CD8⁺ Phagocyte Recruitment in Rat Experimental Autoimmune Encephalomyelitis

Association with Inflammatory Tissue Destruction

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Increasing evidence suggests an important role of CD8⁺ cells in the pathogenesis of multiple sclerosis and its animal model experimental autoimmune encephalomyelitis (EAE). In our present study we analyzed the spatiotemporal expression pattern of the CD8 antigen in various rat EAE models characterized by a different extent of inflammation, demyelination, and axonal injury. Unexpectedly, in chronic demyelinating EAE induced by immunization against myelin oligodendrocyte glycoprotein (MOG) the majority of CD8 immunoreactivity was expressed on ED1⁺ microglia/macrophages whereas only limited CD8⁺ T-cell infiltration was present. CD8⁺ phagocyte recruitment was restricted to sites of severe inflammatory tissue destruction. Contrastingly, macrophages in a perivascular or submeningeal position and in secondarily degenerating fiber tracts were mostly CD8⁻. CD8⁺ phagocytes were absent in myelin basic protein-induced EAE characterized by a purely inflammatory pathology and lack of demyelination. Our data demonstrate significant heterogeneity of lesion-associated phagocytes in rat models of central nervous system autoimmune disease and suggest a specific role of CD8⁺ microglia/macrophages in the pathogenesis of inflammatory tissue damage. (*Am J Pathol* 2003, 163:1517–1524)

Central nervous system (CNS) injury in multiple sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis (EAE) has for a long time been considered to be mediated by autoreactive CD4⁺ helper T cells. Only recently, emerging evidence points to a potentially crucial role of CD8⁺ cytotoxic T cells. Molecular studies of both CNS tissue¹ and cerebrospinal fluid² obtained from MS patients showed clonal expansions of CD8⁺ T cells, sug-

gesting antigen-specific proliferation in response to an as yet unidentified CNS autoantigen. Histopathologically, the extent of axonal damage as the likely cause of chronic disability in MS is correlated with the frequency of CD8⁺ cells in affected CNS tissue.³ Moreover, severe demyelinating EAE could be induced in C57/Bl mice by the transfer of CD8⁺ MOG-reactive T-cell lines.⁴

In rats, distinct disease variants of EAE can be distinguished.⁵ MBP-induced EAE in Lewis rats as the classical model of CD4⁺ helper-cell-mediated CNS inflammation is characterized by strong infiltration of T cells and macrophages into the CNS but an almost complete preservation of myelin and axons. Accordingly, the disease takes a self-limiting monophasic course leading to complete recovery of even severely affected animals. Contrastingly, immunization of DA, BN, and certain MHC-congenic rat strains with a recombinant aminoterminal fragment of myelin oligodendrocyte glycoprotein (MOG) leads to chronic progressive or relapsing disease with substantial permanent disability.^{6,7} Detailed morphological studies of MOG-EAE revealed that this model mimics key features of MS-like neuropathology such as demyelination, glial scarring, and axonal damage.⁶ The relative extent of these histopathological findings in different rat strains is genetically determined by factors both within and outside the MHC.^{7,8}

So far, the specific role of CD8⁺ cells for lesion pathogenesis in MOG-EAE is unknown. In the present study, we performed an immunohistochemical analysis of CD8 expression in chronic demyelinating MOG-EAE in comparison to MBP-induced disease as a model of nondemyelinating CNS inflammation. We report the unexpected finding that the majority of CD8 in MOG-induced EAE is not expressed on T cells but instead on a subset of lesion-associated macrophages.

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Materials and Methods

Rat Experiments

All rat experiments were performed in accordance with institutional guidelines. MOG-EAE was induced in 10- to 14-week-old female BN (RT1ⁿ), DA (RT1^{av1}), and LEW.1N (RT1ⁿ) rats by active immunization with the recombinant MOG protein corresponding to the N-terminal sequence of rat MOG (amino acids 1–125) as described previously.^{6,7} For immunohistochemical analysis, rats were perfused with 4% paraformaldehyde between day 27 and 33 (BN, DA) and on day 61 (LEW.1N) after immunization corresponding to maximum disease severity in the chronic disease stage. MBP-EAE was induced in 8-week-old female Lewis rats (Charles River Laboratories, Wilmington, MA) either by active immunization with an encephalitogenic peptide (25 μ g per rat) comprising amino acids 68–86 of guinea pig myelin basic protein (MBP)⁹ or passive transfer of 10⁶ MBP-specific T-cell line cells.¹⁰ Rats were perfused at the peak of clinical disease severity (day 13 after active immunization, day 6 to 7 after T-cell transfer). Spinal cords were dissected out and routinely embedded into paraffin.

