American Journal of Pathology, Vol. 137, No. 4, October 1990
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Intrathecal Application of Interferon Gamma

Progressive Appearance of MHC Antigens Within the Rat Nervous System

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Intrathecal injection of interferon-γ induced a significant increase of the number of class I and class II major histocompatibility complex (MHC)-expressing cells within the rat nervous system. A progressive appearance of MHC-antigen–positive cells was found by light- and electron microscopic immune histology. The first level comprised cells that constitutively expressed MHC antigens in normal animals (meningeal and endoneural monocytes, some perivascular dendritic cells, and few parenchymal microglia cells, especially in the lumbar spinal cord and in the cerebellar white matter). The second level represented cells readily expressing MHC antigens after stimulation with interferon-γ (all perivascular, dendritic cells, and microglia). The third level included ependymal cells, astrocytes, and Schwann cells. After stimulation with interferon-γ, these neuroectodermal cells expressed MHC antigens inconsistently, usually in a low density and patchy distribution. The progressive appearance of MHC antigens may be reflected by the variances of lesional patterns found in experimental allergic encephalomyelitis of different histologic severity. (Am J Pathol 1990, 137:789–800)

T lymphocytes recognize antigen in context of major histocompatibility (MHC) antigen. Within the central and the peripheral nervous system, the nature of antigen-presenting cells is still on debate. Results from *in vitro* studies suggested astrocytes,[1-3] microglial,[4,5] endothelial,[3,6] and Schwann cells[7] to express MHC-class I and II antigens and to present antigen to T-lymphocytes under certain conditions. Conversely, *in situ* studies focussing on the tissue distribution and the histologic characterization of MHC-antigen–expressing cells are contradictory. Using light microscopic techniques, all cells of nervous system were reported to express MHC antigens in humans or experimental animals,[8-21] although ultrastructural confirmation was lacking. In previous electron microscopic studies on lesions of acute experimental allergic encephalomyelitis (EAE), we were not able to prove the presence of class II MHC antigen on cells other than bone marrow-derived monocytes and on microglia[22-24]; however, other studies suggested Ia-expression on endothelial cells[25] and Schwann cells.[26]

In the present study, we investigated MHC expression within the nervous system under normal conditions and after stimulation by intrathecal injection of interferon-γ. Major histocompatibility antigens were found on bone-marrow–derived monocytic cells, on microglia, and occasionally on neuroectodermal cells. Our results argue for a hierarchical organization and a stepwise activation of MHC expression within the nervous system. This may play an important role in the subtle regulatory mechanisms controlling inflammation within the nervous system.

Materials and Methods

Antibodies and Reagents

For the experiments, recombinant mouse interferon-γ was used (a gift from Dr. G.R. Adolf, Ernst Boehringer Institut für Arzneimittelforschung, Vienna, Austria). Mouse interferon-γ was effective in stimulating class II expression on rat macrophages (data not shown). All primary antibodies (Table 1) were obtained from Sera Labs, Crawley Down, UK. The anti-GFAP (glial fibrillary acidic protein) was from Boehringer-Mannheim (Mannheim, FRG), the lectin bandeirea simplicifolia BS-II (GSA) was from Sigma (St. Louis, MO); the species-specific biotinylated anti-mouse immune globulin (Ig), from Amersham; the alkaline-phosphatase anti- alkaline-phosphatase complex (APAAP), from DAKOPATTS (Copenhagen, Denmark); and the diaminobenzidine, from Sigma.

Supported by the Science Research Fund Austria, project P6438M.
Accepted for publication May 8, 1990.
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Table 1. *Antibodies and Lectin Used for Immunohistochemistry*

Antibody	IgG subclass	Dilution	Labeled cell	Structure recognized	Reference
W3/13	IgG_1	1:200	T cells, granulocytes	Leucosialin	64, 65
Ox19	IgG_1	1:100	T cells	CD5—antigen	66
W3/25	IgG_1	1:200	T cells, monocytes/macrophages	CD4—antigen	64, 67, 68
Ox35	IgG_{2a}	1:25	T cells, monocytes/macrophages	CD4—antigen	68
Ox38	IgG_{2a}	1:25	T cells, monocytes/macrophages	CD4—antigen	68
Ox8	IgG	1:200	T cells	CD8—antigen	67
Ox6	IgG_1	1:200		MHC—class II (I-A)	69
Ox17	IgG_1	1:100		MHC—class II (I-E)	70
Ox18	IgG_1	1:1000		MHC—class I	70
Ox41	IgG_{2a}	1:50	Monocytes/macrophages		49
Ox42	IgG_{2a}	1:50	Monocytes/macrophages	Complement receptor 3	49
Ox43	IgG_1	1:400	Monocytes/macrophages		71
ED1	IgG_1	1:1000	Monocytes/macrophages		72
ED2	IgG_{2a}	1:2000	Monocytes/macrophages		72
ED3	IgG_{2a}	1:50	Monocytes/macrophages		72
Ox39	IgG_1	1:50		Il-2-receptor	73
Anti-GFAP	IgG	1:100	Astrocytes	GFAP	
GSA	—	1:40	Microglia		74

Surgical Procedures

For intrathecal injection of interferon-γ, essentially the procedure described by Lassmann et al[27] was followed. Twenty-one Sprague-Dawley rats (200 g, from Versuchstieranstalt, Himberg) were anesthetized by intraperitoneal injection of Equithesin (containing 42.5 mg chloralhydrate and 9.7 mg pentobarbital per milliliter), the sacral dura was exposed by removing the arc of the 5th lumbar vertebra. Interferon-γ 10^1 to 10^4 units in a total volume of 50 μl was gently injected into the sacral subarachnoide space. Four additional animals were injected with the same volume of 0.9% saline and another seven animals served as uninjected control. The wound was closed and the animals were allowed to awake. At 1, 24, 48 hours later, animals were killed by an overdose of Equithesin. After flushing the blood with 100 ml phosphate-buffered saline (PBS) *via* the aorta, the spinal cord was removed, placed into Tissue-Tek OCT compound (Miles Labs, Elkhart, IN), and immediately frozen in dry-ice–cooled isopentane (Fluca, Buchs, Switzerland). The blocks were stored until further processing at −70°C.

