Early MRI changes in a mouse model of multiple sclerosis are predictive of severe inflammatory tissue damage

Stefan Nessler,1,2,* Susann Boretius,3,* Christine Stadelmann,4,* Alwina Bittner,5 Doron Merkler,4 Hans-Peter Hartung,1 Thomas Michaelis,3 Wolfgang Brück,4 Jens Frahm,3 Norbert Sommer5,∗ and Bernhard Hemmer1,2,†

1Department of Neurology, Heinrich-Heine-University Düsseldorf; 2Clinical Neuroimmunology Group, Department of Neurology, Klinikum rechts der Isar, Technical University Munich; 3Biomedizinische NMR Forschungs GmbH and Max-Planck-Institut for biophysikalische Chemie Göttingen; 4Institute of Neuropathology, Georg-August-University Göttingen and 5Clinical Neuroimmunology Group, Department of Neurology, Philipps-University Marburg

*These authors are the joint First Authors.
†These authors are the joint Last Authors.

Correspondence to: Prof. Dr Bernhard Hemmer, Department of Neurology, Klinikum rechts der Isar, Technische Universität, Ismaninger Str. 21, Munich, Germany
E-mail: hemmer@lrz.tu-muenchen.de

MRI is routinely used for in vivo detection of multiple sclerosis (MS) lesions. Histopathological correlates of MRI signal alterations are still poorly defined. In the present study, we describe a mouse model of MS presenting with inflammatory brain lesions. During the acute disease phase, two independent lesion patterns were identified by T1- and T2-weighted high-resolution 3D MRI: lesions with reduced signal intensity on both T1- and T2-weighted images (type A) and lesions with slightly reduced signal intensity on T1-weighted images and increased signal intensity on T2-weighted images (type B). Type A lesions were characterized by significantly denser inflammatory cell infiltrates and more myelin loss than type B lesions. Lesion cellularity, myelin loss and immunoglobulin deposition correlated with MRI signal intensities in both lesion types. Gd-DTPA enhancement correlated with Ig deposition and spacially matched to areas with abundant activated microglia cells at the lesion border. Using serial MRI, type A lesions revealed a persistent hypointense pattern reflecting axon and myelin loss. Signal intensity increases on T2-weighted images of type B lesions decreased during lesion evolution, and no significant T1 signal alterations developed. Taken together, MRI of mouse EAE models with brain lesions provide new insights into lesion pathology and evolution and may prove useful for the in vivo assessment of new therapeutic strategies in MS.

Keywords: magnetic resonance imaging (MRI); experimental autoimmune encephalomyelitis (EAE); histopathology; T-cell clone; multiple sclerosis

Abbreviations: APC = antigen presenting cell; BBB = blood–brain barrier; CNP = cyclic nucleotide phosphodiesterase; EAE = experimental autoimmune encephalomyelitis; GFAP = glial fibrillary acidic protein; Ig = immunoglobulin; MBP = myelin basic protein; MS = multiple sclerosis; PLP = proteolipid protein; PMN = polymorphonuclear cell


Introduction

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the CNS (Noseworthy et al., 2000). Despite extensive research efforts, its precise aetiology has not yet been elucidated (Hemmer et al., 2006). The complex inflammatory cascade of lesion development includes T-cell and macrophage recruitment, blood–brain barrier (BBB) breakdown, demyelination, axonal damage, remyelination and gliosis (Prineas, 1985; Trapp et al., 1998; Lassmann, 2005; Ludwin, 2006; Frohman et al., 2006). Studying the mechanisms underlying lesion formation is impaired by the inaccessibility of the neuropathological...
MRI is frequently used to assess disease activity in MS and has been correlated with histopathological findings in post-mortem studies and brain biopsies (Bruck et al., 1997; De Groot et al., 2001). Signal intensity on T2-weighted MRI is altered by subtle changes in the macromolecular environment of water protons. Acute lesions which are characterized by inflammation, oedema and demyelination as well as chronic lesions with profound demyelination, axonal loss and gliosis elevate the tissue water content and increase signal intensity on T2-weighted images. Therefore T2-weighted MRI is a sensitive but unspecific marker of increase signal intensity on T2-weighted images. Therefore T2-weighted MRI is a sensitive but unspecific marker of tissue pathology (Werring et al., 2000; Barkhof, 2002). Permanent hypointense lesions on T1-weighted MRI, so called 'black holes', have been correlated with axonal loss and increased disability in MS (Truyen et al., 1996, van Walderveen et al., 1998). The administration of the contrast agent Gd-DTPA improves pathological specificity because areas of enhancement reflect BBB disruption and thus early inflammation (Miller et al., 1988; Bruck et al., 1997). Advances in depicting pathological changes by MRI will be of great value to stratify patients into prognostically and therapeutically relevant subgroups and to improve correlations between MRI and clinical disability (Kalkers et al., 2001).

