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Differential modulation of MHC class I antigen expression on mouse brain endothelial cells by MHV-4 infection

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Summary

Virus-induced modulation of mouse cerebral endothelial cell class I and class II antigens by the neurotropic coronavirus, MHV-4 (JHM), was examined by flow cytometry. In susceptible BALB/c, H-2K^d was downregulated, while H-2D^d was upregulated following infection by MHV-4. In contrast, H-2K and H-2D antigens were both upregulated in either MHV-4-susceptible B10.S and (BALB/c × SJL) F1, or MHV-4-resistant SJL-derived cerebral endothelial cells following infection with this virus. Class II antigen expression was unchanged following MHV-4 infection. Virus-induced MHC class I modulation is genetically regulated, and may influence virus clearance by class I-dependent CTL.

Introduction

Mouse hepatitis virus type 4 (MHV-4) (JHM strain), a member of the coronavirus family, is neurotropic and causes a spectrum of disease in the central nervous system (CNS), ranging from fatal acute encephalomyelitis to demyelination (Bailey et al., 1949). In MHV-4-infected Lewis rats, a late occurring demyelinating disease, with perivascular inflammation and cellular infiltration, can be found (Wantanabe et al., 1983). It is characterized by myelin-reactive lymphocytes and increased class II major histocompatibility (MHC) antigen expression on astrocytes. In studies investigating the immune mechanisms involved in virus-induced demyelination, Massa et al. (1986, 1987a), demonstrated the ability of MHV-4 to directly induce Ia (class II) antigen expression on Lewis rat astrocytes in vitro. Astrocytes are also able to function in antigen presentation to myelin-reactive lymphocytes (Fontana et al., 1984). In contrast, in the C57BL/6 mouse, Suzumura et al. (1986) showed that a related coronavirus yielding demyelination, MHV-A59, induced class I, but not class II, MHC antigens on mouse astrocytes and oligodendrocytes. They postulated that this could lead to a virus-induced cytotoxic lymphocyte (CTL)-mediated mechanism of demyelination following MHV infection.

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Class I antigen-restricted CTL killing is an essential component of the immune response to some viruses (Zinkernagel and Doherty, 1975, 1976). H-2^d-restricted CTL specific for MHV-4 (JHM) have recently been described (Yamaguchi et al., 1988). Downregulation of MHC class I antigen expression, and H2-K^d expression specifically, has been shown following infection with adenovirus (Paabo et al., 1986; Burgert et al., 1987). This was demonstrated to interfere with CTL effectiveness (Burgert et al., 1987), and suggests a potential mechanism by which unchecked virus replication and spread may occur.

We were interested in studying the response of cerebral endothelial cells, a major structural component of the blood–brain barrier (BBB), to MHV-4 infection. These cells play an important role in regulating virus entry into the brain (Johnson, 1974, 1982; Wiley et al., 1986). Cerebral endothelial cells have also been shown to function in antigen presentation (McCarron et al., 1985, 1986), and therefore could potentially modulate immune-mediated events occurring in the CNS following MHV-4 infection. We assessed the expression of class I and class II MHC antigens, following MHV-4 infection, in brain endothelial cells derived from strains of mice that differ in their susceptibility to MHV-4-induced disease.

Our studies were performed using cultured brain endothelial cell lines derived from MHV-susceptible (BALB/c, B10.S and (BALB/c × SJL) F1) and MHV-resistant (SJL) strains of mice (Stohlman and Frelinger, 1978; Knobler et al., 1981). Cytopathic effects, and the degree of expression of class I and class II MHC antigens, were examined after MHV-4 infection of cerebral endothelial cells. Our findings demonstrate that following MHV-4 infection, there was differential modulation of the H-2K and H-2D class I molecules on brain endothelial cells derived from BALB/c, but not SJL, B10.S and (BALB/c × SJL) F1 mice, in which parallel modulation occurred. In contrast, class II molecules did not fluctuate following MHV-4 infection.

Materials and methods

Endothelial cell culture

Cerebral endothelial cells were isolated from the brains of BALB/c (H-2^d), SJL (H-2^s) (BALB/c × SJL) F1 or B10.S (H-2^s) mice using the method of DeBault et al. (1981). Briefly, mouse brain tissue was minced and homogenized with a dounce homogenizer in Hanks' balanced salt solution. The homogenate was then sequentially passed through 110 μm and 55 μm Nitex screens. Capillaries were washed off the second screen, and sedimented through 6% bovine serum albumin by centrifugation at $200 \times g$ for 2.5 min. Capillaries were plated onto 0.1% gelatin-coated plates, and the endothelial cells grew out in about 10 days. Long-term cell lines were established from these initial outgrowths using a modified Lewis medium. This consists of Medium 199 with 20% fetal bovine serum and additional supplements that include basal medium Eagle (BME) amino acids, BME vitamins, glutamine (Gibco), Bacto-peptone (Difco) and penicillin/streptomycin.