Neuropathological Analysis and Immunohistochemistry

To assess inflammation, demyelination, and axonal pathology paraffin sections were stained with hematoxylin and eosin, luxol fast blue, and Bielschowsky silver impregnation, respectively.⁶ For immunohistochemistry, serial 5- μ m paraffin sections were incubated with the following primary antibodies: mAb ED1 against the macrophage-specific CD68 antigen (1:2000; Serotec, Oxford, UK), mAbs Ox-8 (1:1000; Serotec), or 15–11C5 (1:1000; Hycult Biotechnology, Uden, The Netherlands) against the CD8 α chain, and mAb W3/13 against the pan-T cell marker CD43 (1:500; Serotec). Mouse mAb A112–2 (IgG₁) against keyhole limpet hemocyanin (1:1000; PharMingen, Palo Alto, CA) was used as an isotype-matched negative control antibody. Bound antibody was detected using biotinylated horse anti-mouse IgG and the Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA) with diaminobenzidine as substrate according to the manufacturer's protocol.

Double-Labeling Immunofluorescence and Confocal Microscopy

For double-labeling immunofluorescence with two mouse mAbs on the same tissue section we used a sequential staining procedure which is based on the detection of one of the primary antibodies by high-sensitivity tyramide signal amplification.¹¹ In a first step, either mAb Ox-8 or the A112–2 isotype matched negative control mAb were applied at 1:10,000 dilution, followed by donkey anti-mouse horseradish peroxidase (Jackson ImmunoResearch, West Grove, PA) at 1:500 dilution and the TSA Plus Fluorescein System (PerkinElmer Life Sciences, Boston, MA) according to the manufacturer's protocol. In

a second staining round, either mAb ED1 as a macrophage marker (1:500) or mAb 15–6A1 as a pan-T cell marker (1:200; Hycult Biotechnology) were detected by donkey anti-mouse Texas Red (1:100; Jackson ImmunoResearch). Sections were mounted with Vectashield mounting medium (Vector Laboratories) and analyzed using a Leica TCS-NT confocal laser scanning system with an argon-krypton laser on a Leica DM IRB inverted microscope. Images were acquired from two channels at 488 nm and 568 nm wavelength. To analyze the localization of different antigens in double-stained samples, images obtained from the appropriate excitation wavelength were collected and merged.

Results

Distinct Morphology of CD8⁺ Cells in MBP- and MOG-EAE

We first studied the distribution and morphology of CD8⁺ cells in the various models of rat EAE by conventional immunoperoxidase staining with the rat CD8 α -specific mAb Ox-8. In nondemyelinating MBP-EAE, CD8⁺ cells were found with a relatively low frequency and mainly exhibited the morphology of small lymphocyte-like cells which were located in the immediate surroundings of inflamed vessels (Figure 1A). In contrast to MBP-EAE, immunization of DA, BN, or LEW.1N rats with MOG leads to severe demyelination and axonal injury.⁶ In these models, much stronger expression of CD8 was found. Figure 1, B to E, shows typical findings from a DA rat with a large lesion in the dorsal column of the spinal cord. CD8⁺ cells were mainly located in the parenchyma of the demyelinating lesion. At higher magnification, some smaller lymphocyte-like CD8⁺ cells were observed in a perivascular position (arrowheads in Figure 1C). However, the vast majority of CD8⁺ cells were located in the demyelinated parenchyma and had the morphology of large phagocyte-like cells (arrows in Figure 1, C and E). Similar findings were obtained in BN and LEW.1N rats. Control experiments using mAb 15–11C5 as an alternative rat CD8 α -specific mouse mAb yielded identical staining patterns. Conversely, omission of the primary antibodies or their replacement by isotype-matched control mAb A112–2 led to a complete disappearance of immunostaining.