Experimental autoimmune encephalomyelitis (EAE) induced by active immunization with myelin antigens or the adoptive transfer of encephalitogenic T-cells is a widely used animal model of MS (Gold et al., 2006; Steinman and Zamvil, 2006). Until recently, MRI studies have primarily been performed in rat, guinea pig and marmoset EAE (Jordan et al., 1999; Boretius et al., 2006; Blezer et al., 2006). In guinea pig and rat EAE models, hyperintense lesions on T2-weighted images were shown to correspond to regions of macrophage recruitment, demyelination and oedema (Grossman et al., 1987; Duckers et al., 1997). BBB breakdown detected by Gd-DTPA enhancement correlated with macrophage recruitment in rat and guinea pig EAE models (Hawkins et al., 1990; Morrissey et al., 1996). Recent MRI studies in mouse models of MS used iron-oxide-labelled T-cells (Anderson et al., 2004), superparamagnetic antibodies (Pirko et al., 2004) or superparamagnetic iron oxide contrast agents (Xu et al., 1996) for lesion retrieval and characterization in the spinal cord. However, MRI lesion detection in mouse EAE, the model most frequently used for the development of new therapies, is hampered by the lack of easily accessible brainstem and brain lesions.

In the present study, we took advantage of a clonal adoptive transfer EAE model in the SJL/J mouse with lesion formation in the brain to perform high-resolution 3D MRI in vivo. We established a correlation of MRI and histopathology in mice, the species most commonly used for modelling MS.

Materials and methods

Cell culture and generation of T-cell clone 3-1

Cell culture was performed in RPMI 1640 containing 10% foetal calf serum, non-essential amino acids, HEPES, l-glutamine, 2-mercaptoethanol and antibiotics (Sommmer et al., 1997). T-cell clones were generated from PLP139-151 specific cell lines in limiting dilution technique. Briefly, female SJL/J mice were immunized subcutaneously with 200 µg PLP139-151 (HSLGKWLGHPDKF, purchased from Sigma-Genosys, Pampisford, Cambridgeshire, UK) in complete Freund’s adjuvant (CFA, Difco Laboratories, Detroit, MI, USA) substituted with 5 mg/ml inactivated M. tuberculosis H37RA (Difco Laboratories). The draining lymph nodes were removed 11 days later and single cell suspensions were made. These cells were plated in 24 well plates at a concentration of 4 x 10⁶ cells/ml and restimulated with 10 µg/ml PLP139-151. Cells were split every 3 days, and 2 ng/ml recombinant murine IL-2 was added (R&D systems, Minneapolis, MN, USA). After 2 weeks cell lines were cloned by limiting dilution in 96-U-shaped well plates with 2.5 x 10⁶/ml irradiated (30 Gy) syngeneic spleen cells as antigen presenting cells (APC). One week later cells were restimulated with syngeneic irradiated spleen cells, PLP139-151 and IL-2. Growing clones were selected after another week, further expanded and characterized.

Characterization of T-cell clone 3-1

The T-cell receptor was amplified and sequenced using previously published primer pairs (Bell et al., 1993; Zisman et al., 1994). Briefly, for RT-PCR of the T-cell receptor, RNA from 2 x 10⁶ T-cells was isolated with an RNA isolation kit (RNasy, Qiagen, Hilden, Germany). Reverse transcription was performed using Superscript II RNase H Reverse Transcriptase (Invitrogen, Karlsruhe, Germany). One microlitre forward primer, 1 µl reverse primer, 1 µl DNA and 22 µl PCR-SuperMix (Invitrogen) were mixed on ice. The PCR reaction was started with an initial denaturation step at 94°C for 5 min. The amplification was done for 39 cycles with the following conditions: 94°C for 1 min, 56°C for 1 min, 72°C for 1 min followed by a final extension at 72°C for 10 min. PCR products were visualized in SYBRgold (Molecular Probes, Leiden, The Netherlands) stained 1.5% agarose gels and band lengths were compared with a 100 bp DNA ladder.

