analysis of rN-immunized rat brains. The immunized NBI rats were treated with or without RAGE-Fc and sacrificed at 14 days after the immunization (5 weeks p.i.). Serial brain sections from the cerebellum regions were stained with anti-S100B, RAGE, and VCAM-1 antibody. Magnification, ×100. Arrows indicate positive perivascular regions. Chemokine (B) and cytokine (C) expressions in rN-immunized rat brains. Expressions of MIP-1β and MCP-1 were monitored in the cerebellum by semiquantitative RT-PCR at 14 days after the rN immunization. The band intensities were determined by NIH image, and values were normalized to the GAPDH mRNA level. (C) Levels of IL-1β and TNF-α expression in the cerebellum were estimated with ELISA kits at 14 days after the rN immunization. (D) Quantification of VCAM-1 expression in rat brains. The relative pixel intensities of NBI animals at 5 weeks p.i. are shown. (E) Induction of mononuclear cell infiltration by rN-immunized rat cerebellum. Infiltrated mononuclear cells positive for ED-1 are found in perivascular areas (arrows). IHC results of S100B and RAGE are also shown.

**S100B signaling is necessary for the development of mononuclear cell infiltrates in NBI rat brains.** The results above suggested that the downregulation of S100B expression may be responsible for the elimination of the vascular inflammatory responses, as well as the infiltration of encephalitogenic T cells, in persistently infected NBI brains. Therefore, we sought to determine whether the expression level of endogenous S100B is associated with the development of the mononuclear cell infiltrates in the NBI brain. Previous studies have demonstrated that immunization of the viral antigen into BDV-infected animals maintains inflammatory responses in the brains (9, 12). Thus, we immunized NBI rats with rN antigen at 3 weeks p.i. to continuously stimulate the activation of astrocytes, as well as the expression of S100B, in the brain. The immunization at 3 weeks p.i successfully revealed enhanced immunoreactivity of S100B in the perivascular CNS areas even at 5 weeks p.i. compared with that of nonimmunized NBI rats (Fig. 5A and E, arrows). On the other hand, the expression levels of several chemokines and proinflammatory cytokines were quite similar to those of the rN-immunized and nonimmunized, BDV-infected rat brains at 5 weeks p.i. (Fig. 5B and C). In the immunized rat brains, however, an apparently extensive vasodilatation along with intense immunoreactivity of RAGE and VCAM-1 in the vascular endothelium was found (Fig. 5A, arrows, and D). In addition, the immunoreactivity of ED-1 was found in the rN-injected animals, both in the perivascular tissue and the parenchyma (Fig. 5E, arrows). To understand the role of S100B signaling in the development of the vascular responses, we blocked the S100B-RAGE interaction by injecting RAGE-Fc at 2-day intervals from the day of immunization with rN. Previous studies have demonstrated that a low level of endogenous soluble RAGE exists in normal plasma, and the injection of rat RAGE-Fc does not induce any immune responses (34, 51, 52). Interestingly, interference of S100B-RAGE interaction by the repeated i.p. injection of RAGE-Fc drastically repressed the vascular responses, as well as the vasodilatation, in the rN-inoculated rats (Fig. 5A and D), indicating that the S100B signaling may be important for the progression of vascular inflammation in NBI rat brain.

## DISCUSSION

In the present study, we demonstrated that the expression of an astrocyte-derived factor, S100B, is reduced in both the cerebral cortex and cerebellum of Lewis rat brains persistently infected with BDV. Although the level of S100B secreted

within the rat CNS could not be determined in this experiment, the immunoreactivity level appears to correlate with the amount of this protein secreted in the brain (30). The downregulation of S100B expression is likely to affect the expression of RAGE in the persistently infected brains, suggesting that the secretion of S100B could also be disrupted in the CNS. On the other hand, a significant upregulation of GFAP expression, as well as the chronic production of proinflammatory cytokines and chemokines, was demonstrated in the persistently infected brains, indicating that the downregulation of S100B expression is not due to the deletion of astrocytes in the CNS.

