RESEARCH ARTICLE

Expression of Major Histocompatibility Complex Class I Molecules on the Different Cell Types in Multiple Sclerosis Lesions

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Multiple sclerosis is considered to be an immune-mediated disease of the central nervous system, characterized by chronic inflammation, primary demyelination and axonal damage. The mechanisms of demyelination and axonal injury are heterogeneous and complex. One possible mechanism is direct damage of oligodendrocytes and neurons by Class I MHC restricted cytotoxic T-cells. In this study we analyzed the expression of functional MHC class I molecule complex, consisting of α-chain and β2-microglobulin, in a large sample of human autopsy material, containing 10 cases of acute MS, 10 cases of chronic active MS, 10 cases of chronic inactive MS and 21 controls. To examine the expression of MHC class I and II molecules on the different cell-types in brain, we used quantitative immunohistochemical techniques, double staining and confocal laser microscopy scans on paraffin embedded sections. We found constitutive expression of MHC class I molecule on microglia and endothelial cells. A hierarchical up-regulation of MHC class I was present on astrocytes, oligodendrocytes, neurons and axons, depending upon the severity of the disease and the activity of the lesions. MHC class II molecules were expressed on microglia and macrophages, but not on astrocytes. These data indicate that in MS lesions all cells of the central nervous system are potential targets for Class I MHC restricted cytotoxic T-cells.

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INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory disease, leading to large plaques of primary demyelination in the white and gray matter of the central nervous system. Although the mechanisms of demyelination and axonal damage in this disease are complex and heterogeneous (24), cytotoxic T-lymphocytes appear to be one important factor in the induction of the lesions. The T-lymphocyte infiltrates in MS lesions are dominated by the $CD8^+$ subset (8, 14, 16, 30) and clonal expansion is preferentially found in this T-cell subpopulation (4). In addition, acute axonal injury within the lesions correlates with the density of macrophage (13, 21, 41) or $CD8^+$ T-cell infiltration (6), but not with that of other subsets of inflammatory cells.

To exert their cytotoxic action T-cells have to recognize antigenic peptides when they are displayed on the surface of the body's own cell. Antigens, derived from endogenously synthesized proteins in the cytosol, are bound to MHC class I molecules and are recognized by CD8 positive T-cells, whereas peptides internalized by endocytosis and generated in vesicles are bound to MHC class II molecules and recognized by CD4 positive T-cells (23). Thus, intracellularly processed autoantigens and viral antigens are particularly good candidates for recognition by Class I MHC restricted T-cells. However, the central nervous system was long believed to be an immunoprivileged site, protected in part against T-cell-mediated attacks by the lack of MHC class I expression on CNS-resident cells (17, 19, 33).

In vitro, with appropriate stimuli, MHC Class I antigens can be expressed in all glia cells as well as neurons. In contrast, MHC Class II antigen expression is more restricted to microglia, but after profound stimulation can also be induced on astrocytes, ependymal cells or endothelial cells (29). In vivo, in human and experimental brain diseases, the situation is more complex. The expression of MHC-antigens is dependent upon the type of tissue injury, the stage and activity of the lesions and the genetic background (17, 26, 27, 29, 46).

Studies on MHC Class I expression have been performed in normal brain and various inflammatory diseases of the central nervous system (1, 2, 15, 16, 22, 34, 39, 43, 47) and demonstrate expression on endothelial cells and some microglia. Regarding a possible Class I expression in resident glia cells or neurons in inflammatory brain lesions the results are controversial. Due to the lack of suitable markers, which can be used in routinely processed autopsy tissue, previous studies were either restricted to freshly frozen tissue samples or to small brain biopsies.

To overcome these limitations we used newly available markers and techniques for the detection of MHC molecules in routinely fixed and processed brain tissue. This allowed us to study a large cohorts of different controls and of MS patients with different clinical disease manifestations and

	Cases	Mean age (years); range	F:M	Disease duration (weeks) ± SEM
Acute MS	10	47.4; 29-68	5:5	7.0 ± 2.0
Chronic active MS	10	39.5; 28-57	9:2	252.0 ± 47.6
Chronic inactive MS	10	67.2; 50-83	6:4	1349.1 ± 162
Controls	9	68.8; 46-83	4:5	none
Septic controls	12	75.0; 42-95	6:6	not determined

Table 1. Cases.

at different stages of disease development. Our results show that both, MHC Class II as well as Class I molecules are highly expressed in actively demyelinating MS lesions and that oligodendrocytes, astrocytes, axons and neurons are potential targets for Class I MHC restricted cytotoxic T-lymphocytes.

