

INDUCIBILITY OF Ia ANTIGEN ON ASTROCYTES BY  
MURINE CORONAVIRUS JHM IS RAT STRAIN DEPENDENT

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Infection of Lewis rats with the murine coronavirus JHM leads to a persistent infection in the central nervous system (CNS) and a disease process that shares some features with experimental allergic encephalomyelitis (EAE) (1–4). After an incubation period of weeks, a subacute demyelinating encephalomyelitis (SDE) develops that is characterized by paralysis (2). Histologically, the main lesion consists of a primary demyelination associated with perivascular cuffings and a cellular infiltration of macrophages as well as helper and cytotoxic T lymphocytes (1, 2). SDE animals reveal a cell-mediated immune (CMI) response to myelin basic protein (MBP) as documented in adoptive transfer experiments (1). In addition, genetic experiments demonstrate that Brown-Norway (BN) rats are resistant to SDE, similar to what has been observed in EAE (4). These findings indicate an immunopathological reaction in coronavirus-induced SDE in Lewis rats that is lacking in BN rats.

A CMI response is genetically controlled at at least two levels: (a) the ability of the animal to generate an encephalolytogenic T cell response to antigen within the brain (foreign or autoantigen) from its genetically determined T cell repertoire and (b) the degree to which the antigen is presented to these T cells in the context of class I and II (Ia) histocompatibility molecules within a particular tissue. Class II-restricted T cells initiate an immune response to infection and both class I- and II-restricted T cells provide cytolysis of infected cells (5). Since Ia molecules are normally lacking on cells of the brain (6), the induction of Ia within the brain is crucial for the initial local presentation of viral antigen during infection. It may therefore be important that astrocytes of Lewis rat brain can be induced to express Ia directly by JHM virus (7, 8) as well as by other viruses. IFN- $\gamma$  subsequently released by antigen-activated T cells can further induce the antigen-presenting function of astrocytes (9–11).

The direct induction of Ia on astrocytes by JHM virus is also thought to play a crucial role in eliciting the excessive T cell response in susceptible rats. Supporting this notion are recent observations by us (12) showing that astrocytes of rat and mouse strains susceptible to EAE express much higher levels of Ia than astrocytes of resistant strains after treatment with IFN- $\gamma$ . We now extend these studies and analyze JHM virus induction of Ia on astrocytes of rats susceptible or resistant to SDE and show that, unlike astrocytes of susceptible Lewis rats, induction of astrocyte Ia by JHM virus is not detectable in the resistant BN rat.

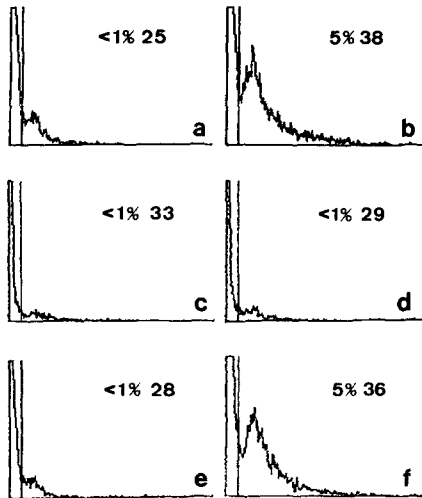


FIGURE 1. Comparison of Ia antigen induction by JHM virus on astrocytes from disease susceptible and resistant strains. (a and b) Lewis rat mixed glial cultures; (c and d) BN rat mixed glial cultures; (e and f) congenic Lewis.BN rat mixed glial cultures. Histograms to the left are analyses of cultures treated with control supernatants without JHM virus and those to the right were treated with JHM virus-containing supernatants for 5 d. Only Lewis (b) and congenic Lewis.BN astrocytes (f) show induction of Ia but not BN astrocytes (d). The percentage of induced cells appears on the figure. The quantity appearing after the percentage represents the mean fluorescence intensity of the sample of 50,000 cells. Abscissa, log integral green fluorescence; ordinate, relative cell number.

### Materials and Methods

**Animals.** Lewis, BN, and congenic Lewis.BN rats were obtained from the Central Institute for Experimental Animal Breeding, Hannover, Federal Republic of Germany.

**Tissue Cultures.** Mixed glial cultures containing astrocytes and oligodendrocytes were prepared from brainstem and cerebella of 1-d-old rat pups (specific pathogen-free) as recently described (8). Cultures were incubated at 37°C with 10% CO<sub>2</sub> at maximum humidity, then fed with fresh medium 4 d after plating. Infection of cultures with wild-type JHM coronavirus began at 5 d after plating. DME with 15% FCS was used as medium.

Pure rat astrocyte cultures were prepared from 1-d-old rat pups as previously described (12). Pure oligodendrocyte cultures were prepared from whole brains of 4 adult Lewis and BN rats (5–6 weeks old) following the procedure of Kim et al., 1983 (13).