Immune Histochemical Procedures

Five-micron–thick frozen sections were fixed in acetone and defatted in chloroform. After preincubation with fetal calf serum (FCS), sections were incubated with the primary antibodies as indicated in Table 1. This step was followed by the species-specific anti-mouse Ig (1:100, additionally preabsorbed by normal rat serum) and by the APAAP complex (1:100). Incubation with anti-mouse Ig followed by the APAAP complex was repeated three times. Alkaline phosphatase then was developed using Fast Blue BB Salt (Sigma) as a chromogen. Sections either stained without or with an irrelevant primary antibody of the same IgG class served as controls. Sections were finally slightly stained with nuclear fast red.

Quantitative Examination and Statistical Analysis

For each animal, at least three levels of the spinal cord (lumbar, thoracic, and cervical) for central nervous system (CNS) and spinal nerve roots for peripheral nervous system (PNS) were examined. Values indicate number of positively labeled cells per square millimeter. Statistical analysis was calculated on an Apple Macintosh computer using the statistical software package StatviewPlus (Brain Power, Inc., Calabasas, CA).[28]

Immune Electron Microscopic Procedures

Paraformaldehyde-fixed spinal cord tissue was cut into 50- to 100-μ-thick sections. For immune electron microscopy, a pre-embedding technique[22,24] was used. After preincubation with FCS, sections were exposed to the primary antibodies (for dilutions, see Table 1), followed by the biotinylated anti-mouse antibody and by peroxidase-labeled avidin. Each incubation step lasted for at least 8 hours and was followed by extensive washing with PBS. After peroxidase was developed using diaminobenzidine as chromogen, sections were osmicated, dehydrated, and embedded in epoxy resin (Epon, Serva, Heidelberg, FRG).

MHC Antigens in Normal CNS

In the meninges, cells positive for both class I and class II MHC antigens were of round or dendritic shape with

Table 2. *Induction of MHC Antigens in the CNS and PNS by Interferon-γ*

	Control		IFN-γ		Antigens additionally expressed by the cells
	Ox6	Ox18	Ox6	Ox18	
Meningeal MO	+	+	+↑	+↑	ED1, ED2, CR3, Ox43, CD4, GSA, Ox17
Perivascular MO	(+)	(+)	+↑	+↑	ED1, ED2, CR3, Ox43, CD4, GSA, Ox17
Microglia	(+)	(+)	+↑	+↑	CR3, CD4, GSA
Ependymal cells	−	−	(+)*	+	
Astrocytes	−	−	(+)*	+/−	GFA
Oligodendrocytes	−	−	−	+/−	
Nerve cells	−	−	−	+/−	
Endoneuronal MO	+	+	+↑	+↑	ED1, ED2, CR3, Ox43, CD4, GSA, Ox17
Schwann cells	−	−	(+)*	+	
Endothelial cells	−	+	−	+	

+: all cells positive
(+): some cells positive
+↑: increased labeling intensity after IFN-γ injection
*: patchy, discontinuous staining
+/−: weak and inconsistent staining
Ox6: class II MHC (I-A)
Ox18: class I MHC

round to oval nuclei and fine, frequently ramifying processes. A similar meningeal cell population was labeled by the macrophage makers ED1, ED2, Ox43, expressed the CR3 complement receptor (Ox42), and the CD4 antigen (W3/25, Ox35) (Table 2).

A cell population with a comparable marker profile also was present in the perivascular space of spinal cord vessels (Figure 1a, c, i through l, open arrows). These cells were either located in the Virchow Robin spaces of larger vessels or appeared to be closely attached to the walls of smaller vessels (arterioles or venoles). In normal rats some of these cells expressed class I (Figure 1c) and class II Figure 1a) MHC antigens.

In addition to meningeal and perivascular cells, a third cell population expressing MHC antigens in the normal CNS was found within the CNS parenchyma. These cells were small with generally bipolar, dendritic processes and rod-shaped nuclei (Figure 1a through h, arrowheads). They were distributed in both the gray and white matter and were consistently labeled by the Ox42 antibody (CR3-antigen, Figure 1g) and the lectin GSA (Figure 1h). A fraction of these cells also expressed the CD4 antigen (W3/25, Ox35, Figure 1f), but were not stained by the other macrophage markers. In normal animals, MHC antigens were only present on a fraction of such microglialike cells. As shown in Figure 2, MHC class II expression on microglial cells differed in various areas of the CNS, and was most pronounced in the lower portions of the spinal cord and the cerebellar white matter.

Whereas class I and class II MHC expression was similar on meningeal and perivascular dendritic cells as well as on microglia, constitutive class I but not class II expression was additionally found on ependymal (Figure 1c) and on endothelial cells (Figure 1c). Immune staining with anti-GFAP in normal rats showed positive reactions in the subpial regions and in some astrocytic processes in the white matter. The staining pattern was completely different from that obtained with the antibodies against MHC antigens (data not shown).