MATERIALS AND METHODS

Cases. Paraffin embedded and formalin fixed archival human autopsy material was collected in the Department of Neurology at the Lainz Hospital in Vienna, the Department of Neuropathology at the Georg-August-University in Göttingen, the Mayo Clinic, Rochester, Minn, and the Brain Research Institute at the Vienna University Medical School, consisting of acute MS cases of Marburg's type (n = 10), chronic active MS (n = 10), chronic inactive MS (n = 10) and age matched controls. Control patients had no evidence for neurological disease or cerebral lesions and included patients dying without (controls I, n = 9) or with terminal inflammatory complications (sepsis, pneumonia, controls II, n = 12). All cases underwent detailed neuropathologic examination (KJ, MS, HL), assessing up to 20 tissue blocks per case.

Primary antibodies. The following well characterized primary antibodies were used: *Monoclonal (mouse)antibodies*: α-glial fibrillary acid protein GFAP (astrocytes; Neomarkers, Fremont, Calif), α-2′3′-cyclic nucleotide 3′ phosphodiesterase CNP (oligodendrocytes, myelin; Sternberger Monoclonals Incorporated, Lutherville, Md), α-myelin oligodendrocyte glycoprotein MOG (32), α-myelin associated glycoprotein MAG (myelin, 12), α-proteolipid protein PLP (myelin; Serotec, Oxford, United Kingdom), α-HC10 (α-chain of MHCclass I; 40), α-CD1d (a 49kD heavy chain, Neomarkers), α-HLA DR,DQ,DP (MHCclass II, DAKO, Glostrup, Denmark). *Polyclonal (rabbit) antibodies*: α-GFAP (astrocytes, DAKO), α-myelin basic protein MBP (myelin, DAKO), α-neurofilament 68kD (neurons, axons; Chemicon, Temecula, Calif), β2-microglobulin (β2-microglobulin, light chain of MHCclass I, DAKO), α-factor VIII related protein (endothelial cells, Neomarkers) and *polyclonal (goat) antibody*: α-GFAP (astrocytes, Santa Cruz Biotechnology, Santa Cruz, Calif).

Immunohistochemistry. Two to 4-µm thick adjacent serial sections were cut on a microtome and stained with hematoxylin/eosin, Luxol fast blue and Bielschowsky's silver impregnation to assess inflammation and demyelination, respectively.

Immunohistochemistry for lightmicroscopy was carried out as follows: the sections were dewaxed in xylol for one hour and transferred to 96% ethanol. Endogenous peroxidase was blocked by incubation in 0.03% hydrogen peroxide for 30 minutes. Sections were rehydrated through a descending ethanol series (96%, 70%, 50%) and rinsed in distilled water. The slides were heated in citrate buffer, rinsed in 0.1M PBS (phosphate buffered saline) and then incubated in 10% FCS (fetal calf serum) in PBS for 20 minutes. After removing the FCS and washing with PBS we incubated the slides at 4°C overnight with primary antibodies (described above). The sections were then washed in PBS and incubated with biotinylated secondary antibodies (1:200; donkey-anti-rabbit; donkey-anti-mouse; Amersham Pharmacia Biotech, Uppsala, Sweden) in FCS/PBS containing 3% normal human serum for 2 hours at room temperature followed by avidin-peroxidase complex (1:100; Sigma, St. Louis, Mo) for one hour. Labelling was visualized with 3,3′ diaminobenzidine-tetra-hydrochloride (Sigma) containing 0.30% hydrogen peroxide. Negative controls were performed by omitting the primary antibody or by using irrelevant isotype control antibodies. All sections were counterstained with Meyer's hematoxylin, dehydrated and coverslipped.

Double/triple fluorescence labeling. Sections for fluorodye labelled stains were pre-treated as described above for light microscopy. In principle, for double/triple labelling primary antibodies from different species (mouse, rabbit) were applied simultaneously at 4°C overnight. After washing with PBS, slides were incubated with compatible secondary antibodies consisting of either biotinylated-sheep-α-mouse or biotinylated-sheep-α-rabbit (both 1:200; Amersham Pharmacia Biotech) and either donkey-α-mouse Cy3 or goat-α-rabbit Cy3 (both 1:100; Jackson Immuno Research Laboratories, West Grove, Pa) for one hour at room temperature. Double labelling was finished by application of Cy2 conjugated streptavidin (1:75; Jackson Immuno Research). In case of triple labelling sections were further incubated with an additional primary antibody from either mouse or rabbit and followed by either donkey-α-mouse Alexa660 (Molecular Probes, Leiden, The Netherlands) or goat-α-rabbit Cy5 (Jackson ImmunoResearch). Finally the slides were mounted with PBS/glycerol (1:9) with 3% DABCO (Sigma).