**Virus.** Wild type murine coronavirus JHM stock virus was isolated and cloned as previously described (3).

**Preparation of Conditioned Supernatants.** Mixed glial cultures grown for 5 d were infected with wild-type JHM virus at 10<sup>5</sup> PFU/ml, then fed fresh medium. Cultures were incubated thereafter for 5 d when cytopathic effects (CPE) covered 50% of the cell culture area. At this time the virus-containing supernatants were titered and aliquots were stored in liquid nitrogen. As a control, supernatants conditioned over the same 5-d period by uninfected glial cultures were similarly handled.

**Muramyl Dipeptide Treatment.** 7-d mixed glial cultures were treated daily with 0.1 µg/ml muramyl dipeptide (Sigma Chemical Co., St. Louis, MO) as recently reported (8), then they were stained for Ia antigens at day 5 after treatment.

**Fluorescence-activated Flow Cytometry.** At 5 d after infection, the glial cultures were prepared for flow cytometry (EPICS V; Coulter Electronics, Hialeah, FL), as previously described (12). Ia and class I molecules were stained using the mAbs Ox6 (14) and Ox18 (15), respectively. Pure astrocyte cultures were also stained for Ran-2 antigen (16) and oligodendrocytes for galactocerebroside (17).

### Results and Discussion

**Strain-specific Ia Induction of Astrocytes by JHM Virus.** Primary rat glial cultures produced from SDE-susceptible (Lewis and congenic Lewis.BN) and SDE-resistant (BN) rats were treated daily with wild-type JHM virus, and analyzed 5 d after infection. As shown in Fig. 1, uninfected control Lewis glial cultures typically contained no Ia<sup>+</sup> cells (Fig. 1 a), but at 5 d after infection, Ia<sup>+</sup> astrocytes became

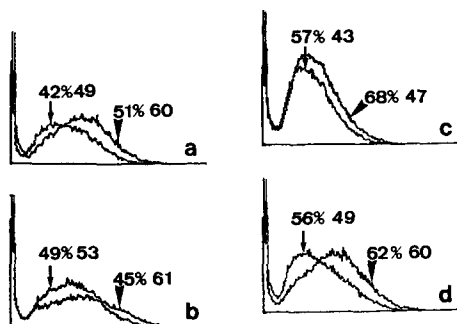


FIGURE 2. Analysis of enhancement of class I antigens in rat mixed glial cultures. Histograms representing conditioned supernatant-treated cultures (with or without JHM virus) are labeled with arrowheads and those with fresh medium are labeled with arrows. (a) Lewis culture treated with conditioned medium from uninfected glial cultures. (b-d) Lewis (b), BN (c), and congenic Lewis.BN cultures (d) treated with conditioned medium from JHM virus-infected glial cultures. The percentage of class I-expressing cells appears in the figure in association with the arrows and arrowheads. The quantity appearing after the percentage indicates the mean fluorescence intensity of the sample of 50,000 cells. Abscissa, log integral green fluorescence; ordinate, relative cell number.

TABLE I  
Spontaneous Increase in Class I Expression on Astrocytes and Oligodendrocytes in Pure Cultures over Time after Plating

Cell type	Time after plating	Number positive	Mean fluorescence intensity
		%	
Oligodendrocytes	0 h	1	26
	18 h	38	61
	2 d	78	81
	1 wk	56	43
Astrocytes	4 d	14	27
	2 wk	64	35
	3 wk	68	40

Oligodendrocyte cultures were >90% positive for galactocerebroside, and astrocytes were >90% positive for Ran-2 antigen stained as described in Materials and Methods.

detectable (Fig. 1 b), as previously reported (7, 8). In contrast, in glial cultures derived from disease-resistant BN rats, Ia was never observed after infection (Fig. 1 d) with JHM virus.

However, infection of cultures of congenic Lewis.BN rats led to astrocyte Ia induction to the same levels seen in the Lewis rat (Fig. 1, e and f). Therefore, responsiveness to JHM virus with respect to Ia induction is controlled by Lewis rat genes located outside the RT-1 locus, and perhaps is related to disease susceptibility of these animals (4).

*Class I Antigen Expression on Glial Cells.* The induction of class I molecules on mouse glial cells by a virus related to JHM virus, coronavirus A59, has recently been reported (18). In the rat, class I antigens were spontaneously expressed in high constitutive levels on numerous glial cells in control cultures (Fig. 2), in agreement with others (19). As shown in Table I, spontaneous induction of class I antigens on pure astrocyte cultures increased over time after plating.