Spatial Pattern of CD8 Expression in MOG-EAE

To study the distribution of CD8⁺ cells in MOG-EAE in more detail we stained serial sections with the cell lineage markers ED1 for phagocytic macrophages and W3/13 for pan-T cells (Figure 2). These studies were done in the LEW.1N strain characterized by particularly strong CD8 expression. Figure 2 is taken from a large demyelinating lesion in the lumbosacral spinal cord. CD8⁺ cell recruitment was most pronounced in central necrotic (dashed line in Figure 2E) and severely demyelinated (dashed line in Figure 2F) lesion areas. Contrastingly, perivascular cuffs (arrows in Figure 2, C and D) and

Figure 1. Distinct pattern of CD8⁺ cell recruitment during nondemyelinating MBP-EAE in Lewis rats (**A**) compared to demyelinating MOG-EAE in DA rats (**B** to **E**). Spinal cord paraffin sections were stained for the CD8 α chain using mAb Ox-8. In MBP-EAE, CD8 expression is overall sparse and localized to small lymphocytes in the immediate surroundings of inflamed vessels (**A**). In MOG-EAE, a large demyelinating lesion developed in the dorsal column of the spinal cord, which is strongly infiltrated by CD8⁺ cells (**B** to **E**). CD8⁺ cell recruitment is not restricted to the perivascular space but extends into the demyelinated CNS parenchyma (**B** and **D**). At higher magnification (**C** and **E**), some small lymphocyte-like CD8⁺ cells can be seen around vessels (arrowheads in **C**) whereas most CD8⁺ cells in the demyelinated lesion area have the distribution and morphology of large round phagocytes (arrows in **C** and **E**).

submeningeal areas (dashed line in in Figure 2C) were densely infiltrated by ED1⁺ macrophages but largely spared from CD8⁺ cell infiltration (Figure 2, E and F). In fiber tracts undergoing secondary Wallerian-like degen-

eration (dashed line in Figure 2D) CD8 expression was also much weaker. This was particularly evident in the most rostral part of the lesion (Figure 3) where Wallerian-like degenerative changes were predominant and T-cell

infiltration absent (Figure 3C). At this level, CD8 expression was greatly reduced (Figure 3B) despite strong expression of the ED1 antigen (Figure 3A). Thus, along the rostrocaudal extension of the lesion CD8⁺ cell recruitment was overall associated with T-cell infiltration as an indicator of ongoing immune-mediated tissue destruction. However, at a given lesion level, both the morphology and spatial distribution of T cells (Figure 2, G and H) was clearly distinct from that of CD8⁺ cells (Figure 2, E and F).

Cellular Localization of CD8 Antigen by Confocal Microscopy

To clarify the cellular localization of the CD8 antigen in the lesions of MOG-EAE we performed double-labeling immunofluorescent staining in combination with confocal microscopy (Figure 4). Since these studies relied on the sequential application of two mouse monoclonal antibodies on the same tissue section we used a special double-staining protocol in which the CD8 antigen was detected by tyramide signal amplification.¹¹ Preliminary experiments were performed to exclude undesired cross-reactions between secondary detection antibodies (not shown).¹¹

In the lesions of MOG-EAE, the vast majority of CD8 immunoreactivity was localized on ED1⁺ microglia/macrophages (Figure 4, A to D). In line with the immunoperoxidase stains, the appearance of ED1⁺/CD8⁺ phagocytes was essentially restricted to severely affected lesion areas whereas perivascular cuffs of ED1⁺ macrophages were mostly CD8⁻ (Figure 4A). The CD8 immunoreactivity exhibited the typical surface staining of a membrane antigen (Figure 4C) whereas the ED1 antigen was localized intracellularly (Figure 4B). Most CD8⁺ cells had the morphology of large round macrophages (Figure 4, A,H,I). However, on several instances a branched morphology of activated microglia was apparent (arrowheads in Figure 4, B to D). Overall, we found only limited CD8 expression on T cells. CD8⁺ T cells were predominantly localized in perivascular inflammatory infiltrates (Figure 4E) whereas little CD8⁺ T-cell infiltration into demyelinated lesion areas was apparent (Figure 4, G to I, arrowheads). In contrast to MOG-EAE, double-staining of sections from MBP-EAE revealed a complete lack of colocalization of the ED1 and CD8 antigens (Figure 4F). Throughout all experiments, replacement of mAb Ox-8 by the isotype-matched control mAb A112-2 led to complete disappearance of the immunostaining.