MHC Antigens in Normal PNS

In the spinal roots, MHC-positive cells were found in the perivascular space and diffusely dispersed in the endoneurium. Compared with the CNS, the number of MHC-expressing cells per square millimeter was considerably higher (Ox6: 54 ± 36, Ox18: 131 ± 84). Similar cell populations were labeled by the macrophage markers ED1 (not shown), ED2 (Figure 1o), Ox43 (Figure 1p), and by the lectin GSA (not shown). They also expressed CD4 (W3/25, Ox35) and CR3 (Ox42, not shown). Thus, these PNS cells were very much akin to the cells found in the meninges and the perivascular spaces of the CNS (Table 2). Endothelial cells of PNS vessels expressed class I MHC antigens.

Intrathecal Injection of Interferon-γ

The animals did not develop signs of disease within the 48 hours' observation time. Whereas intrathecal injection of saline resulted in a staining pattern indistinguishable from that of control animals, interferon-γ, as expected, induced a significant increase in the number of cells expressing MHC antigens and in the intensity of MHC expression (Figure 3). Interferon-γ, however, had no effect on the expression of other macrophage antigens (ED1, ED2, Ox43), of the lectin GSA, of the CR3 (Ox42), and of CD4-antigen (W3/25, Ox35) (data not shown). Induction of MHC antigens by interferon-γ peaked 24 to 48 hours after injection (Table 3) and was dose dependent (Figure 3). The highest numbers of positive cells were found after