Laser scanning confocal microscopy. A Zeiss LSM-410 motorized confocal laser scan microscope (Carl Zeiss, Jena, Germany) equipped with an argon-ion laser source (488 nm excitation and two HeNe lasers [543 and 633 nm excitation]) was used. Operating conditions (contrast, brightness settings) and an appropriate combination of filters (bandpass filters 515-525 nm and 595-615 nm, longpass filter 650 nm) were used to eliminate "bleed-through" from either channel. Fluorescence signals were collected either simultaneously or sequentially in the green (Cy2) and red channels (Cy3, Cy5, Alexa660). In some cases, z-series from 3 to 10 planes with an axial resolution of 0.25 µm were collected for reconstruction and analysed with LSM Image Browser© software from Zeiss. Colocalization of antigens, for example β_2-microglobulin red and GFAP green, appears in yellow.

Classification of lesional activity. Lesional activity was determined by the presence of myelin degradation products for minor myelin proteins (MAG, MOG) in early active and major myelin proteins (MBP, PLP) in late actively demyelinating lesions within foamy macrophages as described in detail previously (9). Inactive lesions either contained macrophages with empty vacuoles (neutral lipid stage of myelin degradation) or were devoid of macrophages. Periplaque white matter (PPWM) was defined as a strip of tissue, extending ten millimeters from the border of the active or inactive lesions into the normal white matter.

Quantitative evaluation of labelled cells. Evaluation of α-chain, β2-microglobulin chain and HLA-D expressing cells was performed on serial sections in large corresponding fields, with an average size of 6.2 ± 0.4 mm^2 in periplaque white matter (PPWM) and 7.4 ± 0.7 mm^2 in MS plaques for MHC class I molecule staining and on an average field of one mm^2 in PPWM and MS plaque for MHC class II molecule staining, each defined by an ocular morphometric grid. The different cell types, consisting of macrophages/microglia, astrocytes, oligodendrocytes and neurons, were identified morphologically, based on their cell size and shape and their cytological features. In addition, the cellular nature of the MHC-expressing cells was further confirmed by double staining and confocal laser microscopy.

Statistical analysis. For statistical analysis nonparametric group tests (Mann-Whitney, Kolmogorov-Smirnov) and regression analysis were applied. All tests were classified as significant if the p-value was <0.05. All values are expressed as means ± standard error of the mean.

RESULTS

We identified the central nervous system cell types expressing MHC class I and II molecules by morphological criteria in light microscopy, using immunohistochemistry with antibodies directed against HLA-D to image MHC class II molecule and antibodies directed against α-chain and β2-microglobulin to detect MHC class I molecules. To confirm our observations from light microscopy we performed double/triple labeling immunofluorescence

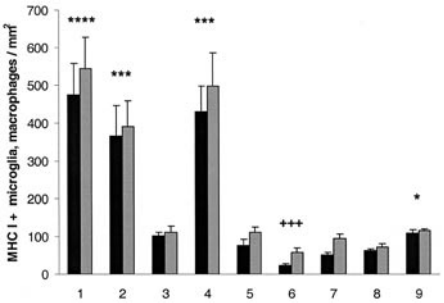

Figure 1. *MHC class I expression (α-chain/β2-microglobulin) on microglia or macrophages.* Significant differences for Class I in active acute MS p<0.0005(****), in inactive acute MS and lesions of chronic active MS p<0.001(***) versus both control groups. MHC Class I expression was significantly different between patients dying from septic complications to controls lacking a systemic inflammatory disease p<0.003(*). In chronic inactive MS lesions the density of β2-microglobulin positive microglia is less compared to controls p<0.0009(+++). Black bars: β2-microglobulin; striped bars: α-chain.

Figures 1 to 4. *X-axis columns.* **1.** *active* lesion acute MS; **2.** *inactive* lesion acute MS; **3.** *PPWM* acute MS; **4.** *active* lesion chronic active MS; **5.** *PPWM* chonic active MS; **6.** *inactive* lesion chronic inactive MS; **7.** *PPWM* chonic inactive MS; **8.** controls without any evidence of systemic inflammation; **9.** controls dying becuase of septic complications.

to co-localize MHC expression with the specific cell type markers in confocal laser microscopy scans. Our studies were performed on patients with Marburg's type of acute MS (10 patients, 16 lesions), on patients with chronic MS with actively demyelinating lesions (chronic active MS; 10 patients, 16 lesions) and in patients who lacked actively demyelinating lesions in the CNS (chronic inactive MS; 10 patients, 21 lesions). In addition we included 2 control populations. The normal control group (9 patients) included patients without clinical evidence for a peripheral inflammatory or infectious complication, the major reason for death in this group was myocardial infarct. The second group were patients, who died in a septic condition, but had no neuropathological evidence for inflammatory brain disease (12 patients, 24 lesions).

