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Journal of Neuroimmunology, 16 (1987) 215–224
Elsevier

JNI 00516

Induction of class I and class II transplantation antigens in rat brain during fatal and non-fatal measles virus infection

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(Received 21 November 1986)
(Revised, received 12 February 1987)
(Accepted 12 February 1987)

Key words: Transplantation antigen, class I and class II; T lymphocyte; Immunohistochemistry; Measles virus infection

Summary

Measles virus induced a marked increase in the expression of MHC-coded class I and class II antigens as detected by immunostaining during both fatal and non-fatal brain infections in rats. The distribution of these molecules in the brain was much more widespread than the occurrence of viral antigen suggesting a soluble factor for their induction. In 14-day-old rats with a non-fatal infection there was a marked infiltration of T lymphocytes of 'cytotoxic/suppressor' phenotype in the brain parenchyma, whereas T 'helper' cell phenotypes mainly were located perivascularly. In brains from newborn rats with a fatal infection no or only few lymphocytes were detected.

Introduction

The outcome of an acute viral infection of the brain may vary from a rapid self-extinguishing fatal or non-fatal disease to a chronic or persistent infection. The

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age of the host animal strongly influences the course of many virus infections (Byington and Burnstein 1973). For instance, neurotropic strains of measles virus cause a fatal necrotizing brain infection in newborn rats, while animals inoculated at 10 days of age or later are refractory to development of clinical signs as a consequence of the virus infection (Percy and Coulter-Mackie 1982). The detailed mechanism(s) for the age dependence are not fully understood. They may involve a more productive virus replication in immature brains (Griffin et al. 1974) or reflect differences in the immune response or other host defence mechanisms with age (Johnson and Norrby 1974).

An important factor for the outcome of a virus infection may also be the occurrence of cells expressing major histocompatibility (MHC) class I and class II antigens, since activation and effects or functions of T cells are dependent on recognition of the virus antigen in context with these antigens. T lymphocytes of 'cytotoxic/suppressor' phenotype recognize antigen on cells that also express MHC class I antigen, whereas T lymphocytes of 'helper' phenotype require that the antigen is located on cells that express MHC class II antigen. Under normal conditions the nervous system has low levels of MHC antigen expression (Vitetta and Capra 1978; Schnitzer and Schachner 1981), but an enhanced expression of both class I and class II transplantation antigens is seen during certain circumstances. Thus, experimental allergic encephalitis (EAE) induces an increased synthesis of class II antigen (Sobel et al. 1984; Craggs and Webster 1985; Traugott et al. 1985; Vass et al. 1986), and γ-interferon and coronavirus infections induce both class I and class II antigen expression on neural cells in vitro (Lampson and Fisher 1984; Wong et al. 1984; Fierz et al. 1985; Massa et al. 1986; Suzumura et al. 1986).

In the present work we have used immunohistochemical techniques to study MHC-coded transplantation antigens and immunocompetent cells in measles virus-infected rat brains. Both animals inoculated during the first week after birth leading to a fatal disease, and animals injected at the age of 14 days with a clinically inapparent infection were examined.

Materials and methods

Rats of the Lewis strain (Alab, Stockholm) were used. Groups of altogether 35 rats, aged 3, 7 and 14 days, were injected intracerebrally with 20 μl of the Hamster Neurotropic (HNT) strain of measles virus. The virus suspension was prepared by homogenizing brains of infected BALB/c mice in phosphate-buffered saline (PBS) (10% w/v, titre $10^{4.7}$ $ICLD_{50/ml}$). Fourteen control rats were injected with suspensions of homogenized 10% uninfected mouse brain in PBS.

At various time points after inoculation rats were killed by decapitation, brains dissected and approximately 4 mm thick coronar slices of the forebrain and parietal region were cut, rapidly frozen in liquid nitrogen and stored at −70 °C until sectioning. Serial 8 μm thick frozen sections were cut, fixed in cold acetone, briefly washed in PBS and subjected to enzyme immunohistochemistry.