A decreased level of S100B is observed in both NBI and ABI brains, suggesting that the downregulation may be associated with the duration of CNS inflammatory responses in the CNS, such as a chronic expression of inflammatory mediators. A recent study found that treatment with an inflammatory cytokine, gamma interferon (IFN-γ), which has been shown to activate astrocytes to acquire immune functions, downregulates S100B gene expression in primary mouse astrocytes, by using a microarray (11). This observation suggested that a negative regulatory effect of cytokines to immune responsive cells contributes to the downregulation of S100B in the brains. In addition, it has been reported that reactive astrocytes are regulated by the signal transducer and activator of transcription 3 (STAT3) and the protein suppressor of cytokine signaling 3 (SOCS3) (27). Considering that the expression of S100B is also controlled by the STAT3-SOCS3 signaling in the CNS (13), the downregulation of S100B may be caused by the aberration of the signaling via sustained activation of astrocytes. It has also been reported that continual release of S100B during chronic brain stresses directly causes S100B deprivation within astrocytes (10), suggesting that depletion of S100B protein may occur in astrocytes of persistently infected brains. Further study will be needed to elucidate the regulatory mechanism underlying S100B downregulation in the persistently infected brain.

Our analysis using immunoblotting demonstrated that the reduction of the S100B level in persistently infected brains is statistically significant, but the overall difference seems to be modest. The NBI rats showed about 30% reduction of S100B in both the cerebral cortex and cerebellum. A previous study revealed that prenatal stresses induced a 25% reduction in hippocampal S100B content in rats, leading to abnormal postnatal brain development (47). In addition, a significant suppression of S100B (a 24% decrease) by arundic acid (ONO-2506) in the brains of transgenic mice, which overproduce a mutant form of amyloid precursor protein, was markedly ameliorated in β-amyloid plaque burden and amyloid-β peptide levels (24). In these experiments, although expression levels of many other genes associated with neurodevelopment or brain damage could also be changed in the brains, some correlations were observed between the expression level of S100B and specific neurological effects in the animals (24, 26, 47). These observations suggested that even at a moderate level, altered expression of S100B could affect astrocyte activities, resulting in specific biological effects in the brains. On the other hand, in this study we examined the effects of S100B in the brains of NBI rats exposed to LPS or MBP. Interestingly, no upregulation of S100B expression was found in the NBI rat brains by the injection of the immune stimuli, while the protein expression increases about 75% in mock-infected brains (Fig. 2B and Fig. 4B). Along with the modest reduction of S100B at the basal level, the unresponsiveness of S100B expression indicated that the level of this protein is considerably lower in the brains of stimulated NBI rats than control rats, suggesting that the reduced level of S100B has potentially important implications for biological significances found in the NBI brains.

S100B is an EF-hand type $Ca^{2+}$-binding multifunctional protein in the CNS. This protein exerts autoparacrine and paracrine effects on neurons and glia and is involved in a variety of cellular responses, such as protein phosphorylation, cell proliferation and differentiation, the structural organization of membranes, cytoskeleton modifications, intracellular $Ca^{2+}$ homeostasis, and the promotion of cell survival (1, 37, 40, 41). In this experiment, we found that the administration of LPS at 5 weeks p.i. does not enhance the expression level of S100B in NBI rat brains and causes significant cell death of both neurons and glia in the cerebellum. The administration of LPS is known to induce CNS stress responses via upregulation of the production of proinflammatory cytokines and nitric oxide (19, 42). The studies using S100B transgenic and knockout mice demonstrated that S100B plays a role in astrocyte proliferation under stressful conditions (3, 6, 32). Considering the neurotrophic effects of reactive astrocytes in inflamed CNS (5, 27, 35), the downregulation of S100B may induce neural cell death as a consequence of impairing astrocyte proliferation in NBI rat brains. In addition, the level of S100B is also known to be directly linked with cellular survival by preventing apoptosis (1, 17, 37, 41). In either mechanism, the brains with downregulated S100B expression might succumb to the stressful environment and cause the apoptosis of neural cells. Previous studies have demonstrated that in BDV-infected neonatal rat brains, reactivation of glial cells may be associated with specific neuronal cell apoptosis rather than viral tropism and virus-specific immune responses (29, 50). These studies support our hypothesis that sustained activation of astrocytes in the persistently infected brains may exhaust the glial functions concerned with cellular survival. Elucidation of how the downregulation of S100B expression is involved in the functional exhaustion of astrocytes may be important to understanding the pathology of neurodegenerative disorders.