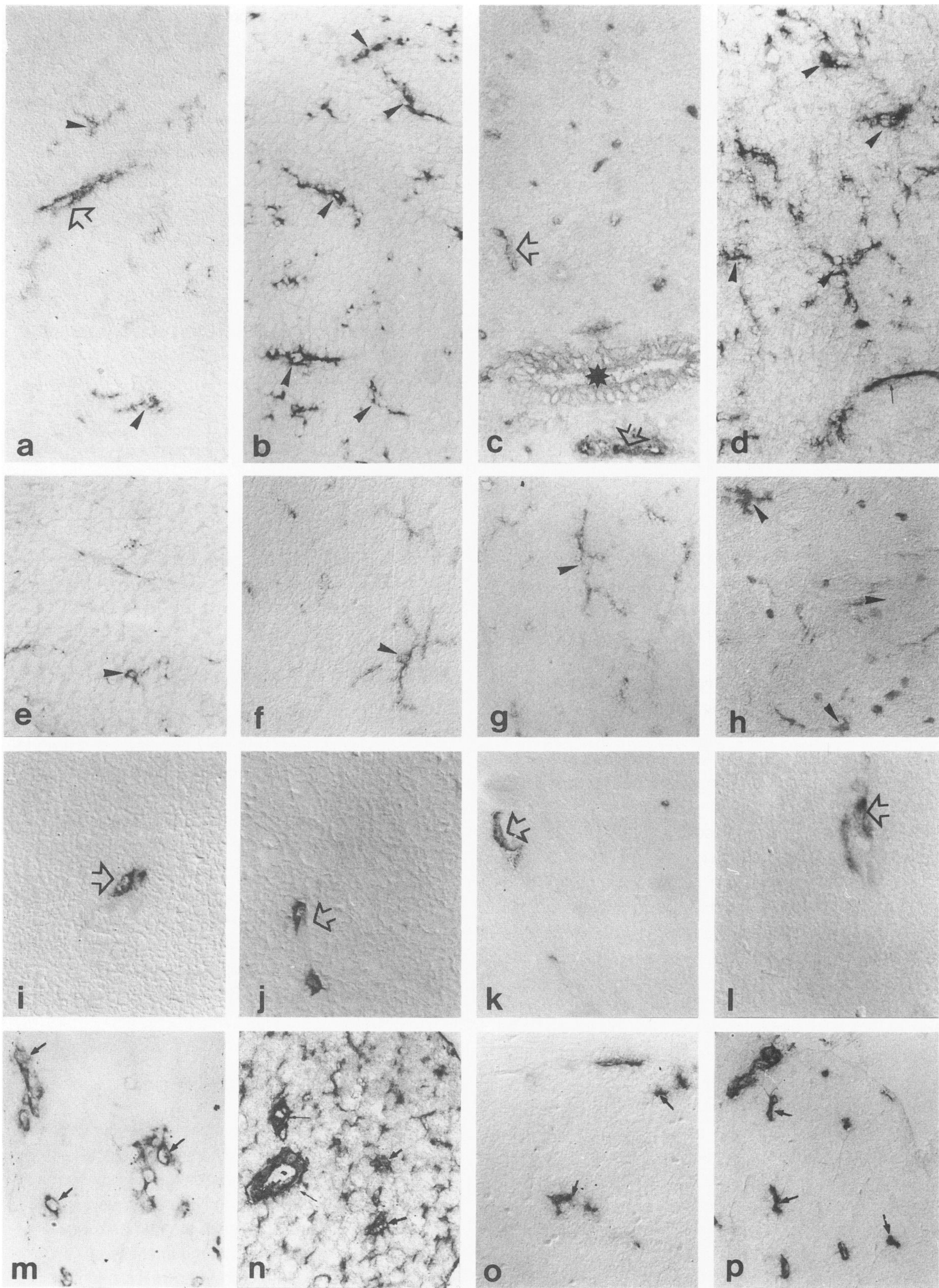

Figure 1. *Upregulation of MHC expression on cells of the nervous system by interferon-γ* (a–d) *and phenotypical characterization of CNS* (e–l) *and PNS* (m–P) *cells by monocyte/macrophage markers.* a: *Ox 6, control;* b: *Ox 6, 10^4 units interferon-γ, 48 hours;* c: *Ox 18, control,* d: *Ox18, 10^4 units interferon-γ, 48 hours;* e: *Ox 17, 10^4 units interferon-γ, 48 hours;* f: *W3/25, control;* g: *Ox42, control;* h: *GSA, 10^4 units interferon-γ, 48 hours;* i: *ED1, control;* j: *ED2, 10^4 units interferon-γ, 48 hours;* k: *ED3, control;* l: *Ox43, control;* m: *Ox6, 10^4 units inteferon-γ, 24 hours;* n: *Ox 18, 10^4 units interferon-γ, 48 hours;* o: *ED2, control;* p: *Ox43, control. Arrow heads: microglial cells; open arrows: perivascular cells; thin arrows: vessels; thick arrows: endoneural macrophages; asterisk: central canal with ependyma. Frozen sections, ×250.*

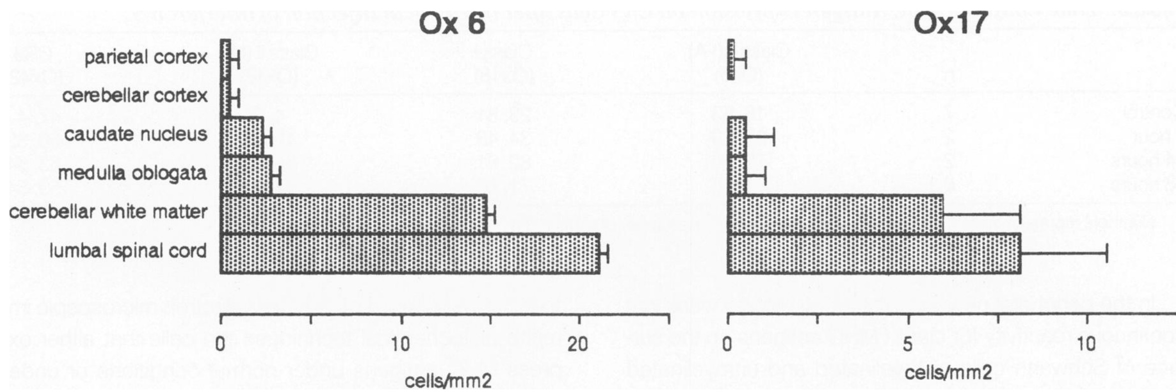

Figure 2. *Number of MHC class II-expressing cells in various regions of the normal rat CNS. Bars indicate numbers of positive cells per mm² ± standard deviation. *P < 0.05 when compared with controls, Dunnett's t-test.*[63]

injection of 10^4 units. Lower doses resulted in high MHC expression in the subpial regions but low numbers of positive cells in the central portions of the spinal cord.

Qualitatively interferon-γ induced and increased MHC expression in cells, similar to those already found positive (in lower numbers) in control animals (Figure 1 a through d). This was especially evident for class II antigens. In the spinal cord, the number of positive parenchymal cells with morphology of microglial cells approached the number of CR3 (Ox42)-positive cells 48 hours after intrathecal injection of interferon-γ. An exact identification of stained glial cells other than microglia, however, was not possible at a light microscopic level. Whereas reactivity for class I MHC antigens was also found on all ependymal cells, class II antigens were detected only on single cells within the ependymal lining (not shown).

In the peripheral nervous system, we did not find a significant increase in the number of MHC-antigen–expressing cells after intrathecal interferon-γ injection (data not shown). The immune-stained cells were morphologically similar to those found in normal animals (Figure 1m, n); however the intensity of the staining reaction was increased compared with controls.

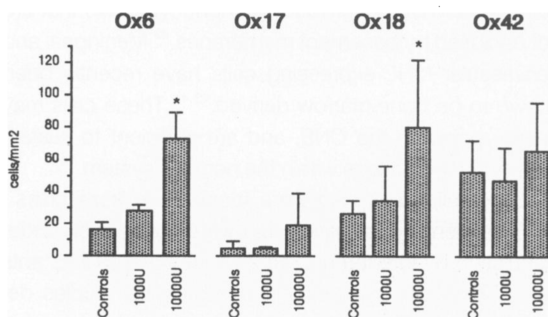

Figure 3. *Intrathecal injection of interferon-γ: effect of various concentrations on the number of MHC-antigen–expressing cells of the spinal cord parenchyma. Bars indicate numbers of positive cells per mm² ± standard deviation. P < 0.05 when compared with controls, Dunnett's t-test.*[63]

Electron Microscopic Characterization of MHC-antigen–Expressing Cells After Intrathecal Interferon-γ Injection

The most intense expression of class I and class II MHC antigens was found on monocytic cells in the meninges and the perivascular spaces, as well as on small, frequently bipolar cells with delicate cell processes distributed diffusely in the gray and white matter of the spinal cord (Figure 4a through c). The latter cells generally contained small rodlike nuclei, a dense cytoplasm, some parallel layers of smooth endoplasmic reticulum, and lysosomes (Figure 4a). These cells thus fulfilled the morphologic criteria of microglia and were ultrastructurally similar to the cells stained with Ox42 (anti-CR3, Figure 4e). Similarly in the peripheral nervous system Ox6- and Ox18-positive cells were found, scattered in the endoneurium, without basement membrane or association with nerve fibers.