Class I MHC molecule expression. *Controls*. Under normal conditions only endothelial cells and microglia showed immunoreactivity for MHC class I molecules. Only exceptionally some scattered astrocytes and even fewer oligodendrocytes revealed weak immunoreactivity. In the CNS of patients with terminal septic complications, we found a significant up-regulation of MHC

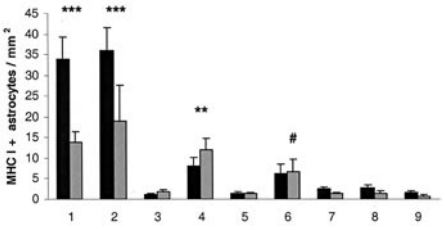

Figure 2. *MHC class I (α-chain/β2-microglobulin) expression on astrocytes in MS lesions.* Significant differences for MHC class I expression (α-chain/β2microglobulin) on astrocytes between both control samples and the respective MS groups are: for active and inactive lesions of acute MS p<0.0003(***) and for lesions of chronic active MS p<0.006(**). Significant differences only for α-chain on astrocytes were found for chronic inactive MS lesions p<0.01(#). Black bars: β2-microglobulin; striped bars: α-chain.

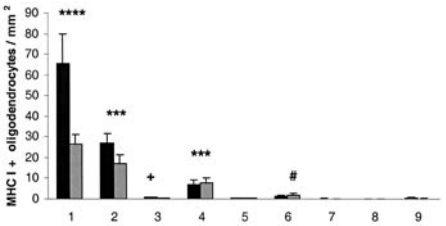

Figure 3. *MHC class I (α-chain/β2-microglobulin) expression on oligodendrocytes in MS lesions.* Significant differences for MHC class I expression (α-chain/β2-microglobulin) on oligodendrocytes between both control samples and the respective MS groups are: for active acute MS p<0.0001 (****), for inactive acute MS and lesions of chronic active MS p<0.0002 (***). Significant differences only for α-chain on oligodendrocytes were found for chronic inactive MS lesions p<0.002(#) and for β2-microglobulin on oligodendrocytes for the PPWM of acute MS cases p<0.004(+). Black bars: β2-microglobulin; striped bars: α-chain.

class I molecules in comparison to normal controls on ramified microglia (Figure 1), but not on astrocytes (Figure 2) or oligodendrocytes (Figure 3).

Multiple sclerosis. In comparison to both control groups, the expression of MHC Class I molecules was significantly higher in astrocytes and oligodendrocytes in active and inactive lesions of acute as well as chronic MS (RR, SP and PP). Overall the expression level was higher in Marburg's type of acute compared to chronic MS (Figures 2, 3). Also the numbers of Class I positive macrophages and microglia were significantly elevated in acute and chronic active MS lesions. Chronic inactive lesions demonstrated MHC class I expression on all microglia, however their total density within the lesion, was significantly lower as compared to normal white matter of either MS patients or controls. There was a trend of more class I in oligodendrocytes

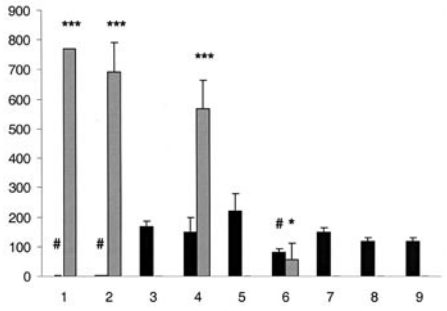

Figure 4. *MHC class II (HLA DR,DQ,DP) expression on microglia and/or macrophages in MS lesions.* Black bars: HLA-DR positive microglia, striped bars: macrophages ± SEM; significant differences for macrophages (*) and for microglia (#) between respective groups and both control groups. High numbers of macrophages were found in active and inactive lesion areas of acute MS and chronic active MS p<0.0001 (***). Chronic inactive MS showed significantly increased numbers of macrophages p<0.04(*) compared to both controls. Within acute MS lesions HLA-D positive cells with typical morphological criteria for microglia were only rarely found p<0.0003 (#). Density of microglia was reduced in chronic inactive cases; p<0.04 (#).

in pattern III and in microglia in pattern II MS lesions (24), however due to small numbers of cases, the results did not reach significant differences (data not shown). While the expression of the α-chain and β2-microglobulin was quantitatively similar on macrophages, microglia (Figure 1) and endothelial cells (data not shown), significantly more oligodendrocytes and astrocytes were stained with antibodies for β2-microglobulin than for the α-chain.

Class II MHC molecules were intensely expressed in lesions from cases with Marburg's type of acute MS, followed by those with active demyelination in chronic disease (Figure 4). In these lesions the vast majority of Class II expressing cells showed a macrophage like phenotype, while HLA-D positive cells with microglia morphology were mainly encountered in the periplaque white matter and—in low incidence—within active and inactive lesions of chronic MS.