Discussion

Until recently, expression of the CD8 antigen had been considered to be specific for certain subpopulations of

Figure 3. Reduced CD8 expression in peripheral parts of MOG-EAE lesions dominated by Wallerian-like degenerative changes. **A to C:** The most rostral part of the large lumbothoracic lesion shown in Figure 2. At this rostral level, strong ED1 expression is associated mainly with degenerating fiber tracts (**dashed lines** in **A**) where only weak CD8 immunostaining is detectable. T cells are essentially absent (**C**).

Figure 2. Distribution of ED1⁺ macrophages (**A,C,D**), CD8⁺ cells (**B,E,F**), and T cells (**G** and **H**) in a demyelinated lesion from a MHC-congenic LEW.1N rat. **C, E, G** and **D, F, H** are higher magnification images from the ventral and dorsal columns, respectively (as indicated in **A**). CD8⁺ cell recruitment is most intense in central necrotic (**dashed line** in **E**) and actively demyelinating lesion areas (**dashed line** in **F**) whereas perivascular cuffs (**arrows** in **C**) submeningeal infiltrates (**dashed line** in **C**) and secondarily degenerating fiber tracts (**dashed line** in **D**) exhibit intense ED1 immunoreactivity but lack significant CD8 expression. Both the morphology and spatial distribution of W3/13⁺ T cells is distinct from that of CD8⁺ cells (**G** and **H**).

lymphocytes, in particular for cytotoxic/suppressor T cells and natural killer cells. However, accumulating evidence from animal models of cerebral ischemia,^{12–14} sciatic nerve axotomy,¹⁵ Bornavirus encephalitis,¹⁶ globoid cell leukodystrophy,¹⁷ irradiation injury,¹⁸ renal allograft rejection,¹⁹ and glomerulonephritis²⁰ as well as examination of alveolar macrophages *in vitro*^{21–23} suggests that a considerable proportion of cells belonging to the monocyte/macrophage lineage exhibits surface expression of CD8. Based on both protein and mRNA data, macrophages express authentic CD8 antigen mainly in form of the $\alpha\beta$ heterodimer.^{12,15,21,22} Functionally, signaling via the CD8 molecule leads to the release of inflammatory mediators such as nitric oxide and tumor necrosis factor- α from cultured alveolar macrophages.^{21,22} Thus, expression of the CD8 antigen is more promiscuous than previously thought and may mediate important functions in inflammatory macrophage activation.

In our present study we show that in demyelinating spinal cord lesions occurring during MOG-EAE in rats the vast majority of CD8 immunoreactivity is expressed on a subset of lesion-associated phagocytes. CD8⁺ T cells were overall rare and mostly restricted to the perivascular space. CD8⁺ phagocytes coexpressed the phagocyte-specific ED1 marker and in most cases exhibited the morphology of round, presumably blood-derived macrophages. However, we additionally found some ED1⁺/CD8⁺ cells with a ramified morphology typical of activated resident microglia. This is in line with previous studies showing CD8⁺ microglia-like cells in rat models of cerebral ischemia¹⁴ and malignant glioma.²⁴ Thus, it seems likely that both hematogenous macrophages and resident microglia contribute to the population of CD8⁺ phagocytes.

In contrast to MOG-EAE, essentially all macrophages infiltrating inflammatory lesions of MBP-EAE were CD8[−]. Thus, when comparing the various EAE models characterized by a different extent of tissue destruction, the appearance of CD8⁺ phagocytes was specifically associated with the development of demyelination and axonal damage characteristic of MOG-EAE whereas a purely inflammatory pathology without structural tissue damage as seen in MBP-EAE did not induce the CD8⁺ phagocyte phenotype. A similar association was found at the level of the individual lesion in MOG-EAE since most macrophages in perivascular cuffs were CD8[−] whereas those in demyelinated lesion areas were CD8⁺. Furthermore, ED1⁺ macrophages in fiber tracts undergoing secondary Wallerian-type degeneration were CD8[−] which is in line with our previous findings in experimental axotomy models.¹⁵ Taken together, these data demonstrate significant

heterogeneity of lesion-associated phagocytes in CNS autoimmunity and suggest a specific role of CD8⁺ macrophages/microglia in the pathogenesis of immune-mediated tissue damage in MOG-EAE.

