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The expression of major histocompatibility complex (MHC) class I antigens in the brain differs markedly in acute and persistent infections with lymphocytic choriomeningitis virus (LCMV)

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Summary

Intracranial inoculation of immunocompetent mice with lymphocytic choriomeningitis virus (LCMV) induces a fatal neurologic illness. In this disease a marked increase in MHC class I expression was found, closely associated with viral antigens and inflammatory infiltrates, in meninges, choroid plexus and ventricular ependyma but not within the brain parenchyma. Immunosuppression prevented MHC induction. Mice inoculated at birth had persistent infections, with LCMV antigens found primarily in neurons, but no inflammatory cells or focal increase in MHC class I. Failure of infected neurons to express MHC class I allows them to escape destruction by cytotoxic T cells (CTL) but may increase their susceptibility to be persistently infected by non-lytic viruses.

Introduction

Infections of mice with lymphocytic choriomeningitis virus (LCMV) provide useful models for virus-induced neurologic disease (Buchmeier et al., 1980; Lehmann-Grube, 1984; Oldstone et al., 1985, 1986). In immunocompetent mice, intracranial inoculation of LCMV leads to an acute fatal neurologic illness which is dependent on LCMV-specific cytotoxic T cells (CTL) (Cole et al., 1972; Zinkernagel and Doherty, 1973; Dixon et al., 1987; Klavinskis et al., 1989). Inoculation of LCMV into neonatal or immunosuppressed mice, on the other hand, establishes a persistent infection in which viral antigens are expressed but not detected by host CTL (Hotchin and Cinits, 1958; Gilden et al., 1972; Mims and Blanden, 1972). The recognition of viruses, such as LCMV, by CTL is critically dependent on major histocompatibility complex (MHC) class I molecules (Doherty and Zinkernagel, 1974; Zinkernagel and Doherty, 1974, 1983). The level of MHC class I expression by infected cells correlates with the degree of effectiveness of CTL-mediated lysis (Plata et al., 1981; Flyer et al., 1985; Gairin et al.,

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1991; Joly et al., 1991). While in the normal CNS only low levels of MHC class I antigens are found, MHC class I expression can be upmodulated on some brain cells by cytokines and viruses (Wong et al., 1984; Main et al., 1985; Lampson and Hickey, 1986; Suzumura et al., 1986, 1988; Hickey and Kimura, 1987; Joly et al., 1991). The current paper compares amount and distribution of MHC class I molecules, inflammatory infiltrates and viral antigens in brains of mice with acute and persistent LCMV infections to assess the roles of these factors in immune-mediated neurologic disease and viral persistence.

Materials and methods

Twenty-four 3- to 4-month-old immunocompetent C57BL/6 (H-2^b) and C3H (H-2^k) mice were untreated or inoculated intracranially (i.c.) with 30 μl of: phosphate-buffered saline (PBS), or 1000 plaque-forming units (PFU) of the Armstrong CA 1371 clone 53B of LCMV (Dutko and Oldstone, 1983) in medium or LCMV inactivated by ultraviolet (UV) irradiation and heating. Total body irradiation (900 rads) from a cobalt source given just before i.c. inoculations was used to suppress CTL responses in eight C57BL/6 mice. Each experimental paradigm was tested on groups of 3–4 mice. Typical signs of acute LCMV infection developed in non-irradiated mice inoculated with live virus after 5–6 days whereas none of the other mice showed signs of illness. All mice were killed 6–7 days after the acute i.c. inoculations together with three age-matched C57BL/6 mice persistently infected with LCMV by i.c. inoculation at birth (Oldstone et al., 1986). Serial cryostat-cut sections of snap-frozen brains (10 μm) were air-dried at room temperature (RT) for 1 h, fixed in ice-cold acetone for 10 min and stored at 4°C overnight. Subsequent incubations and washes were carried out at RT in 2% fetal bovine serum/1 × PBS (pH 7.3). After 30 min of incubation in buffer alone sections were incubated with primary antibodies for 2 h: mouse monoclonal antibody (mAb) B22-249. R1 directed against D^b (Lemke et al., 1979) (dilution 1:50) or mouse mAb 1–1.3 specific for LCMV nucleoprotein (Buchmeier et al., 1981) (dilution 1:100). This was followed by a 1 h incubation with peroxidase-coupled sheep anti-mouse IgG_{2a} secondary antibodies (Bioproducts for Science, Indianapolis, IN, USA) at a 1:200 dilution. Sections were developed with H_2O_2/diaminobenzidine (DAB) for 10–15 min and counterstained with hematoxylin and eosin.