Cellular nature of MHC-expressing cells in MS lesions. Class I MHC molecules. In principle all cell types present within actively demyelinating MS lesions were found to express Class I MHC molecules, although the incidence of positive cells varied in the different cell populations. All inflammatory cells (T-cells, B-cells and macrophages; Figures 5A, I; 6B, C, H, I, J) as well as endothelial cells (Figures 5D, J; 6B, C, G, J) were invariably positive for

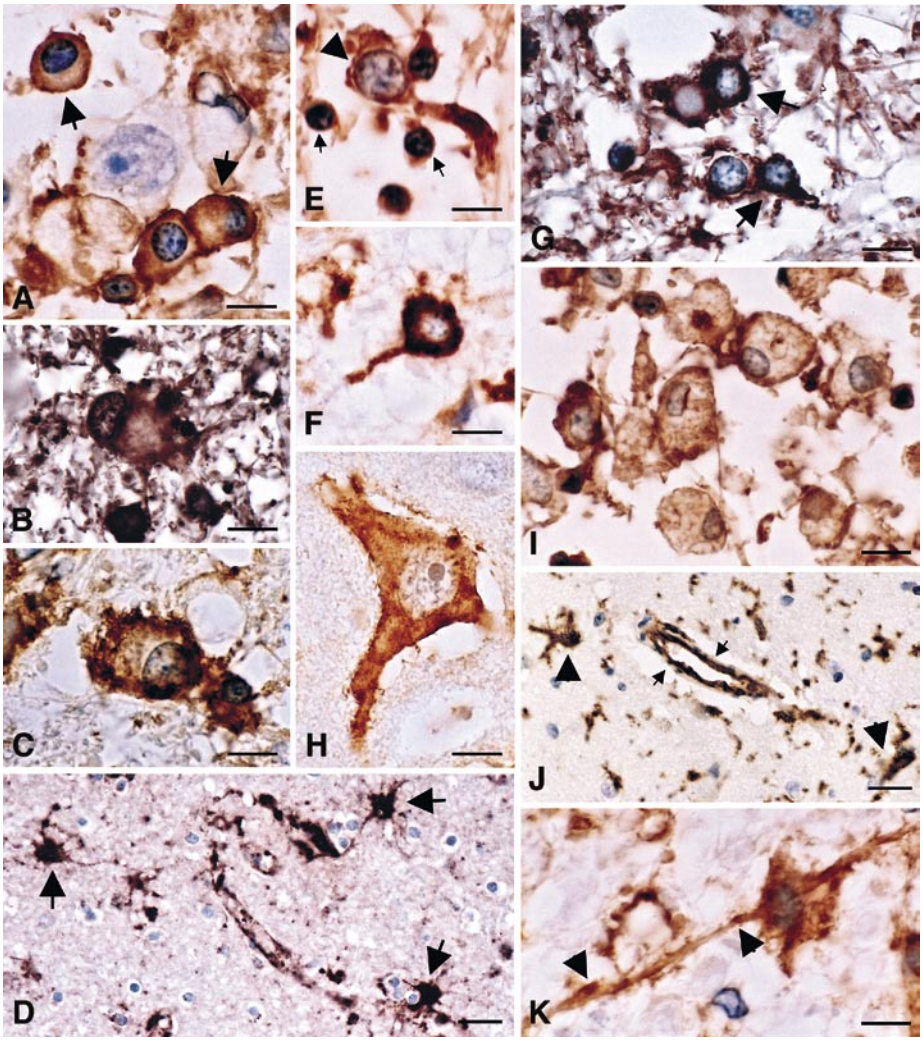

Figure 5. *Immunohistochemistry for MHC Class I molecules on different cell-types in MS lesions.* **A**: plasma-cells (α-chain of MHC class I complex, arrows). **B**: astrocytes (β2-microglobulin). **C**: astrocytes (α-chain of MHC Class I). **D**: astrocytes (β2-microglobulin). **E, F**: oligodendrocytes (α-chain of MHC Class I; arrowhead)—note, small arrows in (**E**) indicating adjacent lymphocytes positive for α-chain as well. **G**: oligodendrocytes (β2-microglobulin; arrows). **H**: neuron (α-chain of MHC Class I). **I**: foamy macrophages (α-chain of MHC Class I). **J**: microglia (arrowheads) and endothelial cells (arrows). **K**: axon within the MS lesion positive for α-chain of MHC Class I complex (arrowhead). Bars: **A-C**, **E-I**, **K**: 10 µm; **D**, **J**: 30 µm.