A prominent role of this protein seems to be the promotion of inflammation through binding to RAGE as an inflammatory cytokine (36, 37, 46, 51). Expression of S100B is associated with the activation of astrocytes followed by damage to the CNS, during which production of proinflammatory cytokines, such as IL-1β, is increased (6, 22). Stimulation of RAGE signaling through the binding of S100B can lead to the activation of MAP kinase and increased NF-κB activity (17, 22). The RAGE-S100B interaction was confirmed to induce MCP-1 expression, which is often associated with localized inflammation (2, 46). Furthermore, the signaling also enhances the expression of cell adhesion molecules, including VCAM-1, on vascular endothelial cells at inflammatory sites, which is recognized by the integrin VLA-4 expressed on lymphocytes and monocytes, resulting in infiltration by the cells (15, 46, 51). Thus, there is a large body of evidence suggesting that S100B is involved in the development and/or amplification of CNS-based inflammatory responses via modification of the expression of the molecules on the vascular endothelium (46, 51),

indicating that the regulation of S100B expression in the injured CNS plays a key role in determining the severity of inflammation in the brain. We found that the downregulation prevents severe EAE in MBP-injected NBI rats. In the rat brain, no significant immunoreactivity of S100B or RAGE was observed in the perivascular areas and vascular endothelium, respectively. The lack of vascular inflammatory reactions may result in a reduction in vascularity and mononuclear cell infiltration in the NBI rat brain. This observation strongly suggests that the activation of S100B is required for the progression of the vascular inflammatory responses, followed by encephalitogenic T-cell infiltration in MBP-immunized rat brains.

In contrast with the MBP immunization, the rN-injected NBI rat brains developed severe vasodilatation and mononuclear cell infiltration in the cerebellum, with the expression of RAGE and VCAM-1 on the vascular endothelium. The role of S100B in the inflammatory reactions in the rN-injected rats was confirmed by the experiment in which the binding of S100B to RAGE was interfered with following repeated administration of RAGE-Fc. These results also implied an important role for S100B signaling in the progression of the vascular inflammatory reaction in the NBI rat brain. Intriguingly, the rN injection into NBI rats successfully upheld the strong immunoreactivity of S100B in the perivascular areas of the cerebellum at 5 weeks p.i. This may be because upon the injection of rN at 3 weeks p.i., astrocytes can continue to retain a phenotype characteristic of astrocytosis with the reactivity of S100B in the rat brain. On the other hand, the MBP immunization at 7 weeks p.i. could not reactivate the astrocytes and S100B expression in the NBI brains. These results may indicate that continuous stimulation with initial antigens is necessary to maintain intact astrocyte activation in the brains. This hypothesis is under investigation using NBI rats at 3 weeks p.i. and immunization with several different antigens.

Our results strongly suggested that downregulation of S100B in NBI rat brains is considerably responsible for the neural cell death and reduced vascular inflammatory responses found in the brains. It should be noted, however, that a large number of different genes could also be altered in BDV-infected rat brains. These altered factors could make it difficult to assess the effects related only to the expression level of S100B in the brains. On the other hand, in this study we focused on NBI instead of ABI brains to observe the effects of S100B downregulation. This is because vascular inflammatory responses by additional stimuli could be easy to detect in NBI brains, in which background infiltration and vascular immune response are totally lacking. As described above, however, it is also certain that NBI rat brain shows a complex environment, with alteration of many different factors associated with abnormal neurodevelopment (16, 53). A comprehensive analysis using a proteomics technique and/or a comparative experiment between NBI and ABI rat brains would be helpful for further evaluation of biological importance of S100B downregulation in persistently infected brains. Such studies will be challenged in the future.

In conclusion, we demonstrated that the expression of an astrocyte-derived inflammatory cytokine, S100B, was downregulated in rat brains persistently infected with BDV. At present, we can only speculate as to the impact of this downregulation, owing to the multiple functions of this protein in the CNS. However, our results are consistent with observations from previous reports that the expression of S100B is involved in the induction of vascular immune responses and the infiltration of encephalitogenic T cells in the CNS of experimental animals (46, 51), implying that BDV may evade CNS-based immune responses through the regulation of astrocyte functions by downregulating the expression of S100B in the brain, resulting in a persistent infection. Given that the downregulation of S100B expression is established as a common course of chronic activation of astrocytes in virus-infected brain, the phenomenon may also be involved in the mechanism of persistent infection of other CNS viruses, such as lymphocytic choriomeningitis virus (28). Interestingly, in patients with human immunodeficiency virus and human T-cell leukemia virus type 1 infections, both of which show severe degenerative effects on brain functions, an increased level of S100B release from activated astrocytes is observed (33, 44). These findings may represent a depletion of S100B protein in the reactive astrocytes of patient brains. The analysis of the regulation of S100B expression in astrocytes would be important to understand the role of this protein in the neuropathogenesis of persistent virus infections. Recently, a remarkable therapeutic approach, referred to as immunocytotherapy, has been discussed as a treatment to eliminate persistent viral infection from the CNS (23). Although the direct role of S100B in the regulation of T-cell responses remains to be elucidated, it might be possible that the abnormal level of S100B influences antiviral functions of transferred T lymphocytes in the CNS immunocytotherapy. Further experiments would be necessary to understand the involvement of the downregulation of S100B in the persistent mechanism of CNS viruses, as well as the therapeutic approach for persistent viral infection, in the brain.