Results and discussion

In unmanipulated and sham-injected (uninfected) C57BL/6 mice (H-2^b) only low levels of MHC class I were identified in restricted brain regions. Specifically, faint immunostaining of cell surfaces for D^b was seen in choroid plexus, meninges and blood vessel walls but not on neurons, astrocytes, oligodendrocytes or microglia. No inflammatory infiltrates or immunostaining for LCMV antigens were found in the brains of control animals. Mice infected acutely by i.c. inoculation with LCMV showed dense inflammatory infiltrates of the meninges (Fig. 1*A* and *C*). Inflammatory cells were also seen along the ependymal lining of the ventricles and around the choroid plexus. The brain parenchyma showed no obvious abnormalities. Viral antigens were identified in the choroid plexus, the meninges, the ependymal epithelium and in some of the inflammatory cells that infiltrated these structures (Fig. 1*B*). These findings are all consistent with previous observations (Gilden et al., 1972; Walker et al., 1975; Doyle and Oldstone, 1978).

Immunostaining for D^b in the acute infection revealed a substantial increase in the expression of MHC class I over control levels in specific sites. Intense D^b expression was found in choroid plexus, meninges and ventricular ependyma as well as on the cells that comprised the inflammatory infiltrates whereas no increase in MHC class I expression was found within the brain parenchyma (Fig. 1*C* and *D*). In some areas a close topologic overlap was documented between the expression of viral antigens and host MHC class I molecules in adjacent sections (Fig. 1*B* and *D*).

Total body irradiation prevented the marked increase in MHC class I expression, the neurologic illness and the inflammatory infiltrates that