The following mouse monoclonal antibodies (MCA) were used: Ox19 (Dallman et al. 1982, 1984) and W3/13 (Williams et al. 1977), which label all peripheral T cells; W3/25 which labels T 'helper' cells and some macrophages (Williams et al. 1977; Barclay 1981); Ox8 which labels T 'cytotoxic/suppressor' cells (Brideau et al. 1980); Ox22 which labels some B cells and a subpopulation of T cells in which both T 'helper' and T 'cytotoxic/suppressor' cells may be contained (Spickett et al. 1983); Ox6 which labels class II transplantation antigens (McMaster and Williams 1979) and Ox18 which labels class I transplantation antigens (Fukumota et al. 1982). The Ox19, W3/13, W3/25, Ox8 and Ox6 hybridomas were provided by Dr. Alan Williams, University of Oxford, U.K. Antibodies were purified from culture supernatants (Holmdahl et al. 1985). Ascites fluid containing the Ox18 and Ox22 MCAs were purchased from Seralab, Crawley-Down, U.K. Two different polyclonal rabbit anti-MHC class I antigen antisera were used. Both these reagents were directed against the heavy chain and the β_2-microglobulin (Trägårdh et al. 1979). For visualization of measles virus antigen a mouse monoclonal antibody (16 AC5) directed against the nucleoprotein (NP) was used (Norrby et al. 1982).

A modified peroxidase-antiperoxidase (PAP) method (Sternberger 1974) was used. Briefly, endogenous peroxidase was blocked by preincubation in 0.3% H_2O_2. The sections were then incubated with appropriate dilutions of different specific antibodies. After washing, a goat anti-mouse IgG (Dakopatts, Denmark) was applied. This antiserum had been adsorbed with rat serum coupled to Sepharose (Pharmacia, Sweden) prior to use. After washing in PBS, mouse PAP (Dakopatts) was added. To visualize immunoglobulins and B cells, sections were incubated with a rabbit anti-rat immunoglobulin. These sections and the specimens incubated with the polyclonal anti-MHC class I antigen were then sequentially incubated with anti-rabbit IgG (Dakopatts) and rabbit PAP (Dakopatts). Possible cross-reactivity of the anti-rabbit antiserum with rat Ig was eliminated by extensive adsorption by Sepharose-coupled rat immunoglobulins. The specificity of the antibodies was tested on frozen sections of normal rat lymph nodes and spleens. Omission of the first layer of antibody was used as a control.

The peroxidase reaction was made according to Kaplow (1975). Sections were counterstained with Mayer's haematoxylin. Slides were coded, read blindly and the mean number of cells per visual field was estimated at a magnification × 200.

Results

Effect of age on disease development

Of 16 rats inoculated 3 days after birth six were sampled before signs of disease had appeared 7 days post-infection (p.i.). All other rats in this group were moribund or were dead at 9 days p.i. Of 11 rats inoculated 7 days after birth six became ill and died 7 days p.i., while the rest appeared healthy until 17 days p.i. when two rats became moribund. Eight 14-day-old rats showed no overt signs of disease after virus inoculation.