## ACKNOWLEDGMENTS

This research was partially supported by the Program of Founding Research Centers for Emerging and Reemerging Infectious Diseases from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan; by Grants-in-Aid for Scientific Research (B) 15390148 and 18390139; a Grant-in-Aid from the Zoonosis Control Project of the Ministry of Agriculture, Forestry and Fisheries of Japan; and a research grant for Nervous and Mental Disorders from the Ministry of Health, Labor and Welfare of Japan.

## REFERENCES

1. **Baudier, J., C. Delphin, D. Grunwald, S. Khochbin, and J. J. Lawrence.** 1992. Characterization of the tumor suppressor protein p53 as a protein kinase C substrate and a S100b-binding protein. Proc. Natl. Acad. Sci. USA **89**:11627–11631.
2. **Bucciarelli, L. G., T. Wendt, W. Qu, Y. Lu, E. Lalla, L. L. Rong, M. T. Goova, B. Moser, T. Kislinger, D. C. Lee, Y. Kashyap, D. M. Stern, and A. M. Schmidt.** 2002. RAGE blockade stabilizes established atherosclerosis in diabetic apolipoprotein E-null mice. Circulation **106**:2827–2835.
3. **Chang, M. S., L. M. Ariah, A. Marks, and E. C. Azmitia.** 2005. Chronic gliosis induced by loss of S-100B: knockout mice have enhanced GFAP-immunoreactivity but blunted response to a serotonin challenge. Brain Res. **1031**:1–9.
4. **Chavakis, T., A. Bierhaus, N. Al-Fakhri, D. Schneider, S. Witte, T. Linn, M. Nagashima, J. Morser, B. Arnold, K. T. Preissner, and P. P. Nawroth.** 2003. The pattern recognition receptor (RAGE) is a counterreceptor for leukocyte integrins: a novel pathway for inflammatory cell recruitment. J. Exp. Med. **198**:1507–1515.
5. **Chen, Y., and R. A. Swanson.** 2003. Astrocytes and brain injury. J. Cereb. Blood Flow Metab. **23**:137–149.
6. **Craft, J. M., D. M. Watterson, A. Marks, and L. J. Van Eldik.** 2005. Enhanced susceptibility of S-100B transgenic mice to neuroinflammation and neuronal dysfunction induced by intracerebroventricular infusion of human beta-amyloid. Glia **51**:209–216.
7. **Dong, Y., and E. N. Benveniste.** 2001. Immune function of astrocytes. Glia **36**:180–190.