All ependymal cells were labeled by Ox18 on their luminal surfaces, and less intensely on their lateral and basal cell membranes (Figure 5d). In contrast, class II antigen was present only on single ependymal cells, embedded in the otherwise negative ependymal layer (Figure 5c). Furthermore a continuous but weak labeling for class I MHC antigens was found on all cells and cell processes in areas that were well penetrated by the antibodies. On the contrary, class II MHC staining was more restricted. Ox6 reactivity was found on the surface of some astrocytes (Figure 5a, b). On these cells, however, the antigen distribution was patchy, resulting in small spots of immune reactivity on the otherwise unstained surface of the astrocytes. Interestingly class II reaction product on astrocytes was mainly present on the cell membranes covering the cell body and the proximal processes, but was hardly detected on the membranes of astrocytic foot processes, which are oriented toward the vascular basement membrane. We did not find class II reactivity on oligodendrocytes and neurons.

Table 3. *Time Course of MHC Antigen Expression on CNS Cells After Intrathecal Injection of Interferon-γ.*

	n	Class II (I-A) (Ox6)	Class I (Ox18)	Class II (I-E) (Ox17)	CR3 (Ox42)
Control	7	16, 53	23, 81	4, 31	47, 4
1 hour	2	22, 69	34, 43	10, 56	56, 32
24 hours	2	75, 50	82, 91	46, 01	63, 54
48 hours	6	72, 76	71, 86	17, 04	59, 54

Numbers represent means values of labeled cells per mm^2; n: number of experiments.

In the peripheral nervous system, we found weak but continuous reactivity for class I MHC antigens on the surface of Schwann cells of myelinated and unmyelinated nerve fibers (Figure 6d). Similarly as on astrocytes, class II MHC antigens were present after intrathecal injection of interferon-γ on the surface of Schwann cells of myelinated fibers in a discontinuous, patchy distribution (Figure 6a, b). In the latter, weak reactivity was also found in the mesaxon and the periaxonal space of unmyelinated nerve fibers (Figure 6a, asterisk).

Intense class I immune reactivity was found on the luminal surface of all endothelial cells in blood vessels of the CNS and PNS (Figure 4d). On the contrary, no single class-II–positive endothelial cells was encountered in all immune electron microscopic samples.

Control Experiments

Only exceptional T lymphocytes, mainly located in the leptomeninges, were found in interferon-γ–injected animals as well as in control animals when the tissue was stained for light and electron microscopic immune histochemistry with the antibodies W3/13, Ox8, Ox19 and Ox39. In cryostat sections, as described by others before, W3/13 predominantly stained nerve cells of the spinal cord.[29] No increase in the number of T cells (W3/13, Ox8, Ox19, Ox39) or macrophages (ED1, ED2, Ox42, Ox43) were found after intrathecal injection of interferon-γ or saline. No immune staining was found, when irrelevant monoclonal antibodies of the same Ig class were used as primary layer or when the primary monoclonal antibodies were omitted in the staining procedure.

Discussion

The cellular distribution of MHC antigens within the nervous system may serve as important clues for the understanding of regulatory events in inflammatory conditions. Although MHC antigen expression has been studied in detail before on isolated cells of the CNS and PNS *in vitro*,[1,2,4–6,30–35] only *in situ* studies in normal and diseased nervous system may offer answers on the role of individual cell types in the dynamics of inflammation. We thus tried to characterize by light and electron microscopic immune histochemical techniques the cells that either express MHC antigens under normal conditions or under maximal stimulation by interferon-γ.

Interferon-γ is a glycoprotein and its stimulatory properties on macrophages and monocytes, but also on numerous other cell types, are well documented.[36–39] Enhancement of class I and class II MHC antigen expression not only on macrophages but also on astroglia has been described.[1,2] Under stimulation of interferon-γ, in addition to macrophages, microglia, astrocytes, Schwann cells, and endothelial cells are reported to present antigen to T lymphocytes.[2,4,6,7,40] *In vivo*, systemic injection of extremely high doses of interferon-γ results in an increase in the number of MHC-expressing cells within the nervous system.[20] Moderate doses even over a longer period had no effect.[36] Conversely, Wong et al[41] described elevation of MHC expression after intracerebral injection of this cytokine. Thus we injected interferon-γ directly into the cerebrospinal fluid.

Our present study indicates a hierarchical organization of MHC expression within the nervous system, which may determine antigen recognition during different phases of an inflammatory process. The first level of antigen recognition is represented by cells that express MHC antigens constitutively. These cells include, for both class I and class II antigens, meningeal, perivascular, and endoneural monocytic cells. These cells show comparable antigenic profiles and express the complete panel of monocytic antigens investigated in our study. Perivascular cells include ED2-positive, pericytelike cells, which have to be distinguished from other perivascular monocytic cells that are not enclosed by basement membranes.[42] Meningeal and perivascular MHC-expressing cells have recently been shown to be bone-marrow derived.[43,44] These cells may quickly populate the CNS, and are sufficient to sustain autoimmune reactions within the nervous system.[44]

Endothelial cells also were found to express class I MHC antigens constitutively, but were never, even under stimulation by interferon-γ positive for class II MHC antigens. There are several light microscopical studies describing endothelial class II MHC antigen expression within the nervous system,[9,10,13,16,20,30,45,46] although these results lack ultrastructural confirmation. Electron microscopic evidence of endothelial class II MHC expression