Likewise, purified rat oligodendrocytes became strongly class I antigen-positive within 18 h after plating, had increased positivity at 2 d, and were stainable at least 7 d after plating (Table I). This indicates that pure rat oligodendrocytes rapidly become class I-positive by conditions associated with cultivation and also

TABLE II  
*Induction of Ia Antigen Expression on Astrocytes by  
 Muramyl Dipeptide*

Rat strain	Medium with muramyl dipeptide	Medium without muramyl dipeptide or with inactive D-D isomer
Lewis	+	-
BN	-	-
Lewis.BN	+	-

+, Detectable by immunofluorescence microscopy (induction of Ia on at least 2,500–5,000 cells/cm<sup>2</sup>); -, undetectable by immunofluorescence microscopy (expression of Ia on 0–10 cells/cm<sup>2</sup>). Mixed glial cultures were prepared as described in Materials and Methods and plated onto polyornithine coated glass coverslips. The total number of cells in the cultures was, on average, 10<sup>5</sup> cells/cm<sup>2</sup>. 7 d after plating and every day thereafter, the cultures were treated with 0.1 µg/ml muramyl dipeptide. Control cultures were fed daily with fresh media without muramyl dipeptide or with inactive D-D isomer of muramyl dipeptide (Sigma Chemical Co.). 5 d after treatment the cultures were stained for Ia using the OX6 mAb, then were examined by FITC fluorescence microscopy.

that soluble class I inducers originating from other cell types were not involved in the rat, as they are in the mouse (18).

With respect to possible enhanced expression of the existing levels of class I antigens as a result of infection (18), Fig. 2 shows that control glial cell-conditioned supernatants containing no JHM virus induced class I antigens on naive cultures (Fig. 2a) to the same degree as virus containing supernatants in Lewis (Fig. 2b), BN (2c), and congenic Lewis.BN cultures (Fig. 2d). Therefore, no increase in either the percentage or fluorescence intensity of class I antigen-positive cells could be attributed to JHM virus. The shift of the entire population of fluorescing cells to higher fluorescence intensity channels produced by conditioned supernatant is consistent with the enhancement of class I antigens primarily on spontaneously expressing cells (Fig. 2).

*Strain-specific Induction of Ia Molecules by Muramyl Dipeptide.* We have recently reported (8) the capacity of the bacterial immune adjuvant, muramyl dipeptide (adjuvant peptide) to induce Ia molecules on astrocytes in a manner identical to that seen with JHM virus. As presented in Table II, muramyl dipeptide induces astrocyte Ia in the same strain-specific pattern as does JHM virus. This gives a further indication that the inducibility of Ia antigens on astrocytes of disease-susceptible animals compared with resistant animals is not dependent on any one viral or bacterial inducer but related to strain-specific regulation of Ia molecules expressed at the level of the astrocyte.

In this report we have analyzed the effect of JHM virus on Ia and class I antigen expression on glial cells of different rat strains. We show that JHM virus induces astrocyte Ia expression in strains susceptible to JHM virus-induced demyelinating disease but not in a resistant strain. Expression of class I antigens was different in the sense that class I genes were expressed before and after infection. Moreover, the expression of class I-positive cells of all rat strains was not appreciably increased by JHM virus above already high levels seen on control glial cells.

The similar Ia-inductive capacity seen between JHM virus and muramyl dipeptide might provide clues to the role of JHM virus in eliciting autoimmune

disease to myelin in susceptible animals. Muramyl dipeptide is the minimal structural component of mycobacteria in Freund's adjuvant that still provides immune adjuvancy to antigens. Moreover, the use of bacterial adjuvants are essential in experimental induction of various autoimmune diseases, including EAE. Similarly, it may be that JHM virus acts as an adjuvant in inducing and/or potentiating an autoimmune reaction against myelin.

Demyelination caused by JHM virus infection of Lewis rat brain is mediated by T lymphocytes sensitized to both myelin basic protein and viral antigens (1, 4). According to our findings, the ability of brain cells to present antigen in the context of class I antigens would appear to be similar between strains, not differing in relation to genetically controlled susceptibility to demyelinating disease. However, the differing degree to which JHM virus induces Ia antigens on astrocytes in susceptible and resistant strains, as described here, would have a major influence on Ia-restricted T cell activation and tissue damage.

### Summary

Inducibility of Ia molecules on cultivated astrocytes by JHM virus (7) correlates with demyelinating disease susceptibility of animals from which these astrocytes are derived. On the contrary, class I induction of both astrocytes and oligodendrocytes occurs as a consequence of normal cultivation procedures in both susceptible and resistant strains. Increased expression of class I antigens on rat astrocytes and oligodendrocytes is not related to JHM viral infection as it is in the mouse (18). These data indicate that strain differences in Ia inducibility, rather than inducibility of class I antigens, by JHM virus may explain higher levels of T cell-mediated damage to myelin during infection in susceptible rat strains compared with resistant strains.

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