For determination of cytokine production 1 x 10⁶/ml T-cell clone cells were stimulated with 4 x 10⁹/ml irradiated APC with or without 10 µg/ml PLP139-151. Supernatants were removed after 48 h and tested for IL-2, IL-4, IL-5, IL-10, IL-17, IL-23, IFN-γ and TNF-α with commercially available ELISA kits (R&D systems) according to the manufacturer’s instructions.

Thymidine incorporation assays were conducted in duplicate wells containing 20 000 T-cells and 200 000 APCs with or without PLP139-151 in a concentration range of 0.1 to 25 µg/ml. At 40 h, cells were pulsed with 0.5 µCi/well ³H-Thymidine (Amersham-Buchler, Braunschweig, Germany) for 8 h and harvested onto glass fibre filter mats. ³H-Thymidine incorporation was analysed by liquid scintillation counting (TopCount, PerkinElmer, Wellesley, USA).

Induction of EAE

Female SJL/J mice were obtained from Harlan–Winkelmann, Borchen, Germany. Mice had access to water and chow ad libitum and were used between 7 and 12 weeks of age. EAE was induced
Clinical signs of EAE were rated from 0 (healthy) to 5 (dead) and mice were used as controls. All mice received an i.p. injection of medetomidine (1.5 mg/kg) and ketamine (150 mg/kg) for anaesthesia induction. Subsequently, the animals were intubated with a polyethylene endotracheal tube and artificially ventilated. Anaesthesia was maintained using 0.5–1.0% isoflurane in oxygen. Respiration was monitored by a signal derived from a home-made pressure transducer fixed to the animal’s chest. The animals were placed in a prone position on a purpose-built holder with an adjustable nose cone. The rectal temperature was kept constant at 37 ± 1°C by water blankets.

All MRI studies were conducted at 2.35 T using an MRBR 4.7/400-mm magnet (Magnex Scientific, Abingdon, UK) equipped with a DBX system (Bruker Biospin, Ettlingen, Germany). For excitation a Helmholtz coil (100 mm) was used and combined with an elliptical surface coil (20 × 12 mm²) for signal detection. Three-dimensional, T1-weighted (3D FLASH, TR/TE = 17/7.58 ms, α = 25°) and T2-weighted images (3D FSE, TR/TE = 3000/98.25 ms, 16 echos, inter-echo-spacing = 12.5 ms) were obtained.

by the adoptive transfer of 3 to 20 × 10⁶ PLP139-151-specific 3-1 T-cells in 300 μl PBS intraperitoneally (Nessler et al., 2006). Clinical signs of EAE were rated from 0 (healthy) to 5 (dead) and were cross-checked by independent observers. The present study was approved by the regional Animal Care Committee, and all experiments were performed according to institutional guidelines.

**MRI**

Ten mice were studied during the acute disease phase at day 1 to 3 after disease onset (mean day 2.0 ± 0.8; mean disease score: 3.75 ± 0.5). Follow-up MRI studies were performed on five additional animals (Table 1). Three healthy age-matched SJL/J mice were used as controls. All mice received an i.p. injection of medetomidine (1.5 mg/kg) and ketamine (150 mg/kg) for anaesthesia induction. Subsequently, the animals were intubated with a polyethylene endotracheal tube and artificially ventilated. Anaesthesia was maintained using 0.5–1.0% isoflurane in oxygen. Respiration was monitored by a signal derived from a home-made pressure transducer fixed to the animal’s chest. The animals were placed in a prone position on a purpose-built holder with an adjustable nose cone. The rectal temperature was kept constant at 37 ± 1°C by water blankets.