8. **Engelhardt, K. R., K. Richter, K. Baur, P. Staeheli, and J. Hausmann.** 2004. The functional avidity of virus-specific CD8+ T cells is down-modulated in Borna disease virus-induced immunopathology of the central nervous system. Eur. J. Immunol. **35**:487–497.
9. **Fassnacht, U., A. Ackermann, P. Staeheli, and J. Hausmann.** 2004. Immunization with dendritic cells can break immunological ignorance toward a persisting virus in the central nervous system and induce partial protection against intracerebral viral challenge. J. Gen. Virol. **85**:2379–2387.
10. **Gerlach, R., G. Demel, H.-G. König, U. Gross, J. H. M. Prehn, A. Raabe, V. Seifert, and D. Kögel.** 2006. Active secretion of S100B from astrocytes during metabolic stress. Neuroscience **141**:1697–1701.
11. **Halonen, S. K., T. Woods, K. McInnerney, and L. M. Weiss.** 2006. Microarray analysis of IFN-γ response genes in astrocytes. J. Neuroimmunol. **175**: 19–30.
12. **Hausmann, J., W. Hallensleben, J. C. de la Torre, A. Pagenstecher, C. Zimmermann, H. Pircher, and P. Staeheli.** 1999. T cell ignorance in mice to Borna disease virus can be overcome by peripheral expression of the viral nucleoprotein. Proc. Natl. Acad. Sci. USA **96**:9769–9774.
13. **He, F., W. Ge, K. Martinowich, S. Becker-Catania, V. Coskun, W. Zhu, H. Wu, D. Castro, F. Guillemot, G. Fan, J. de Vellis, and Y. E. Sun.** 2005. A positive autoregulatory loop of Jak-STAT signaling controls the onset of astrogliogenesis. Nat. Neurosci. **8**:616–625.
14. **Hofer, M., J. Hausmann, P. Staeheli, and A. Pagenstecher.** 2004. Cerebral expression of interleukin-12 induces neurological disease via differential pathways and recruits antigen-specific T cells in virus-infected mice. Am. J. Pathol. **165**:949–958.
15. **Hofmann, M. A., S. Drury, C. Fu, W. Qu, A. Taguchi, Y. Lu, C. Avila, N. Kambham, A. Bierhaus, P. Nawroth, M. F. Neurath, T. Slattery, D. Beach, J. McClary, M. Nagashima, J. Morser, D. Stern, and A. M. Schmidt.** 1999. RAGE mediates a novel proinflammatory axis: a central cell surface receptor for S100/calgranulin polypeptides. Cell **97**:889–901.
16. **Hornig, M., H. Weissenbock, N. Horscroft, and W. I. Lipkin.** 1999. An infection-based model of neurodevelopmental damage. Proc. Natl. Acad. Sci. USA **96**:12102–12107.
17. **Huttunen, H. J., J. Kuja-Panula, G. Sorci, A. L. Agneletti, R. Donato, and H. Rauvala.** 2000. Coregulation of neurite outgrowth and cell survival by amphoterin and S100 proteins through receptor for advanced glycation end products (RAGE) activation. J. Biol. Chem. **275**:40096–40105.
18. **Ikuta, K., K. Hagiwara, H. Taniyama, and N. Nowotny.** 2002. Epidemiology and infection of natural animal hosts. p. 87–123. *In* K. M. Carbone (ed.), Borna disease virus and its role in neurobehavioral disease. ASM Press, Washington, DC.
19. **Iravani, M. M., C. C. Leung, M. Sadeghian, C. O. Haddon, S. Rose, and P. Jenner.** 2005. The acute and the long-term effects of nigral lipopolysaccharide administration on dopaminergic dysfunction and glial cell activation. Eur. J. Neurosci. **22**:317–330.
20. **Kamitani, W., Y. Shoya, T. Kobayashi, M. Watanabe, B.-J. Lee, G. Zhang, K. Tomonaga, and K. Ikuta.** 2001. Borna disease virus phosphoprotein binds a neurite outgrowth factor, amphoterin/HMG-1. J. Virol. **75**:8742–8751.
21. **Lewis, A. J., J. L. Whitton, C. G. Hatalski, H. Weissenbock, and W. I. Lipkin.** 1999. Effect of immune priming on Borna disease. J. Virol. **73**:2541–2546.
22. **Liu, L., Y. Li, L. J. Van Eldik, W. S. Griffin, and S. W. Barger.** 2005. S100B-induced microglial and neuronal IL-1 expression is mediated by cell type-specific transcription factors. J. Neurochem. **92**:546–553.