Class I MHC. Furthermore, all microglia were stained with Class I markers in active, but not in inactive lesions. In contrast the percentage of astrocytes (Figures 5B, C, D; 6A, B, C) and oligodendrocytes (Figures 5: E, F; 6D, E), which were stained for MHC Class I molecules, was significantly lower, but showed a similar gradient as that for microglia (acute MS→active chronic MS→inactive chronic MS). Since the lesions investigated were mostly located in the white matter the number of neurons in the plaques was low. However, some neurons and a variable number of axons, located within the actively demyelinating plaque areas, expressed Class I molecules too (Figures 5H, K; 6F, G). This was mainly the case in Marburg's type of acute MS.

Class II MHC molecules. Nearly all cells, which expressed HLA-D molecules were identified as ramified microglia or foamy macrophages. Their relative contribution varied between different lesion types. In active or inactive lesions from acute MS patients nearly all HLA-D positive cells showed a phenotype of foamy macrophages (Figure 6H), while in active and inactive lesions of chronic MS a variable proportion of the cells were microglia with ramified processes (Figure 6I, J). In the periplaque and "normal" white matter of all MS patients foamy macrophages were nearly absent, but HLA-D expressing microglia were abundant.

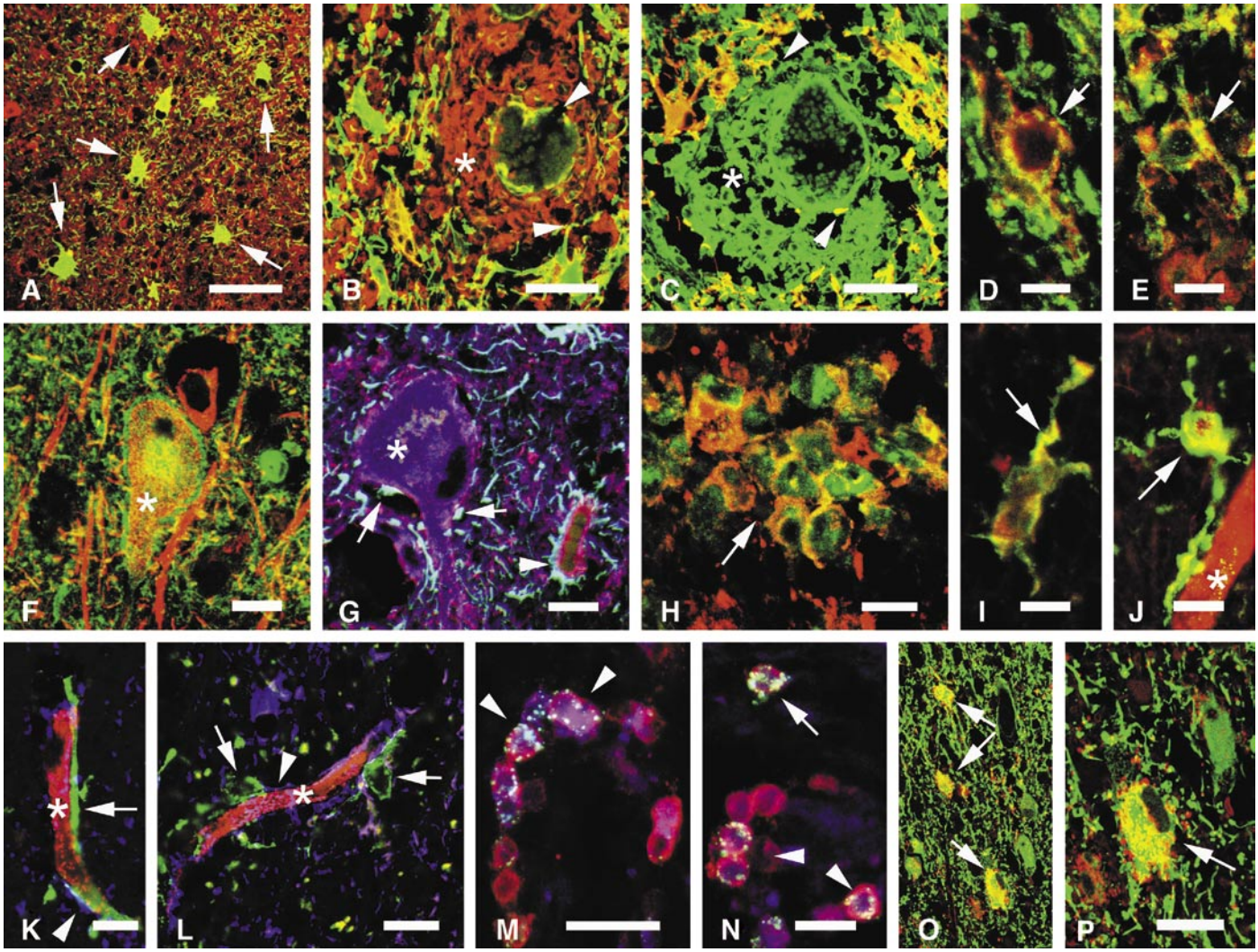

Figure 6. *Confocal laser microscopy scans: MHC Class I expression in actively demyelinating lesions of acute MS* (**A**: 35-year-old male, disease duration 6 weeks; **B-G**: 46-year-old female, 16 weeks disease duration). **A, B**: astrocytes (GFAP: green; β2-microglobulin: red), **C**: astrocytes (GFAP: red; α-chain of MHC Class I: green; colocalization yellow: arrows). Note, astroglial foot processes were partly disrupted, retracted or lost contact to the endothelial cell lining indicating severe damage of the blood brain barrier (arrowheads); asterisks: massive perivascular inflammation - inflammatory cells with expression of light (**B**) and heavy chain (**C**) of MHC Class I. **D**, **E**: oligodendrocytes (CNP: green; β2-microglobulin red; arrows). **F**: neurons (neurofilament: red; α-chain of MHC Class I: green; asterisk: co-labeling). **G**: neurons: (α-chain of MHC Class I: blue; β2-microglobulin: red; GFAP: cyan)—note, in triple labeling (**G**) astrocytes enwrap with their processes either neurons (arrows) or endothelial cells (arrowheads). Neurons co-expressed (pink colored neuronal surface) light and heavy chain of MHCclass I complex (cytoplasm, asterisk).