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<table>
<thead>
<tr>
<th>Mouse no.</th>
<th>EAE lesions detected histopathologically</th>
<th>EAE lesions detected by T2w MRI</th>
<th>EAE lesions detected by T1w MRI</th>
<th>EAE lesions detected by T1/Gd-DTPA</th>
<th>Lesion type</th>
<th>Time of MRI (day after disease onset)</th>
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<td>1 : A</td>
<td>3</td>
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<tr>
<td>3</td>
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<tr>
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<td>3 −</td>
<td>3 −</td>
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</table>

Note: +: detected; −: not detected; +/-: questionable; n/d: not done; T1w: T1-weighted MRI; T2w: T2-weighted MRI; T1/Gd-DTPA: T1-weighted MRI with Gd-DTPA contrast enhancement; n/c: not classifiable.
with an isotropic spatial resolution of 117 μm (Natt et al., 2002). Four animals additionally received an intravenous injection of 0.5 mmol/kg body weight gadolinium-DTPA (Magnevist™, Schering AG, Berlin, Germany). Post-contrast T1-weighted images were acquired after 10 min of circulation. After the final MRI examination, the animals were sacrificed and prepared for histology.

**Histopathology and immunohistochemistry**

Mice were anaesthetized and perfused transcardially with PBS and 4% paraformaldehyde. Brains and spinal cords were dissected and embedded in paraffin. Inflammation, demyelination and axonal loss were assessed by haematoxylin and eosin, Luxol fast blue and Bielschowsky’s silver impregnation, respectively. Giemsa staining was used to assess infiltration by eosinophilic and neutrophilic granulocytes. Prussian blue staining was performed to exclude iron deposition. In adjacent serial sections, immunohistochemistry was performed with antibodies against macrophages/activated microglia (Mac-3, clone M3/84, Pharmingen, San Diego, CA, USA), T-cells (CD-3, clone CD3-12, Serotec, Oxford, UK) and B-cells (CD45R/B220; clone RA3-6B2, Pharmingen) as described previously (Nessler et al., 2006). Furthermore, antibodies against myelin proteins [myelin basic protein (MBP); rabbit polyclonal; Dako, Glostrup, Denmark] and cyclic nucleotide phosphodiesterase (CNP); clone SM94, Sternberger Monoclonals Inc., Lutherville, MD, USA), against mouse immunoglobulin G and M (Vector Laboratories, Burlingame, CA, USA), axons (phosphorylated high molecular weight neurofilaments; SMI31; Sternberger Monoclonals Inc.), damaged axons (amyloid precursor protein (APP), clone 22C11; Chemicon, Temecula, CA, USA) and astrocytes [glial fibrillary acidic protein (GFAP) rabbit polyclonal; Dako] were applied. Tissues were pretreated with microwaving in 10 mM citrate buffer (pH 6.0) for 3 min. Bound antibody was visualized using an avidin–biotin technique. Control sections were incubated in the absence of primary antibody, with isotype control antibodies or with non-immune sera. Slides were counterstained with haematoxylin and coverslipped.

**Morphometry and statistical evaluation**

A comparison of MRI findings and histopathology was performed for 15 representative lesions in 10 animals. Acute inflammatory lesions with a diameter of at least 0.8 mm were selected for detailed immunohistological assessment. Lesion centres and perilesional areas (one microscopic visual field away from the lesion border) were evaluated separately. A quantitative region-of-interest (ROI) analysis was based on MRI signal intensities in the lesion centre \( n = 17 \), two very large lesion centres (animals no. 7 and no. 9) were evaluated by two ROIs and perilesional areas (\( n = 12 \)) normalized by the intensity of normal appearing surrounding tissues equidistant to the MRI receiver coil.

Cellular densities were determined on Mac-3 stained sections, where Mac-3 positive cells (macrophages/activated microglia) and Mac-3 negative inflammatory cells, i.e. polymorphonuclear granulocytes and lymphocytes were counted using an ocular morphometric grid at a magnification of 1000× (oil immersion). Perilesional microglia activation was counted at a magnification of 400×. Cell densities are given as cells/mm². To assess the relative density of myelin fibres and axons, intersections of immunostained fibres with an ocular 25-point grid at a magnification of 1000× were determined. Lesional and perilesional Ig deposition was evaluated semiquantitatively (0: no Ig deposition; 1: little Ig deposition; 2: substantial Ig deposition). The relationship between histopathological parameters and MRI signal intensities was tested by a bivariate correlation analysis including calculation of the Pearson correlation coefficient (\( r \)). For comparison of different MRI patterns we performed an independent samples two-tailed \( t \)-test. Welch correction was applied, if standard deviations were significantly different between groups. Statistical significance was defined as \( P < 0.05 \).