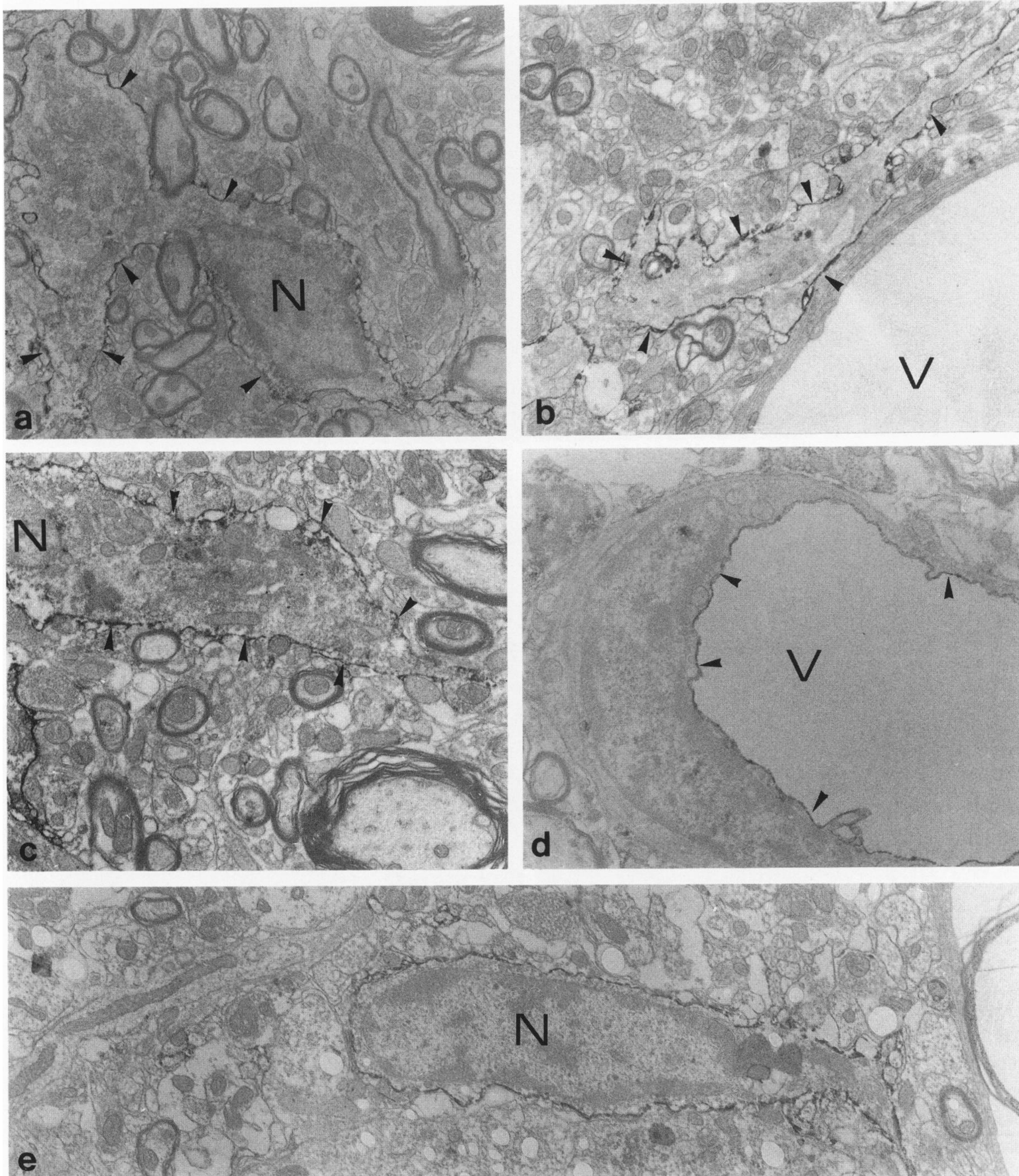

Figure 4. *MHC antigen expression within the CNS after intrathecal injection of interferon-γ.* **a, b**: *Ox6;* **a**: *microglial cell, ×5600;* **b**: *perivascular microglia cell process, ×7000.* **c, d**: *Ox18;* **c**: *microglial cell, × 7000;* **d**: *endothelial cell, ×11,000.* **e**: *Ox42, microglial cell, ×8400. Arrow heads: positive labeling; N: nucleus; V: vessel.*

was recently presented by Sobel et al[25] in the guinea pig. This discrepancy to our results may be explained by species differences. Furthermore, extremely low (morphologically undetectable) MHC class II antigen expression on endothelial cells could still initiate antigen-specific lymphocyte recruitment in combination with cytokine-induced adhesion molecules.[47]

Microglia may be regarded as the second level of antigen recognition within the CNS. Under resting conditions, a fraction of cells fulfilling the phenotypic and electron microscopic morphologic criteria of microglia was positive for class I and class II MHC antigens and the number of MHC-expressing microglial cells increased dramatically after application of interferon-γ. In an *in vitro* study, MHC