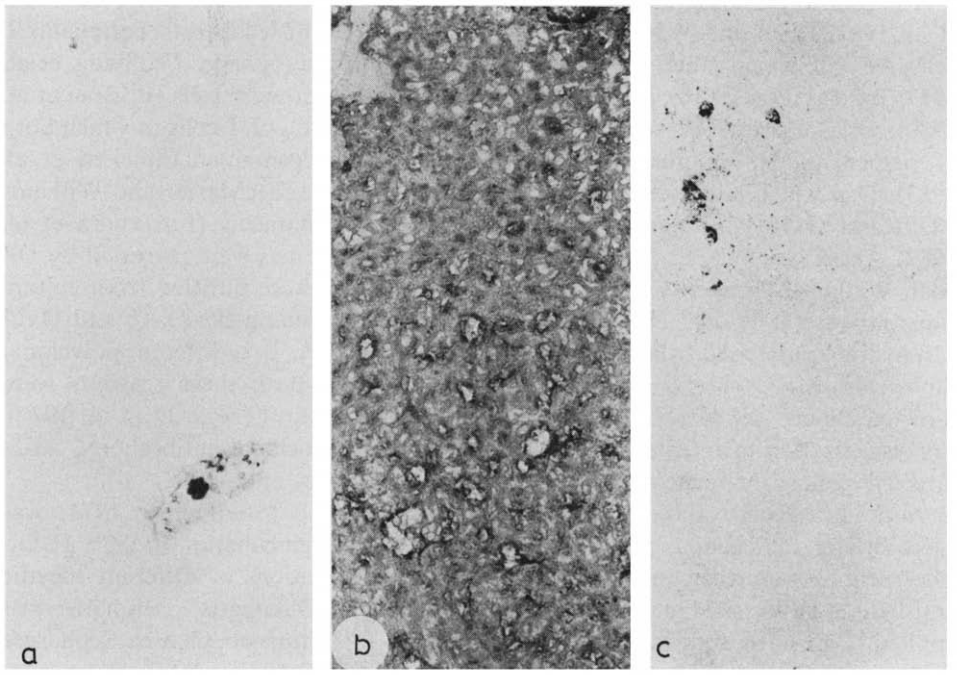

Fig. 1. MHC reactivity (Ox18 antibody) limited to intracerebral vessels in a control rat (*a*). Nine days after infection of a 14-day-old rat there is a strong induction of immunoreactivity in the brain parenchyma (*b*). The diffuse distribution of the Ox18 antibody reactivity is in contrast to the focal clusters of infected neuronal-like cells in this animal (*c*, labelled with an antibody to the measles nucleocapsid antigen). (*a*, *b*, *c* ×120).

Appearance of measles virus antigen

In the youngest rats sampled at the different time points p.i., anti-NP reactivity was seen in clusters of neuronal-like profiles in the grey matter. Immunoreactivity was also present in larger, confluent areas which were partly necrotic. In rats inoculated 14 days p.i. several scattered foci of neuronal-like cells were seen in the grey matter 9 days p.i. (Fig. 1c). A few cells in a perivascular position were also infected. Seventeen days p.i. virus antigen was less abundant. Thirty-two and 49 days p.i. immunoreactivity was found only in some perivascular lymphocyte-like cells.

Class I antigen

In control animals reactivity with Ox18 antibody or rabbit anti-class I antibodies was seen on surfaces of endothelial cells of intracerebral vessels, where the staining was most prominent on the luminal side (Fig. 1a). The antibody also stained surfaces of choroid plexus epithelial and ependymal cells, and of leptomeningeal cells. The brain parenchyma was consistently unstained.

In virus-infected rats a strong reaction with the same antibodies was seen diffusely throughout the brain tissue (Fig. 1b). This was seen to a similar extent in the younger rats, which developed a fatal disease, and in the older rats, which had no overt signs of disease. In the latter, labelled mononuclear inflammatory cells were identified, otherwise the strong widespread staining precluded judgements as to which cell type in the brain parenchyma expressed the antigen. However, staining occurred also in the immediate vicinity of neuronal cell surfaces and appeared to be stronger in the grey than in the white matter. The immunoreactivity extended far beyond areas, which in parallel sections showed viral antigen. The Ox18 and rabbit anti-class I antibody immunoreactivity persisted although at reduced levels 32 and 49 days p.i. in rats infected at 14 days of age.

Class II antigen

No reactivity with the Ox6 antibody was present in the brains of uninfected animals. In brains from measles virus-infected rats of the youngest age groups reactivity with this antibody was present patchwise on cells mainly located in or in the vicinity of necrotic areas in the brain. The Ox6 reactive cells had an irregular