Interestingly, a similar association between CD8⁺ phagocyte recruitment and the specific type of tissue damage can be delineated in focal brain ischemia.^{12–14} Transient occlusion of the middle cerebral artery leads to the development of heterogeneous cerebral infarctions in which the densely ischemic infarct core undergoes pan-necrotic tissue damage comprising both neurons and glia whereas at the lesion periphery selective neuronal death with relative preservation of glia ensues.²⁵ In this model, the infiltration of CD8⁺ macrophages is restricted to the severely damaged infarct core whereas in peripheral lesion areas microglial induction of CD4 but not CD8 antigen can be seen.¹⁴ In addition, secondarily degenerating subcortical fiber tracts are likewise spared from CD8⁺ phagocyte infiltration.¹³ Under functional aspects, it remains an open question if CD8⁺ phagocytes actively contribute to the process of tissue destruction or are part of a secondary wound healing response concerned with extracellular matrix remodeling, induction of gliosis, and the resulting demarcation of the lesion. In cerebral ischemia, the recruitment of CD8⁺ phagocytes occurs slightly delayed and is correlated with the expression of anti-inflammatory and growth-promoting factors rather than pro-inflammatory cytokines. The precise sequence of pro- versus anti-inflammatory cytokine expression in MOG-EAE is currently unknown.

Dendritic cells (DCs) are recruited into inflammatory CNS lesions^{26,27} and can express ED1 and/or CD8 antigens.²⁸ Therefore, we cannot rule out the possibility that DCs contribute to the population of CD8⁺ cells in MOG-EAE lesions. A more comprehensive analysis of DCs in MOG-EAE was not possible in our study since there is currently no rat pan-DC marker for use in paraffin-embedded tissue. However, the specific phenotype of the CD8⁺ cells identified in MOG-EAE and other lesion paradigms^{12,15} and their association with a chronic destructive lesion type characterized by little T-cell infiltration seems to be compatible with a phagocytic rather than dendritic cell lineage.

CD8⁺ cells constitute a considerable component of the inflammatory infiltrate in demyelinating MS lesions and are significantly correlated with the extent of demyelination and axonal injury.^{1,3,29} However, based on single-color immunohistochemistry, CD8 immunoreactivity in MS lesions appears to be mainly associated with small lymphocyte-like cells.²⁹ The interpretation of these results is complicated by the fact that the currently available CD8-specific antibodies exhibit considerable epitope

Figure 4. Cellular localization of CD8 antigen in MOG-induced (**A to E, G to H**) and MBP-induced EAE (**F**) by double-labeling immunofluorescence and confocal microscopy. **A to D** and **G to H** show representative findings from a MOG-immunized LEW.1N rat whereas **E** was taken from a DA rat. mAb Ox-8 against CD8 (throughout in green) was combined with cell-type-specific markers recognizing ED1⁺ microglia/macrophages (red in **A,B,D,F**) and T cells (red in **E,G,I**). **B** and **C** as well as **G** and **H** are single-channel registrations at the respective wavelengths. **A,D,E,F,I** are superimposed images in which sites of colocalization appear yellow. In MOG-EAE, a considerable proportion of microglia/macrophages identified by the presence of intracellular ED1⁺ immunoreactivity co-express the CD8 antigen on their surface (**A** and **D**). Note that macrophages around inflamed vessels are mostly CD8[−] (red in **A**, vessel lumen marked by asterisks) whereas ED1⁺ phagocytes in the lesioned parenchyma are CD8⁺. Most of these cells have a round macrophage-like shape, but some cells with a branched microglia-like morphology are also present (arrowheads in **B** to **D**). A significant proportion of perivascular T cells is CD8⁺ (**E**) whereas in lesioned parenchyma only single CD8⁺ T cells are present (**G** to **I**, denoted by arrowheads). In MBP-EAE, no coexpression of the CD8 antigen on ED1⁺ phagocytes was found (**F**).