MHC Class II Expression in MS lesions (**H**: acute MS, 61-year-old female, 22-week disease duration; **I**, **J**: chronic inactive MS, 71-year-old male, disease duration over decades; **K**, **L**: patient dying from terminal septic complications, 79-year-old male; **M**, **N**: acute MS, 45-year-old female, 1-week disease duration; **O**, **P**: acute MS, 35-year-old male, disease duration 6 weeks). **H**: mononuclear phagocytes (HLA-D: green; β2-microglobulin: red; arrows); **I**: parenchymal microglia (HLA-D: green; β2-microglobulin: red; arrows); **J**: perivascular microglia (HLA-D: green; β2-microglobulin: red; co- labeling: arrow)—note: endothelium with MHCclass I expression (red; asterisk). **K**, **L**: endothelial cells (factor VIII related protein: red - asterisk; HLA-D: green; GFAP: blue) lacked any MHC Class II expression and were encircled partly by perivascular MHC Class II positive migroglia (arrows) or partly by astroglial processes (blue, arrowheads). **M**, **N**: cytotoxic T-cells (CD3: red; CD8: blue; Granzyme B positive granules: green) were found in perivascular infiltrates (arrowheads) and in the parenchyma (arrow). **O**, **P**: CD1d-Expression on astrocytes (CD1d: red; GFAP: green): endolysosomal and surface staining for CD1d molecules (co-expression: yellow: arrows). Bars: **A-C**, **F**: 50 µm; **D**, **E**, **G**, **H**, **L**, **M**, **P**: 20 µm; **I**: 5 µm; **J**, **K**, **N**: 10 µm.

In sections, stained by conventional light microscopic immunocytochemistry, some vascular structures were HLA-D positive, which closely resembled staining of cerebral endothelial cells. However, by analysis of such structures with confocal laser microscopy double staining the HLA-D reactivity was always found on cell processes, which were clearly separated from Factor VIII positive endothelial cells, thus representing fine processes of perivascular macrophages (Figure 6K, L). We did not find any HLA-D staining on astrocytes, oligodendrocytes, neurons or axons.

CD 1d. As described before (4) CD 1d reactivity was found on reactive astrocytes and some microglia (Figure 6O, P).

DISCUSSION

Recent studies suggest that MHC Class I restricted T-lymphocytes may play a prominent role in the pathogenesis of MS. This view is supported by the dominance of CD8 positive T-cells and their preferential clonal expansion in MS lesions (4, 14) . In addition, all cell types of the CNS have been shown to be possible targets for Class I restricted killing, either in vitro or in ex-

perimental in vivo models (11, 26, 27, 29). To what extent they may be relevant for the destruction of myelin sheaths, oligodendrocytes or axons in MS lesions critically depends on the ability of T-cells to recognize their specific antigen on the surface of the respective CNS cells. It is, thus an important question, when and where MHC Class I molecules, which are essential in this recognition process, are expressed in MS lesions.

Previous studies on this question, were limited by small case numbers or relied on frozen material in which tissue preservation and cell identification can be problematic. These studies agree that endothelial cells and infiltrating leucocytes are principal sources of MHC Class I molecules in the CNS, both in normal and diseased tissue. To what extent, however, resident glia cells including microglia and neurons or their processes express MHC Class I molecules remained controversial.