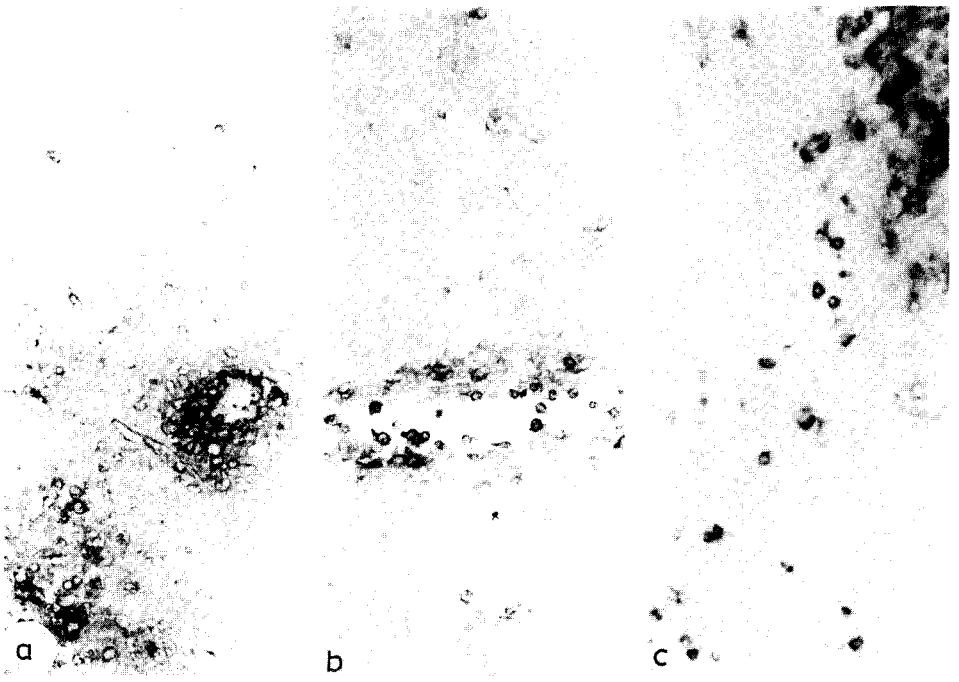

Fig. 2. (*a*) MHC class II reactivity (Ox6 antibody) in perivascular mononuclear cells 9 days after infection of a 7-day-old rat. Nine days after infection of a 14-day-old rat T 'helper' cells (W3/25 antibody) are seen perivascularly (*b*), while T 'cytotoxic/suppressor' cells (Ox8 antibody) occur in large numbers in the brain parenchyma (*c*). (*a*, *b*, *c* ×120).

TABLE 1

DETECTION OF DIFFERENT ANTIGEN-CONTAINING CELLS BY USE OF A PANEL OF MONOCLONAL ANTIBODIES AND IMMUNOPEROXIDASE STAINING

Each row shows observations from 2–4 animals. The results are expressed as the average number of cells per visual field as evaluated on whole sections of the brains (−: no; +: 1–2; ++: 3–4; +++: > 5 labelled cells). For the diffuse anti-class I immunoreactivity the reaction intensity and extension were graded.

Infected day post-partum	Killed day p.i.	Monoclonal antibody specificity							
		16AC5 Virus nucleocapsid antigen	Ox18 Class I	Ox6 Class II	W3/13 Pan T cells	Ox19 Pan T cells	Ox8 Cytotoxic suppressor subset	W3/25 T helper	Ox22 T inducer
3	4	++	++	+	+	−	+	+	−
3	7	++	+++	++	+	−	+	+	−
3	9	++	+++	++	+	−	+	+	+
7	9	++	+++	++	++	−	++	++	++
7	17	++	+++	++	+	−	+	+	+
14	9	++	+++	++	++	+	+++	++	++
14	17	+	++	++	++	++	+++	++	+
14	32	+	+	+	+	++	++	++	+
14	49	+	+	+	−	+	−	+	−
Controls	4–49	−	− [a]	−	−	−	−	−	−

[a] Brain parenchyma was unstained, while endothelial, choroid plexus and ependymal cells showed anti-class I immunoreactivity.

form. Many had a foamy cytoplasm and represented probably scavenger cells, while the origin of others could not be determined. In the older age group, rounded mononuclear inflammatory cells, mainly in a perivascular position, reacted with the antibody (Fig. 2a). Also, irregularly formed cells were stained, although cells with a foamy cytoplasm were scarce. There was no obvious staining of luminal endothelial cell surfaces.

Lymphocytes

No or only occasional lymphocytes were seen with the different cell-specific mononuclear antibodies within the brains of control rats. This was the case also for animals infected with measles virus at day 3 after birth. Occasional lymphocytes were seen in brains of two rats infected 7 days after birth and sacrificed 17 days later when they were moribund. In contrast, brains of all animals infected at day 14 after birth showed conspicuous staining with the different anti-T cell antibodies. The pan T cell markers (W3/13 and Ox19) stained cells with a round lymphocyte-like appearance, mainly located in perivascular cuffs, but also in the parenchyma. The Ox8 reactive T 'cytotoxic/suppressor' lymphocytes occurred perivascularly, but were also spread out in large numbers in the brain parenchyma (Fig. 2c). Ox22 antibody reactive lymphocytes had a similar distribution, but were present only at the earlier time points after infection (Table 1). W3/25 reactivity was noted on rounded, lymphocyte-like cells confined to perivascular cuffs (Fig. 2b). In all groups of animals irregularly formed, non-lymphocytic cells reacted with the Ox8 and W3/25 antibodies. In the youngest rats they included cells with a foamy cytoplasm in necrotic areas.