23. **McGavern, D. B.** 2006. Immunotherapeutic relief from persistent infections and amyloid disorders. Neurology **66**:S59–S64.
24. **Mori, T., T. Town, J. Tan, N. Yada, Y. Horikoshi, J. Yamamoto, T. Shimoda, Y. Kamanaka, N. Tateishi, and T. Asano.** 2006. Arundic acid ameliorates cerebral amyloidosis and gliosis in Alzheimer transgenic mice. J. Pharmacol. Exp. Ther. **318**:571–578.
25. **Nakamura, Y., H. Takahashi, Y. Shoya, T. Nakaya, M. Watanabe, K. Tomonaga, K. Iwahashi, K. Ameno, N. Momiyama, H. Taniyama, T. Sata, T. Kurata, J. C. de la Torre, and K. Ikuta.** 2000. Isolation of Borna disease virus from human brain tissue. J. Virol. **74**:4601–4611.
26. **Ohtani, R., H. Tomimoto, H. Wakita, H. Kitaguchi, K. Nakaji, and R. Takahashi.** 2007. Expression of S100 protein and protective effect of arundic acid on the rat brain in chronic cerebral hypoperfusion. Brain Res. **1135**: 195–200.
27. **Okada, S., M. Nakamura, H. Katoh, T. Miyao, T. Shimazaki, K. Ishii, J. Yamane, A. Yoshimura, Y. Iwamoto, Y. Toyama, and H. Okano.** 2006. Conditional ablation of Stat3 or Socs3 discloses a dual role for reactive astrocytes after spinal cord injury. Nat. Med. **12**:829–834.
28. **Oldstone, M. B.** 2006. Viral persistence: parameters, mechanisms and future predictions. Virology **344**:111–118.
29. **Ovanesov, M. V., C. Sauder, S. A. Rubin, J. Richt, A. Nath, K. M. Carbone, and M. V. Pletnikov.** 2006. Activation of microglia by Borna disease virus infection: in vitro study. J. Virol. **80**:12141–12148.
30. **Petzold, A., M. J. Eikelenboom, D. Gveric, G. Keir, M. Chapman, R. H. C. Lazeron, M. L. Cuzner, C. H. Polman, B. M. J. Uitdehaag, E. J. Thompson, and G. Giovannoni.** 2002. Markers for different glial cell responses in multiple sclerosis: clinical and pathological correlations. Brain **125**:1462–1473.
31. **Plata-Salaman, C. R., S. E. Ilyin, D. Gayle, A. Romanovitch, and K. M. Carbone.** 1999. Persistent Borna disease virus infection of neonatal rats causes brain regional changes of mRNAs for cytokines, cytokine receptor components and neuropeptides. Brain Res. Bull. **49**:441–451.
32. **Reeves, R. H., J. Yao, M. R. Crowley, S. Buck, X. Zhang, P. Yarowsky, J. D. Gearhart, and D. C. Hilt.** 1994. Astrocytosis and axonal proliferation in the hippocampus of S100b transgenic mice. Proc. Natl. Acad. Sci. USA **91**:5359–5363.
33. **Regner, A., O. Bianchini, C. Jardim, and M. Menna-Barreto.** 2002. HTLV-I-associated myelopathy: are ferritin, S100β protein, or guanine nucleotides CSF markers of disease? J. Neurovirol. **8**:64–67.
34. **Renard, C., O. Chappey, M. P. Wautier, M. Nagashima, E. Lundh, J. Morser, L. Zhao, A. M. Schmidt, J. M. Scherrmann, and J. L. Wautier.** 1997. Recombinant advanced glycation end product receptor pharmacokinetics in normal and diabetic rats. Mol. Pharmacol. **52**:54–62.
35. **Ridet, J. L., S. K. Malhotra, A. Privat, and F. H. Gage.** 1997. Reactive astrocytes: cellular and molecular cues to biological function. Trends Neurosci. **20**:570–577.
36. **Rong, L. L., S. F. Yan, T. Wendt, D. Hans, S. Pachydaki, L. G. Bucciarelli, A. Adebayo, W. Qu, Y. Lu, K. Kostov, E. Lalla, S. D. Yan, C. Gooch, M. Szabolcs, W. Trojaborg, A. P. Hays, and A. M. Schmidt.** 2004. RAGE modulates peripheral nerve regeneration via recruitment of both inflammatory and axonal outgrowth pathways. FASEB J. **18**:1818–1825.
37. **Rothermundt, M., M. Peters, J. H. Prehn, and V. Arolt.** 2003. S100B in brain damage and neurodegeneration. Microsc. Res. Tech. **60**:614–632.
38. **Rott, R., and H. Becht.** 1995. Natural and experimental Borna disease in animals. Curr. Top. Microbiol. Immunol. **190**:17–30.