fine specificity. In the rat, CD8⁺ macrophages can be detected using mAb Ox-8 directed against the hinge region of the CD8 α chain,^{12,15,21} mAbs R1–10B5 and 15–11C5 against yet undefined epitopes in the CD8 α chain,^{12,15,30} and mAb 341 against the CD8 β chain.^{12,15,19–21} By contrast, mAb G-28 specific for the IgV-like domain of CD8 α recognizes T lymphocytes but not macrophages.^{21,23} Most human CD8-specific mAbs, on the other hand, have been raised against C-terminal peptide fragments of the CD8 α molecule whereas no hinge-region specific antibodies are available. Thus, it remains an open question if CD8⁺ macrophages likewise exist in humans. Of note, considerable species specificity has been shown with respect to the CD4 antigen which is expressed on human and rat, but not mouse macrophages.³¹ It is therefore conceivable that the CD8 antigen has its value as a marker for specific subsets of lesion-associated phagocytes in the rat whereas corresponding marker antigens for these cells in other species remain to be identified.

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References

- Babbe H, Roers A, Waisman A, Lassmann H, Goebels N, Hohlfeld R, Friese M, Schroder R, Deckert M, Schmidt S, Ravid R, Rajewsky K: Clonal expansions of CD8(+) T cells dominate the T-cell infiltrate in active multiple sclerosis lesions as shown by micromanipulation and single cell polymerase chain reaction. *J Exp Med* 2000, 192:393–404
- Jacobsen M, Cepok S, Quak E, Happel M, Gaber R, Ziegler A, Schock S, Oertel WH, Sommer N, Hemmer B: Oligoclonal expansion of memory CD8+ T cells in cerebrospinal fluid from multiple sclerosis patients. *Brain* 2002, 125:538–550
- Kuhlmann T, Lingfeld G, Bitsch A, Schuchardt J, Bruck W: Acute axonal damage in multiple sclerosis is most extensive in early disease stages and decreases over time. *Brain* 2002, 125:2202–2212
- Sun D, Whitaker JN, Huang Z, Liu D, Coleclough C, Wekerle H, Raine CS: Myelin antigen-specific CD8+ T cells are encephalitogenic and produce severe disease in C57BL/6 mice. *J Immunol* 2001, 166:7579–7587
- Wekerle H, Kojima K, Lannes-Vieira J, Lassmann H, Linington C: Animal models. *Ann Neurol* 1994, 36:S47–S53
- Storch MK, Steffler A, Brehm U, Weissert R, Wallstrom E, Kerschensteiner M, Olsson T, Linington C, Lassmann H: Autoimmunity to myelin oligodendrocyte glycoprotein in rats mimics the spectrum of multiple sclerosis pathology. *Brain Pathol* 1998, 8:681–694
- Weissert R, Wallstrom E, Storch MK, Steffler A, Lorentzen J, Lassmann H, Linington C, Olsson T: MHC haplotype-dependent regulation of MOG-induced EAE in rats. *J Clin Invest* 1998, 102:1265–1273
- Storch MK, Weissert R, Steffler A, Birnbacher R, Wallstrom E, Dahlman I, Ostensson CG, Linington C, Olsson T, Lassmann H: MHC gene-related effects on microglia and macrophages in experimental autoimmune encephalomyelitis determine the extent of axonal injury. *Brain Pathol* 2002, 12:287–299
- Jander S, Pohl J, D'Urso D, Gillen C, Stoll G: Time course and cellular localization of interleukin-10 mRNA and protein expression in autoimmune inflammation of the rat central nervous system. *Am J Pathol* 1998, 152:975–982
- Berger T, Weerth S, Kojima K, Linington C, Wekerle H, Lassmann H: Experimental autoimmune encephalomyelitis: the antigen specificity of T lymphocytes determines the topography of lesions in the central and peripheral nervous system. *Lab Invest* 1997, 76:355–364
- Shindler KS, Roth KA: Double-immunofluorescent staining using two unconjugated primary antisera raised in the same species. *J Histochem Cytochem* 1996, 44:1331–1335