Use of anti-immunoglobulin antiserum revealed staining within blood vessels of controls. In infected animals, staining in addition occurred in the brain parenchyma outside vessels, and was extensive within necrotic areas of the brain in young animals. B cells and plasma cells occurred rarely.

Discussion

In the present study anti-class I immunoreactivity was detected in intracerebral vessels, but not in the brain parenchyma, and anti-class II immunoreactivity was absent within the brains of uninfected rats. However, in the brains of animals infected with the HNT strain of measles virus a strong immunoreactivity with both class I- and class II-specific antibodies was induced. This induction occurred both in younger animals with a fatal brain infection and in older animals with a clinically inapparent course of infection. The anti-class I immunoreactivity was distributed diffusely, probably involving most types of cells in the brain, while anti-class II immunoreactivity occurred mostly on cells around intracerebral vessels and in small foci in the brain parenchyma. Some of these cells represent mononuclear inflammatory cells and some scavenger cells, but the nature of the other irregularly shaped cells could not be accurately determined in the present study. A marked induction of class II antigen has previously been observed in EAE, where the immunoreactive

cells include lymphocytes, macrophages, endothelial cells and astrocytes (Sobel et al. 1984; Craggs and Webster 1985; Hickey et al. 1985; McCarron et al. 1985; Traugott et al. 1985; Sakai et al. 1986; Vass et al. 1986). Class II antigen immunoreactivity has also been shown on astrocytes in brains from patients with multiple sclerosis (Hofman et al. 1986).

It is of interest that the distribution of the class I antigen was not limited to the cells or the areas of the brain where viral antigens were demonstrated. This observation suggests that MHC antigens may be induced by some soluble signal substance released during early phases of the viral infection. One such substance may be γ-interferon which in vitro can induce both MHC class I and II antigens on various neuroectodermal cells (Hirsch et al. 1983; Lampson and Fischer 1984; Wong et al. 1984; Fierz et al. 1985). In addition, virus-induced soluble factors, not related to γ-interferon or other lymphokines, and direct interactions of viral particles with cell surfaces can enhance levels of class I (Suzumura et al. 1986) and class II (Massa et al. 1986) antigens, respectively, in vitro.

Although monoclonal antibody surface markers originally were presented as being lymphocyte-specific, several of these markers have turned out also to label other cell types presenting the same or similar antigens. For example, W3/25 antibodies label macrophages in addition to T 'helper' cells (Barclay 1981). In accordance with a previous study (Strigård et al. 1986) we now detected Ox8 antibody labelling also on non-lymphocytic irregularly shaped cells. Certain heterogeneities in the distribution of the different lymphocyte phenotypes in the brain tissue were observed. There was a conspicuous perivascular occurrence both of Ox8 antibody reactive lymphocytes of the T 'cytotoxic/suppressor' phenotype and of W3/25 antibody T 'helper' phenotype in brains of animals inoculated at 2 weeks of age. However, the Ox8 antibody reactive T lymphocytes were also distributed more widely in the brain parenchyma as was their MHC restriction element, namely the class I antigen. These cells might therefore be engaged in a cytotoxic elimination of infected cells. In vitro W3/25 and Ox22 antibody-positive cells function as inducers of T cytotoxic cells (Mason et al. 1983). It is therefore of interest to note that the Ox22 antibody-positive lymphocytes occurred early during the infection.

In conclusion, our study emphasizes that the levels of class I and class II transplantation antigens can be readily enhanced in the brain during a virus infection. In measles virus-infected brains from rats with a non-fatal course of infection a prominent infiltration in brain of T lymphocytes was noted. Observations on different lymphocyte phenotypes within the brain provide a basis for further studies using selective manipulations of these cells in vivo to examine their relative importance for the outcome of the viral infection.

Acknowledgements

This study was supported by grants from the Swedish Medical Research Council (project Nos. 12X-04480 and 16X-00116) and the Norwegian Research Council for Science and the Humanities (to J.M.). We thank Mrs. Lena Karlsson for excellent technical assistance and Mrs. Inga-Lisa Wallgren for typing the manuscript.

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