**Results**

**Characteristics of T-cell clone 3-1**

T-cell clone 3-1 (TCC 3-1) is specific for PLP139-151. The adoptive transfer of in vitro activated 3-1 T-cells into syngeneic SJL/J mice leads to clinical disease 6–10 days after injection (Fig. 1A). The TCC has a Th0 phenotype secreting small amounts of IL-4 and IL-17, moderate amounts of IL-2, TNF-\( \alpha \) and IFN-\( \gamma \) and high amounts of IL-10 and IL-5 (Fig. 1B). TCC 3-1 expresses the T-cell receptor (TCR)-AV8D1/AJ58 and TCR-BV31/BJ1-1 chain. In contrast to most of our SJL/J PLP139-151-specific T-cell clones, clone 3-1 induces EAE lesions in the upper brainstem, cerebellum and pons in addition to spinal cord and optic nerve lesions.

**MRI lesion distribution and conspicuity**

Ten acute diseased mice were studied by high-resolution 3D MRI and histopathology. In these animals, 25 EAE brain lesions could be retrieved by MRI. The majority of lesions (20/25) were found in the pons with three of them extending into the cerebellum (Table 1). Three lesions were located in the diencephalon, one lesion was detected around the third ventricle and one in the basal hypothalamus. Twenty-seven acute EAE lesions were identified by histopathology (Table 1). Two very small subpial EAE lesions in the lower pons (mouse nos. 7 and 8) could not be identified in the MR images corresponding to the histological sections. None of the control animals showed abnormalities by MRI.

**MRI contrast patterns in acute disease**

Although EAE was induced by transfer of one pathogenic TCC, high-resolution T1- and T2-weighted 3D MRI revealed different types of EAE brain lesions with characteristic contrast patterns in the acute disease phase. Type A lesions were characterized by very low signal intensities on both T1- and T2-weighted images (Fig. 2A, B, J and K) and represented the most common lesion type in our model (3 lesions in 9 animals). Type B lesions (7 lesions in 5 animals) presented with slightly reduced or isointense signal intensities on T1-weighted images and hyperintensities on T2-weighted images (Fig. 3A and B). One remarkable lesion showed a strong signal increase on the T2-weighted image and a moderate signal decrease on
the T1-weighted image (Fig. 3G and H). Six MRI lesions could not be classified into type A or type B because they were not unequivocally detected on both T1- and T2-weighted images. In type A lesions, a rim of increased signal intensity surrounding the lesion could be seen in 7 of 12 lesions on the T2-weighted images (Figs 2A and 4A), while no perilesional changes were observed in the remaining 5 lesions (Fig. 2J). In type B lesions the lesion border could not be differentiated from the lesion centre, due to the increased T2-signal intensity of the whole area (Fig. 3A).

A pronounced Gd-DTPA enhancement occurred at the hyperintense lesion borders but not in the lesion centres of type A lesions (Fig. 4A and B). Lesions without perilesional signal increases on T2-weighted images showed only slight Gd-DTPA enhancement (Fig. 4E and F). Gd-DTPA enhancement of type B lesions was observed throughout the lesion (data not shown).

**MRI contrast patterns during lesion evolution**

Serial MRI data were obtained in five animals (Table 1). A total number of four type A lesions (mouse nos. 12, 13 and 14), two type B lesions (mouse nos. 11 and 15) and one atypical lesion (mouse no. 14) were followed by MRI. Serial MRI data are shown in Fig. 5 for an exemplary case (mouse no. 14). A typical type A brainstem lesion was observed on day 3 after disease onset (Fig. 5A–H), and an additional lesion adjacent to the lateral ventricle appeared on day 9 (Fig. 5M–T). The strongly reduced T1- and T2-signal intensities in the centre of the brainstem lesion were preserved until day 21 (Fig. 5A–H). On the other hand, the signal increase on T2-weighted MRI at the lesion border decreased over time (Fig. 5A, C, E and G). The brain lesion close to the lateral ventricle presented with strongly increased signal intensity on T2-weighted MRI and moderately reduced signal intensity on T1-weighted MRI, possibly due to CSF leakage (Fig. 5O and P). On day 21, this lesion was heterogeneous with areas of increased and reduced signal intensity on T2-weighted MRI and moderately reduced signal intensity on T1-weighted MRI (Fig. 5S and T).