39. **Sauder, C., and J. C. de la Torre.** 1999. Cytokine expression in the rat central nervous system following perinatal Borna disease virus infection. J. Neuroimmunol. **96**:29–45.
40. **Scotto, C., J. C. Deloulme, D. Rousseau, E. Chambaz, and J. Baudier.** 1998. Calcium and S100B regulation of p53-dependent cell growth arrest and apoptosis. Mol. Cell. Biol. **18**:4272–4281.
41. **Selinfreund, R. H., S. W. Barger, W. J. Pledger, and L. J. Van Eldik.** 1991. Neurotrophic protein S100 beta stimulates glial cell proliferation. Proc. Natl. Acad. Sci. USA **88**:3554–3558.
42. **Semmler, A., T. Okulla, M. Sastre, L. Dumitrescu-Ozimek, and M. T. Heneka.** 2005. Systemic inflammation induces apoptosis with variable vulnerability of different brain regions. J. Chem. Neuroanat. **30**:144–157.
43. **Sheng, J. G., K. Ito, R. D. Skinner, R. E. Mrak, C. R. Rovnaghi, L. J. Van Eldik, and W. S. T. Griffin.** 1996. In vivo and in vitro evidence supporting a role for the inflammatory cytokine interleukin-1 as a driving force in Alzheimer pathogenesis. Neurobiol. Aging **17**:761–766.
44. **Stanley, L. C., R. E. Mrak, R. C. Woody, L. J. Perrot, S. Zhang, D. R. Marshak, S. J. Nelson, and W. S. Griffin.** 1994. Glial cytokines as neuropathogenic factors in HIV infection: pathogenic similarities to Alzheimer's disease. J. Neuropathol. Exp. Neurol. **53**:231–238.
45. **Tramontina, F., S. Conte, D. Gonçalves, C. Gottfried, L. V. Portela, L. Vinade, C. Salbego, and C. A. Gonçalves.** 2002. Developmental changes in S100B content in brain tissue, cerebrospinal fluid, and astrocyte cultures of rats. Cell. Mol. Neurobiol. **22**:373–378.
46. **Valencia, J. V., M. Mone, J. Zhang, M. Weetall, F. P. Buxton, and T. E. Hughes.** 2004. Divergent pathways of gene expression are activated by the RAGE ligands S100b and AGE-BSA. Diabetes **53**:743–751.
47. **Van den Hove, D. L., H. W. Steinbusch, M. Bruschettini, D. Gazzolo, R. Frulio, A. Scheepens, J. Prickaerts, and C. E. Blanco.** 2006. Prenatal stress reduces S100B in the neonatal rat hippocampus. Neuroreport **17**:1077–1080.
48. **Watanabe, M., Q. Zhong, T. Kobayashi, W. Kamitani, K. Tomonaga, and K. Ikuta.** 2000. Molecular ratio between Borna disease viral-p40 and -p24 proteins in infected cells determined by quantitative antigen capture ELISA. Microbiol. Immunol. **44**:765–772.
49. **Watanabe, M., B. J. Lee, W. Kamitani, T. Kobayashi, H. Taniyama, K. Tomonaga, and K. Ikuta.** 2001. Neurological diseases and viral dynamics in the brains of neonatally Borna disease virus-infected gerbils. Virology **282**:65–76.
50. **Weissenbock, H., M. Hornig, W. F. Hickey, and W. I. Lipkin.** 2000. Microglial activation and neuronal apoptosis in Bornavirus infected neonatal Lewis rats. Brain Pathol. **10**:260–272.
51. **Yan, S. S., Z. Y. Wu, H. P. Zhang, G. Furtado, X. Chen, S. F. Yan, A. M. Schmidt, C. Brown, A. Stern, J. LaFaille, L. Chess, D. M. Stern, and H. Jiang.** 2003. Suppression of experimental autoimmune encephalomyelitis by selective blockade of encephalitogenic T-cell infiltration of the central nervous system. Nat. Med. **9**:287–293.
52. **Yonekura, H., Y. Yamamoto, S. Sakurai, R. G. Petrova, M. J. Abedin, H. Li, K. Yasui, M. Takeuchi, Z. Makita, S. Takasawa, H. Okamoto, T. Watanabe, and H. Yamamoto.** 2003. Novel splice variants of the receptor for advanced glycation end products expressed in human vascular endothelial cells and pericytes, and their putative roles in diabetes-induced vascular injury. Biochem. J. **370**:1097–1109.
53. **Zocher, M., S. Czub, J. Schulte-Monting, J. C. de La Torre, and C. Sauder.** 2000. Alterations in neurotrophin and neurotrophin receptor gene expression patterns in the rat central nervous system following perinatal Borna disease virus infection. J. Neurovirol. **6**:462–477.