Our present study clarifies some of these issues. We show that in principle all cells of the CNS can express MHC Class I molecules in MS lesions and that both components, the α-chain as well as β2-microglobulin is present. The levels of expression, however, follow a hierarchal pattern. Constitutive expression of Class I molecules is present in endothelial cells, perivascular macrophages and some microglia. In the periplaque white matter and in inactive demyelinated plaques, it is mainly the microglia population, in which MHC expression is up-regulated. Only in active lesions of chronic MS and in particular in the fulminate lesions of Marburg's type of acute MS, Class I molecules are also present on neuroectodermal cells of the CNS, such as astrocytes, oligodendrocytes and neurons. A similar hierarchy of MHC expression has previously been shown in rats after intrathecal application of γ-interferon, in which MHC expression on neuroectodermal cells has also been seen only after very profound stimulation (46). In contrast to this experimental study, however, we as others (7) did not find MHC Class II on cells other than leucocytes and microglia. However, this does not exclude that in patients with even more severe disease or by applying more sensitive detection systems, some Class II antigen could be present on astrocytes or cerebral endothelial cells (42, 45).

In our study, MHC class I molecule was represented by antibodies against both the α-chain and β2-microglobulin. In vitro, the alpha chain is more widely expressed, while the appearance of β2-microgloulin on the cell surface is very tightly regulated (27). In contrast,, we find clear over-expression of the β_2-microglubulin chain in astrocytes and oligodendrocytes. As astrocytes have been shown to be the main cell type expressing non-classical MHC-like molecules, such as CD1, which also require the co-expression of β2-microglobulin, this excess of β2-microglobulin may be associated with non-classical MHC molecules (5; Figure 6O, P). Alternatively, β2-microglobulin, which is a small molecule present in soluble form in the blood compartment, may be passively taken up by injured glial cells in active MS lesions with blood brain barrier damage.

In patients with Marburg's type of acute MS terminal septic complications are frequent. For this reason we expanded our study with an additional control group of patients with systemic inflammatory complications, such as sepsis. Both, the inflammatory mediators as well as bacterial products, such lipopolysaccharide, liberated in septic conditions, are known to alter MHC expression in the central nervous system (10, 44). In our septic control group, a significant up-regulation of MHC class I molecules in comparison to the non-inflammatory control group was found on microglia and macrophages, but not on neuroectodermal cells, such as astrocytes, oligodendrocytes or neurons. In addition, the extent of MHC class I reactivity on microglia and macrophages observed in MS lesions was significantly higher as in both control groups. Therefore, the inflammation within the CNS compartment in MS apparently is a much more potent activator of MHC class I expression compared to the systemic inflammatory response in sepsis.

MHC expression within nervous tissue is regulated by several different mechanisms. Neuronal activity, possibly through the secretion of neurotrophic factors, exerts a negative signal, actively suppressing the genetic program for antigen presentation (27, 28}. In addition, the positive signals mediated through pro-inflammatory cytokines, such as γ-interferon, are absent in the normal CNS. In active MS lesions, pro-inflammatory cytokines are abundantly secreted by inflammatory cells. Furthermore, axons in active MS lesions are affected by acute conduction block, both through the acute loss of the myelin sheath (31) and through toxic inflammatory mediators, such as nitric oxide (20, 38). Thus in active MS lesions the pro-inflammatory environment occurs in parallel with a dysinhibition of the antigen presentation program. This may explain the very high levels of MHC expression in this disease and in particular its expression in all neuroectodermal cell types. The local synthesis of MHC molecules can further be augmented by steroid treatment (25).

The importance of Class I MHC restricted cytotoxic T-cells in mediating clinical disease and tissue damage in the central nervous system is highlighted in the model of Theiler's virus induced demyelinating encephalomyelitis. Studies on the genetic susceptibility to this disease (3, 36) as well as on disease induction in β2-microglobulin deficient mice (35) showed that clinical disease and demyelination depends upon a Class I MHC restricted T-cell response. Disease can also be blocked by treatment with anti-CD8 antibodies (37) and specific deletion of a virus peptide specific class I restricted T-cell response preserves motor function in infected animals (18). All these data strongly suggest, that Class I MHC restricted T-cells can directly be involved in the induction of demyelination and axonal injury, possibly by recognizing their specific antigen on glia cells and neuronal cell processes.

Thus, in summary our study shows abundant MHC Class I and Class II expression in active lesions of MS patients. Its expression follows a hierarchy, dependent upon the type and severity of the disease process. We further show that all cells of the CNS can express Class I molecules and are possible targets of Class I restricted T-cell cytotoxicity. Our study, further supports the view that Class I restricted cytotoxic T-cells may play a role in destruction of myelin and axons in MS lesions.

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