Thus, on serial scans type A lesions presented with a persistent signal reduction on T1-weighted MRI at day 21 (mouse no. 14, Fig. 5H) or at day 40 (mouse no. 12) Lesion borders hyperintense on T2-weighted images decreased during lesion development. Similarly, the increased signal intensity on T2-weighted images of type B lesions decreased during lesion evolution. Signal alterations on T1-weighted images of type B lesions were no longer detectable at the given time-points (day 5, mouse no. 11; day 17 mouse no. 15), supplementary Fig. 1.

**Acute type A lesions are more densely infiltrated and destructive than type B lesions**

Acute type A lesions showed a significantly higher density of inflammatory cells than type B lesions (Fig. 6A, $P = 0.0001$). The inflammatory infiltrate in these lesions mainly consisted of polymorphonuclear cells (Fig. 2D). Myelin loss was significantly more pronounced in type A than in type B lesions (Fig. 6B, $P = 0.0069$). The difference in axon density did not reach statistical significance (Fig. 6C, $P = 0.0875$). Prominent Ig deposition in the lesion centre was observed only in 1 of 12 type A lesions compared to 5 of 5 type B lesions, which showed strong central Ig deposition.
Inflammatory infiltrates and myelin loss in the lesion centre correlate with reduced signal intensity on T1- and T2-weighted images. Correlations of MRI signal intensities and histopathological parameters are summarized in Table 2. As a key result, a strong negative correlation could be established between the density of cellular infiltrates and signal intensities on T1- (Fig. 7A, \(P = 0.0048, r = -0.6489\)) and T2-weighted (Fig. 7B, \(P = 0.0007, r = -0.7368\)) images. Myelin fibre density correlated positively with signal intensities on T1- (Fig. 7C, \(P = 0.0214, r = 0.5691\)) and T2-weighted images (\(P = 0.0052, r = 0.6642\)).
was found for a positive correlation with signal intensities on T1-weighted MRI (Fig. 7D, $P = 0.0548$, $r = 0.4736$). The correlation between axon density and T2-weighted signal intensity also did not reach significance in our model ($P = 0.0899$, $r = 0.4239$). Ig deposition correlated positively with signal intensities on T1- ($P = 0.0108$, $r = 0.6008$) and T2-weighted images ($P = 0.0018$, $r = 0.6995$). No correlation was observed between Gd-DTPA enhancement and histological parameters for the lesion centre (Table 2).

Densities of perilesional activated microglia cells and reactive astrocytes positively correlate with T2 and negatively correlate with T1 signal intensities

As a further main finding, the numbers of activated microglia cells and reactive astrocytes in perilesional areas significantly correlated with signal alterations on T1- and T2-weighted images. In particular, the density of activated microglia cells (Fig. 8C, $P = 0.0066$, $r = 0.7338$; for summary see Table 2), of reactive astrocytes (Fig. 8D, $P = 0.0303$, $r = 0.6234$), and the extent of Ig deposition ($P = 0.0043$, $r = 0.7583$) positively correlated with signal intensities on T2-weighted images. Negative correlations were found for microglia cell densities (Fig. 8A, $P = 0.0180$, $r = -0.6661$) and astrocytes (Fig. 8B, $P = 0.0178$, $r = -0.6669$) and T1-weighted signal intensities. At the lesion borders, Gd-DTPA enhancement positively correlated with Ig deposition ($P = 0.0102$, $r = 0.9162$; see Table 2). Significance was not quite achieved for Gd-DTPA enhancement and microglia activation ($P = 0.1193$, $r = 0.7029$). We found no correlation between Gd-DTPA enhancement and astrocyte cell density ($P = 0.6008$, $r = 0.2729$; Table 2).
Immunohistological correlates of serially imaged animals