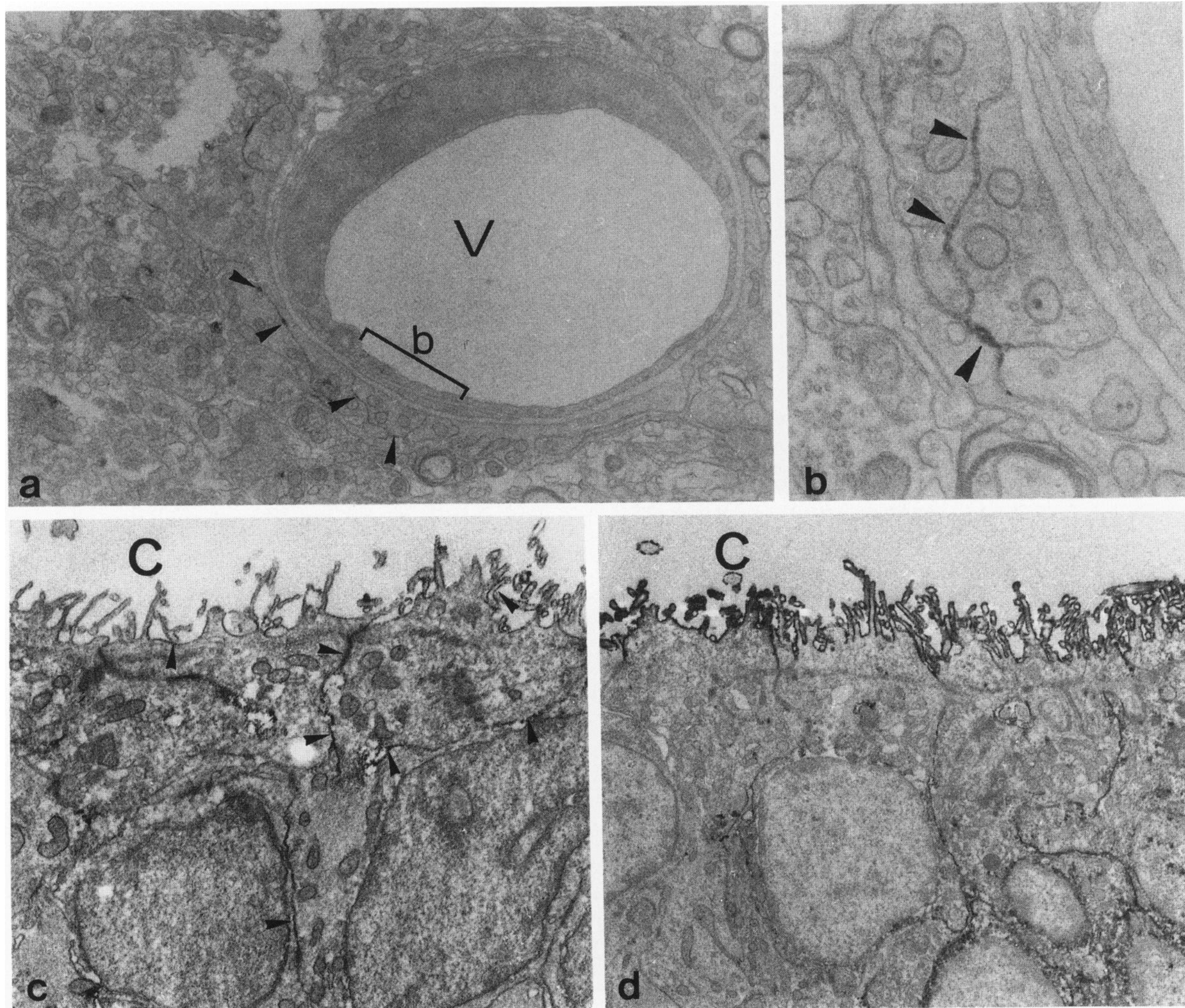

Figure 5. *MHC antigen expression on glia cells of the CNS after intrathecal injection of interferon-γ.* **a, b**: *astrocytes; Ox6;* **a**: *×7000;* **b**: *higher magnification of* **a**, *×15,000.* **c, d**: *ependymal cells;* **c**: *Ox6, ×8400;* **d**: *Ox18, ×4100. Arrow heads: positive labeling; V: vessel; C: central canal.*

class I expression was found on unstimulated 'macrophage-microglia.'[5] Major histocompatibility class II antigens were either not found[5] or only on a small number of cultured glial cells.[35] It remains unclear whether 'perivascular cells'[48] or true 'parenchymal' microglia were isolated in these experiments. Major histocompatibility class II expression in 'normal' autopsy brains[18] and in brain biopsies[16] has been reported, but on unstimulated, resting microglia of naive, untreated control animals neither MHC class I and nor class II expression has been described so far. Microglia cells were characterized additionally by the constitutive expression of CR3 (Ox42[49]). Most of these cells were also positively labeled by the lectin GSA, and some of these cells expressed the CD4 antigen (W3/25, Ox35) but were negative for the ED1, ED2, Ox43 antigens, as described by others.[42,50,51] These results, although contradicted by recent autoradiographic studies,[52] suggest that microglia cells are a specialized subtype of the bone-marrow–derived monocyte/macrophage cell population similar to 'tissue macrophages' described in many other organs. In tissue cultures, microglial cells were shown to present antigen to T lymphocytes and to have cytotoxic properties.[4] Therefore microglial cells probably play the key role in immune regulatory mechanisms within the CNS.[44,53,54]

The regional differences in the number of MHC class II antigen-expressing microglial cells in normal animals is of considerable interest. We found the highest density in the lumbar spinal cord and the cerebellar white matter, whereas in other regions, such as the forebrain, only very few positively labeled cells were detected. The areas of high constitutive microglial MHC class II expression correspond with the preferential sites of inflammatory infiltrates found in EAE,[55] a disease typically mediated by CD4-posi-