- Jander S, Schroeter M, D'Urso D, Gillen C, Witte OW, Stoll G: Focal ischaemia of the rat brain elicits an unusual inflammatory response: early appearance of CD8+ macrophages/microglia. *Eur J Neurosci* 1998, 10:680–688
- Schroeter M, Jander S, Witte OW, Stoll G: Heterogeneity of the microglial response in photochemically induced focal ischemia of the rat cerebral cortex. *Neuroscience* 1999, 89:1367–1377
- Schroeter M, Jander S, Huitinga I, Stoll G: CD8+ phagocytes in focal ischemia of the rat brain: predominant origin from hematogenous macrophages and targeting to areas of pannecrosis. *Acta Neuropathol (Berl)* 2001, 101:440–448
- Jander S, Lausberg F, Stoll G: Differential recruitment of CD8+ macrophages during Wallerian degeneration in the peripheral and central nervous system. *Brain Pathol* 2001, 11:27–38
- Weissenbock H, Hornig M, Hickey WF, Lipkin WI: Microglial activation and neuronal apoptosis in Bornavirus-infected neonatal Lewis rats. *Brain Pathol* 2000, 10:260–272
- Wu YP, McMahon EJ, Matsuda J, Suzuki K, Matsushima GK, Suzuki K: Expression of immune-related molecules is downregulated in twitcher mice following bone marrow transplantation. *J Neuropathol Exp Neurol* 2001, 60:1062–1074
- Kaffenberger W, Gruber DF, MacVittie TJ: Rat monocytes in a model of combined injury express the OX8 antigen. *J Leukoc Biol* 1987, 42:181–187
- Scriba A, Grau V, Steiniger B: Phenotype of rat monocytes during acute kidney allograft rejection: increased expression of NKR-P1 and reduction of CD43. *Scand J Immunol* 1998, 47:332–342
- Tam FW, Smith J, Morel D, Karkar AM, Thompson EM, Cook HT, Pusey CD: Development of scarring and renal failure in a rat model of crescentic glomerulonephritis. *Nephrol Dial Transplant* 1999, 14:1658–1666
- Hirji N, Lin TJ, Befus AD: A novel CD8 molecule expressed by alveolar and peritoneal macrophages stimulates nitric oxide production. *J Immunol* 1997, 158:1833–1840
- Hirji N, Lin TJ, Bissonnette E, Belosevic M, Befus AD: Mechanisms of macrophage stimulation through CD8: macrophage CD8 α and CD8 β induce nitric oxide production and associated killing of the parasite *Leishmania major*. *J Immunol* 1998, 160:6004–6011
- Hirji NS, Lin TJ, Gilchrist M, Nault G, Nohara O, Grill BJ, Belosevic M, Stenton GR, Schreiber AD, Befus AD: Novel CD8 molecule on macrophages and mast cells: expression, function and signaling. *Int Arch Allergy Immunol* 1999, 118:180–182
- Morioka T, Baba T, Black KL, Streit WJ: Immunophenotypic analysis of infiltrating leukocytes and microglia in an experimental rat glioma. *Acta Neuropathol (Berl)* 1992, 83:590–597
- Stoll G, Jander S, Schroeter M: Inflammation and glial responses in ischemic brain lesions. *Prog Neurobiol* 1998, 56:149–171
- Fischer HG, Reichmann G: Brain dendritic cells and macrophages/microglia in central nervous system inflammation. *J Immunol* 2001, 166:2717–2726
- Reichmann G, Schroeter M, Jander S, Fischer HG: Dendritic cells and dendritic-like microglia in focal cortical ischemia of the mouse brain. *J Neuroimmunol* 2002, 129:125–132
- Banuls MP, Alvarez A, Ferrero I, Zapata A, Ardavin C: Cell-surface marker analysis of rat thymic dendritic cells. *Immunology* 1993, 79:298–304
- Bitsch A, Schuchardt J, Bunkowski S, Kuhlmann T, Bruck W: Acute axonal injury in multiple sclerosis. Correlation with demyelination and inflammation. *Brain* 2000, 123:1174–1183
- Jander S, Kraemer M, Schroeter M, Witte OW, Stoll G: Lymphocytic infiltration and expression of intercellular adhesion molecule-1 in photochemically induced ischemia of the rat cortex. *J Cereb Blood Flow Metab* 1995, 15:42–51
- Crocker PR, Jefferies WA, Clark SJ, Chung LP, Gordon S: Species heterogeneity in macrophage expression of the CD4 antigen. *J Exp Med* 1987, 166:613–618