Immunohistology (at day 21 after disease onset) of the serially imaged type A brainstem lesion (mouse no. 14) revealed a moderate density of inflammatory cells mainly composed of Mac-3 positive macrophages/activated microglia cells (Fig. 5I). No iron containing phagocytes were observed by Prussian Blue staining (data not shown). Myelin and axon loss (Fig. 5J and K) as well as gliosis (Fig. 5L) were prominent, whereas Ig deposition was absent (data not shown). The T2 hyperintense brainstem lesion (type B lesion) in mouse no. 15 revealed several foci of Mac-3 positive cells at day 17 after disease onset. Axon and myelin loss was only found immediately around vessels infiltrated by lymphocytes. Dense gliosis, but no deposition of Ig was observed (data not shown).

Discussion

In the present study, we describe a T-cell-clone-mediated mouse model of MS with reproducible lesions in the brain well amenable to non-invasive assessment by MRI. Two main MRI-defined lesion types were detected in the acute disease phase and correlated with distinct histopathological findings: strongly hypointense lesions on T1- and T2-weighted images (type A) and isointense or slightly hypointense lesions on T1-weighted images which were hyperintense on the corresponding T2-weighted images (type B). Type A lesions were characterized by a significantly higher inflammatory cell density, more myelin loss and a tendency towards more axonal loss than type B lesions. Ig deposition was less pronounced than in type B lesions. Statistical analysis revealed significant correlations for (1) cell density which was inversely correlated with T1- and T2-signal intensities, (2) myelin density, which positively correlated with T1- and T2-signal intensities and (3) Ig deposition which also correlated positively with signal intensities on T1- and T2-weighted images. In the perilesional area, there was a strong positive correlation between T2-hyperintensity and the density of activated microglia cells, reactive astrocytes and the extent of Ig deposition.

The MRI hallmarks of MS are multiple hyperintense lesions on T2-weighted images (Fazekas et al., 1999; Filippi et al., 2002) which reflect a variety of pathological processes such as inflammation, oedema, demyelination, axon loss and gliosis (Bruck et al., 1997; van Walderveen et al., 1998; van Waesberghe et al., 1999; Schmierer et al., 2003). Therefore, T2-signal abnormalities are of limited value in assessing disease progression and therapeutic responses. Furthermore, the T2 lesion load shows only a moderate correlation with clinical disability (Filippi et al., 1995; Barkhof, 2002). The type B lesions detected in our EAE model harbour MRI characteristics frequently found in early MS lesions, such as T2-hyperintensity, iso- or mild-hypointensity on T1-weighted images and diffuse enhancement with Gd-DTPA. In our model, histopathological correlates of these lesions were a moderate density of inflammatory cell infiltration, a moderate amount of myelin and axonal loss and prominent Ig deposition.

Early inflammatory lesions induced by the PLP139-151-specific T-cell clone 3-1 in SJL/J mice are mainly composed of densely packed polymorphonuclear cells (PMNs). EAE lesions that harbour substantial numbers of granulocytes in the early inflammatory infiltrate have been described in a number of EAE models (Maatta et al., 1998; Abromson-Leeman et al., 2004; Wensky et al., 2005). The dense
accumulations of PMNs in our model mapped spatially with the occurrence of acute type A lesions that are hypointense on T1- and T2-weighted images. This goes in line with recent findings in a delayed type hypersensitivity model in the rat, where hypercellularity caused by infiltrating macrophages/activated microglia cells led to decreased signal intensities on T1- and T2-weighted images (Broom et al., 2005).