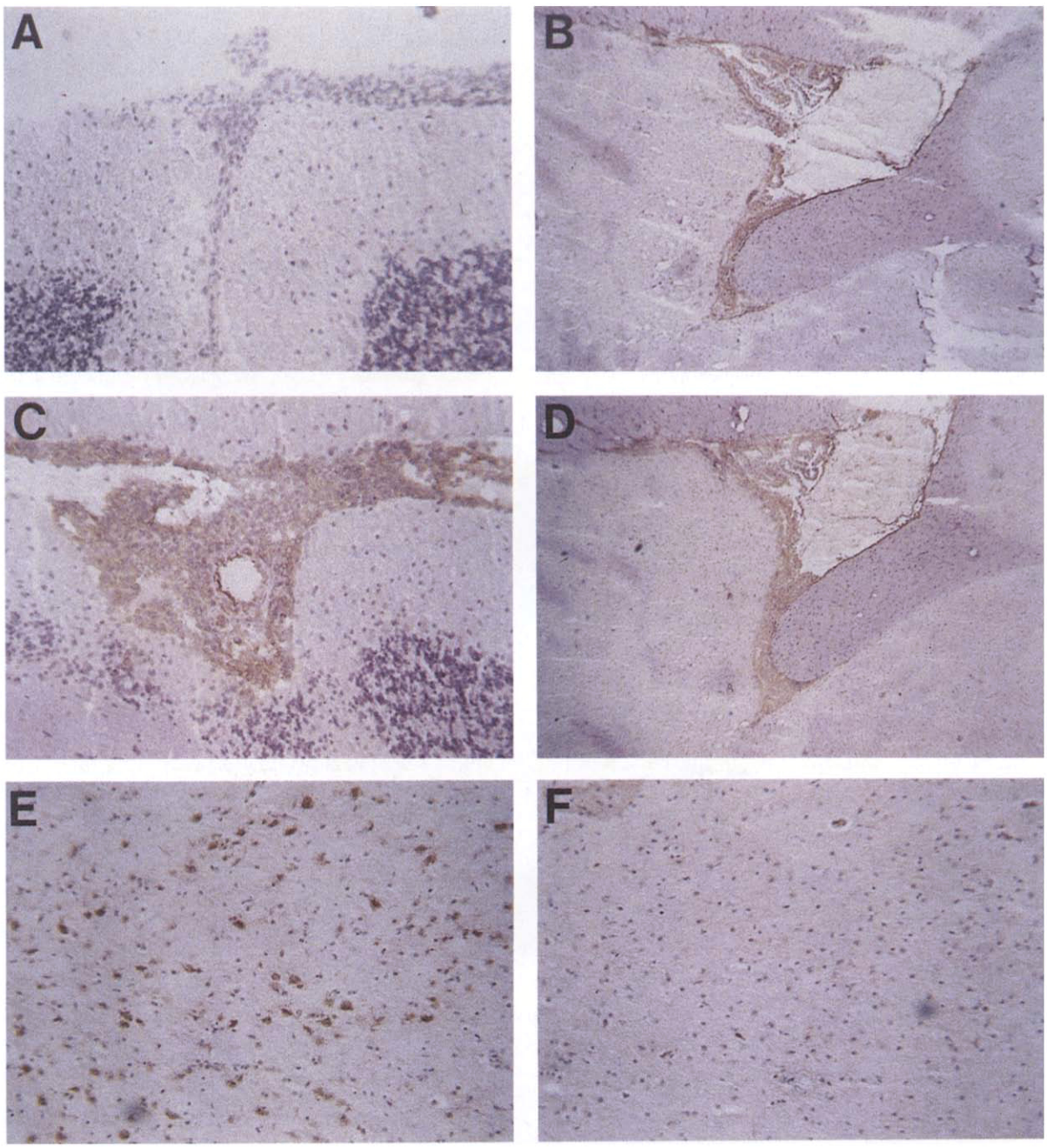

Fig. 1. Expression of MHC class I and LCMV antigens in the brain during acute and persistent infections with LCMV. Sagittal brain sections were obtained from mice acutely (*A–D*) or persistently (*E* and *F*) infected with LCMV and stained with mAbs specific for D^b (*A*, *C*, *D* and *F*) or for LCMV nucleoprotein (*B* and *E*) as described in Materials and methods. Antigens are revealed by brown immunostaining. The meninges overlying the cerebella of acutely infected mice are densely infiltrated by inflammatory cells (*A* and *C*). In C57BL/6 mice ($H\text{-}2^b$) the D^b-specific mAb reveals that this infiltration is accompanied by strong MHC class I expression (*C*) whereas no cross-reactivity is seen in C3H mice ($H\text{-}2^k$) which lack D^b (*A*). In acutely infected mice, choroid plexus, ventricular ependyma and inflammatory infiltrates are stained for both LCMV (*B*) and D^b (*D*) antigens in adjacent serial sections whereas neurons in the surrounding parenchyma are not labeled. In persistently infected mice thalamic neurons are densely labeled with the LCMV mAb (*E*). However, staining of adjacent sections for D^b showed no accompanying increase in MHC class I expression (*F*). Magnifications: 60 × (*A*, *C*, *E* and *F*), 20 × (*B* and *D*).

were seen in acutely infected immunocompetent mice whereas the amount of viral antigen in irradiated and non-irradiated mice was similar (Table 1). This suggests that the upmodulation of MHC class I by acutely infected host cells is induced primarily by the cellular immune response rather than directly by the LCMV infection itself. It is tempting to speculate that the increased MHC class I expression is mediated by cytokines released from infiltrating immune cells. The increased expression of MHC molecules by acutely infected cells would be expected to enhance their interaction with immune cells and may contribute to the immunopathology.

In brains of mice persistently infected with LCMV no inflammatory cells were found despite the presence of LCMV antigens in most brain regions. Consistent with previous observations (Buchmeier et al., 1980; Rodriguez et al., 1983) LCMV antigens were seen primarily in cells with neuronal morphology. In some cases LCMV antigen was also found in cells of the choroid plexus and the ventricular ependyma confirming earlier results (Gilden et al., 1972; Lampert and Oldstone, 1974; Fazakerley et al., 1991). In contrast to the acute infection, in which LCMV antigens co-localized with upmodulation of D^b, the presence of LCMV antigens was not accompanied by focal increases in the expression of host MHC class I molecules (Fig. 1*E* and *F*).

LCMV can be cleared from the brain parenchyma of persistently infected mice by adoptive transfer of LCMV-specific CTL from immunocompetent H-2 matched donors (Oldstone et al., 1986). However, this clearance is not accompanied by inflammatory infiltrates and there is no evidence that LCMV-infected neurons can be lysed by LCMV-specific CTL in vivo (Klavinskis et al., 1989; Joly et al., 1991). In vitro LCMV-infected neuronal cells can be lysed by LCMV-specific CTL but only after treatment with interferon-γ or following expression of exogenous MHC class I (Joly et al., 1991). Our current results suggest that LCMV infection by itself does not induce detectable MHC class I expression on neuronal cells in vivo. Why LCMV-infected neurons fail to express MHC class I molecules in vivo is unclear. While activated T cells are able to traverse the blood–brain barrier (Wekerle et al., 1986, 1987) they may not be able to interact with infected neurons in sufficient numbers because of the relatively sheltered location of neurons within the neuropil. It is also possible that other brain cells, such as astrocytes, produce factors that interfere with MHC induction on neurons. Further, differentiated neurons in vivo may be inherently unable to express MHC class I at significant levels as suggested by recent studies on cultured neuronal cells (Joly et al., 1991). This may provide a selective advantage by excluding these irreplaceable cells from destruction by CTL. The escape of neurons from CTL surveillance would, however, also allow non-lytic viruses to persist in neurons over the animal's life span.

TABLE 1

THE MARKED INCREASE IN MHC CLASS I EXPRESSION ASSOCIATED WITH LCMV INFECTION OF THE CNS DEPENDS ON THE PRESENCE OF LIVE VIRUS AS WELL AS ON THE IMMUNOCOMPETENCE OF THE HOST

Results reflect immunocytochemical findings in brain sections from 3-month-old female C57BL/6 mice ($n = 4$/group for Nos. 1–3; $n = 3$/group for No. 4). (+) Dense immunostaining by peroxidase reaction (see brown labeling in Fig. 1*B–E*). (−) No immunostaining above background levels (see Fig. 1*A* and *F*). Groups 1–3 were acutely inoculated as adults whereas group 4 was persistently infected at birth. Mice were killed for histologic analysis 7 days after the acute i.c. inoculations. At this time all mice in group 1 were ill, whereas all the other mice appeared normal.

Group No.	Live LCMV	Inactivated LCMV	Total body irradiation	Increase in MHC class I	LCMV Ag detected
1	+	−	−	+	+
2	+	−	+	−	+
3	−	+	−	−	−
4	+ (at birth)	−	−	−	+

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