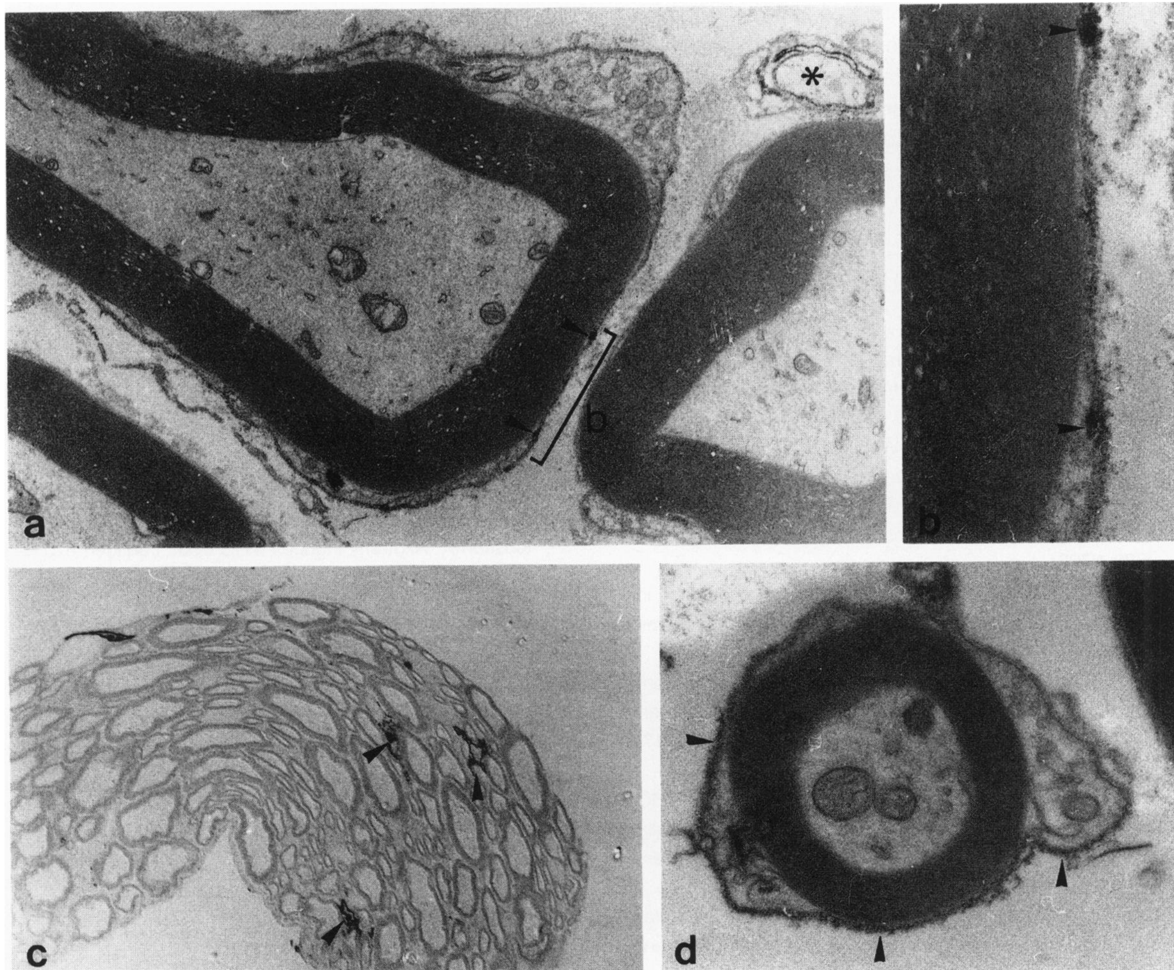

Figure 6. *MHC antigen expression within the PNS after intrathecal injection of inteferon-γ.* **a, b:** *Schwann cells of myelinated and unmyelinated nerve fibers (asterisk), Ox6,* **a:** *×7000;* **b:** *higher magnification of* **a**, *×28,000; arrowheads: positive labeling.* **c:** *endoneuronal macrophages (arrowheads), Ox6, ×600.* **d:** *Schwann cell, Ox18, ×20,000.*

tive T cells. A strict correlation of regional MHC class II expression with the distribution of infiltrating inflammatory cells recently has been shown.[56]

The third level of antigen presentation is represented by true neuroectodermal cells. We found MHC expression on ependymal cells and astrocytes within the CNS and on Schwann cells within the PNS. These cells were found to express class II MHC antigens only after stimulation by interferon-γ. Astrocytes and Schwann cells exhibited patchy MHC expression similar to that known from tissue culture experiments.[1] The role of astroglia in the course of inflammatory lesions is not yet settled. From *in vitro* studies it is known that astrocytes synthesize lymphokines,[57,58] present antigen to T lymphocytes,[2,59] and may become the target of a cytotoxic attack.[60] The latter finding could explain for the widespread necrotic lesions with almost complete disappearance of astrocytes sometimes found in EAE.[61] Ependymal MHC expression[20] points to the importance of the CNS/CSF interface. Ependymal cells are easily accessible for CSF T lymphocytes and could be stimulated to express MHC antigens by cytokines synthesized by these cells. This may activate further T lymphocytes populating the CSF. T-cell activation by ependymal cells may be involved in the formation of periventricular lesions typically found in EAE as well as in multiple sclerosis (MS).[62] Thus we speculate that neuroectodermal cells, although playing a subordinate role for the initiation, are of great importance for propagating inflammatory lesions within the nervous system.

Finally we think the hierarchy of MHC expression in the nervous system, constitutive expression on meningeal, perivascular, and endoneuronal monocytes/macrophages, frequent and strong facultative expression on microglia, and rare and weak expression on true neuroectodermal cells, are also reflected by the pathology of EAE and EAN: First, very mild disease, when a low number of T cells are engaged, is only characterized by meningitis. Second, in moderate disease, perivascular cuffings of in-

flammatory cells and tissue infiltrates in areas with high incidence of class II MHC antigens on microglia (ie, lumbar spinal cord and cerebellar white matter) are encountered. Third, in severe disease massive tissue infiltration by inflammatory cells, with tissue destruction, is found.

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Acknowledgments

The authors thank H. Breitschopf, S. Katzensteiner, A. Kury, and M. Leiszer for technical and photographic assistance.