Fig. 5 Serial MRI of two distinct EAE lesions in one animal at days 3, 9, 11 and 21 after disease onset. A typical evolution of a type A brainstem lesion with a persistent hypointense centre on both T1- and T2-weighted MRI and a fading hyperintense rim on T2-weighted MRI is depicted in A–H (left columns). Final histological examination demonstrates infiltration by foamy macrophages/microglia (I; Mac-3), myelin loss (J; MBP), marked reduction in axonal density (K; Bielschowsky) and perivascularly accentuated gliosis in the area of most prominent tissue damage (L; GFAP). The atypical lesion adjacent to the lateral ventricle (right columns, M–T) is first seen at day 9 (O, P). Part of the signal intensity changes may be related to CSF leakage (O–R). Histopathology reveals infiltration with macrophages/microglia at day 21. Perivascular lymphocytic infiltrates are visible at the right lower image border (U; Mac-3). A circumscribed area of myelin loss is apparent (V; MBP). Axon density is reduced (W; Bielschowsky), and astrocytic gliosis is prominent around the lesion (X; GFAP). Rectangles in G, H, S and T indicate the respective areas depicted in histology (I–L and U–X). Original magnifications: I–L, U–X: ×100; scale bar: X: 200 µm.
In human CNS disease, strong reductions of signal intensities on T2-weighted images as seen in our acute type A lesions have mainly been observed in highly cellular or partially necrotic processes such as tuberculous granuloma, lymphoma and metastasis (Carrier et al., 1994; Kim et al., 1995; Koeller et al., 1997). In chronic disease, persisting T1-hypointensities (‘black holes’) usually indicate severe tissue destruction (Hiehle, Jr et al., 1995; van Waesberge et al., 1998; van Waesberge et al., 1999; Levesque et al., 2005). In the present study, myelin density positively correlated with signal intensities on T1-weighted images, which is in agreement with previous results in humans (Bitsch et al., 2001; Mottershead et al., 2003; Barkhof et al., 2003). Furthermore, axon density correlated with signal intensities on T1-weighted images in human correlative studies (van Waesberge et al., 1999; van Walderveen et al., 1999; Mottershead et al., 2003) and showed a similar tendency in our mouse model ($P=0.0548$). In our study, lesions with severe myelin and axon loss showed a persistent reduction in T1 signal intensity in the chronic disease phase. In contrast, signal alterations on T1-weighted images of type B lesions did neither persist nor develop, and on histological assessment type B lesions were less cellular and destructive than type A.

The majority of acute type A lesions harboured a rim of increased signal intensities on T2-weighted images around the hypointense lesion core which correlated positively with the density of cellular infiltration ($P=0.0001$) (A) and the extent of myelin loss ($P=0.007$) (B). There is a trend towards more axonal damage in lesions with reduced signal intensity ($P=0.0875$) (C).

In our clonal EAE model, Gd-DTPA enhancement was most prominent in the perilesional area of type A lesions and positively correlated with Ig deposition. BBB breakdown therefore occurred in areas where activated microglia cells and reactive astrocytes were located. This observation is in agreement with Morrissey et al. (1996) who found Gd-DTPA enhancement associated with microglia/macrophage activation in an adoptive transfer rat EAE model. Interestingly, we did not observe Gd-DTPA enhancement in the densely infiltrated, granulocyte-dominated lesion centres in type A lesions, possibly due to a local lack of perfusion or reduction in extracellular space. No correlation of Gd-DTPA enhancement with demyelination was observed as described in the marmoset EAE model (Hart et al., 1998). This may be due to the fact that
myelin loss was most pronounced in the non Gd-DTPA enhancing, highly cellular lesion centre in this EAE model.

In summary, the present study correlates MRI findings with lesion pathology in a clonal mouse EAE model. We were able to detect the majority of acute EAE lesions by the combination of conventional high-resolution 3D MRI. Two MRI-defined lesion types were induced by the adoptive transfer of a PLP-specific T-cell clone. Strongly reduced signal intensities on T1- and T2-weighted images were predictive of severe inflammatory tissue damage with persistent prominent T1-hypointensities as confirmed by serial MRI. Thus, the established EAE model not only proved useful for an MRI assessment of lesion pathology in acute and chronic disease, but also promises to become a versatile tool for the evaluation of novel therapeutic strategies using cellular and molecular imaging techniques.

Supplementary material
Supplementary material is available at Brain online.

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Fig. 8 MRI—histopathology correlations of type A lesions at the lesion border. We found inverse correlations between microglia and astrocyte density and signal intensities on T1-weighted MRI [microglia (A): \( r = -0.6661, P = 0.018 \); astrocytes (B): \( r = -0.6669, P = 0.0178 \)]. Significant positive correlations could be established for microglia and astrocyte density and signal intensities on T2-weighted images [microglia (C): \( r = 0.7338, P = 0.0066 \); astrocytes (D): \( r = 0.6234, P = 0.0303 \)].

**References**