Fat pad-derived endothelial cells were isolated as described by Wagner and Mathews (1975). Mouse epididymal fat pads were treated with 0.2% collagenase for 40 min at 37 °C. The resulting slurry was then centrifuged at $100 \times g$ for 10 min. The adipocytes separated from the capillaries during centrifugation, floating to the top. A pellet of capillaries was obtained. Further purification was achieved by velocity sedimentation on 6% bovine serum albumin in phosphate-buffered saline (PBS), that removes most single cells (fibroblasts and macrophages). The pellets of capillary cells were plated in the same medium described above for cerebral endothelial cells. Endothelial cells spread out from the capillary explants and primary cultures thus obtained were used for the virus infection studies.

Endothelial cell identity was established by the uptake of DiI-Ac-LDL (acetylated low density lipoprotein labeled with 1,1′-dioctadecyl-1-3,3,3′,3′-tetramethyl-indocarbocyanine perchlorate) (Biomedical Technologies, Stoughton, MA, U.S.A.) and specific binding of *Bandeiraea simplicifolia* BSI-B_4 (Voyta et al., 1984; Schelper et al., 1985).

Infection of cultures

Endothelial cells grown in T-25 Primaria (Falcon) flasks (1×10^6 cells/flask) were infected for 1 h at 37 °C, with agitation using a multiplicity

of infection (MOI) of 0.1, for MHV-4, which was grown as a stock containing 5×10^5 plaque forming units/ml as previously described (Knobler et al., 1981). Alternatively, cultures were infected with MHV-A59 (Knobler et al., 1984), also at an MOI of 0.1. After 1 h, the supernatant was removed and the cultures were washed with PBS 3 times before being refed with fresh media. Some cultures were exposed to recombinant rat gamma interferon (Amgen Biologicals, Thousand Oaks, CA, U.S.A.) at a concentration of 200 international units (IU) per milliliter.

Antibody labeling and flow cytometry

Cells from infected or paired uninfected cultures were scraped off the flask or removed by exposure to 2.5% trypsin-EDTA 4 days after infection with MHV-4. Aliquots of 5×10^5 cells were labeled with antibody to either H2-K^d, H2-D^d (both are mouse monoclonal antibodies of the IgM isotype, obtained from Organon Teknika, Malvern, PA, U.S.A.), H2-K^s, H2-D^s (both are mouse alloantisera obtained from Dr. Chella David, Mayo Clinic, Rochester, MN, U.S.A.), I-A^d (a mouse monoclonal antibody of the IgG2a isotype, obtained from Becton-Dickinson, Mountainview, CA, U.S.A.), or I-A^s (a rat monoclonal antibody obtained from Dr. Larry Steinman, Stanford University, Stanford, CA, U.S.A.). Fluorescein-conjugated sheep anti-mouse or anti-rat IgG F(ab)$'_2$ fragments (Cappel Labs, Cochranville, PA, U.S.A.) were used as the secondary reagents. Specificity of these reagents was determined by their ability to react with endothelial cells of the appropriate haplotype, and lack of reactivity with cells of irrelevant haplotypes (data not shown).

Cells were studied at 4 days after infection, the time point previously studied (Massa et al., 1986). The percentage of positive cells and mean fluorescence intensity with each reagent were determined by analysis on the flow cytometer (EPICS C, Coulter Diagnostics, Hialeah, FL, U.S.A.), equipped with an argon laser turned to 488 nm. Only live cells were analyzed, using ethidium bromide as an exclusion method for non-viable cells (Crissman and Steinkamp, 1982). All values in Tables 1–5 of the Results section are presented by subtracting the reading measured on cells labeled with the secondary reagent alone (fluorescein-conjugated sheep anti-mouse or anti-rat IgG F(ab)$'_2$ fragments).

Results

The cell populations were identified as brain endothelial cells by their uptake of Dil-Ac-LDL (Voyta et al., 1984), and labeling with the lectin *Bandeiraea simplicifolia* BSI-B$_4$ (Schelper et al., 1985). Characteristic MHV-4-induced cytopathic

TABLE 1

DIFFERENTIAL MODULATION OF CLASS I ANTIGENS ON BALB/c ENDOTHELIAL CELLS FOLLOWING MHV-4 OR MHV-A59 INFECTION

	Class I		Class II
	H-2K^d	H-2D^d	I-A^d
Cerebral endothelial cells			
Experiment 1 [a]			
Untreated	54.26 [b]	11.5	−0.13
MHV-4	25.72	20.77	−0.2
Experiment 2			
Untreated	68.26	8.0	−0.94
MHV-4	45.75	14.3	−4.77
Experiment 3			
Untreated	49.77	0.43	ND [c]
MHV-4	17.54	11.03	ND
Experiment 4			
Untreated	32.9	2.1	−2.3
Gamma interferon [d]	60.18	7.6	42.63
Experiment 5			
Untreated	32.81	1.79	0.04
MHV-4	19.72	5.5	1.23
UV-inactivated MHV-4	32.23	1.54	0.08
Experiment 6			
Untreated	68.26	8.0	−0.13
MHV-A59	56.37	9.14	−0.22
Fat pad endothelial cells			
Experiment 7			
Untreated	67.17	3.14	−1.23
MHV-4	61.91	27.47	1.01

[a] Each data point represents at least duplicate sample analysis for any given measurement.

[b] This number signifies the percent number of positive cells as analyzed by the flow cytometer.

[c] ND, not done.

[d] This data point was obtained after 72 h of exposure to 200 IU of recombinant rat gamma interferon (Amgen).

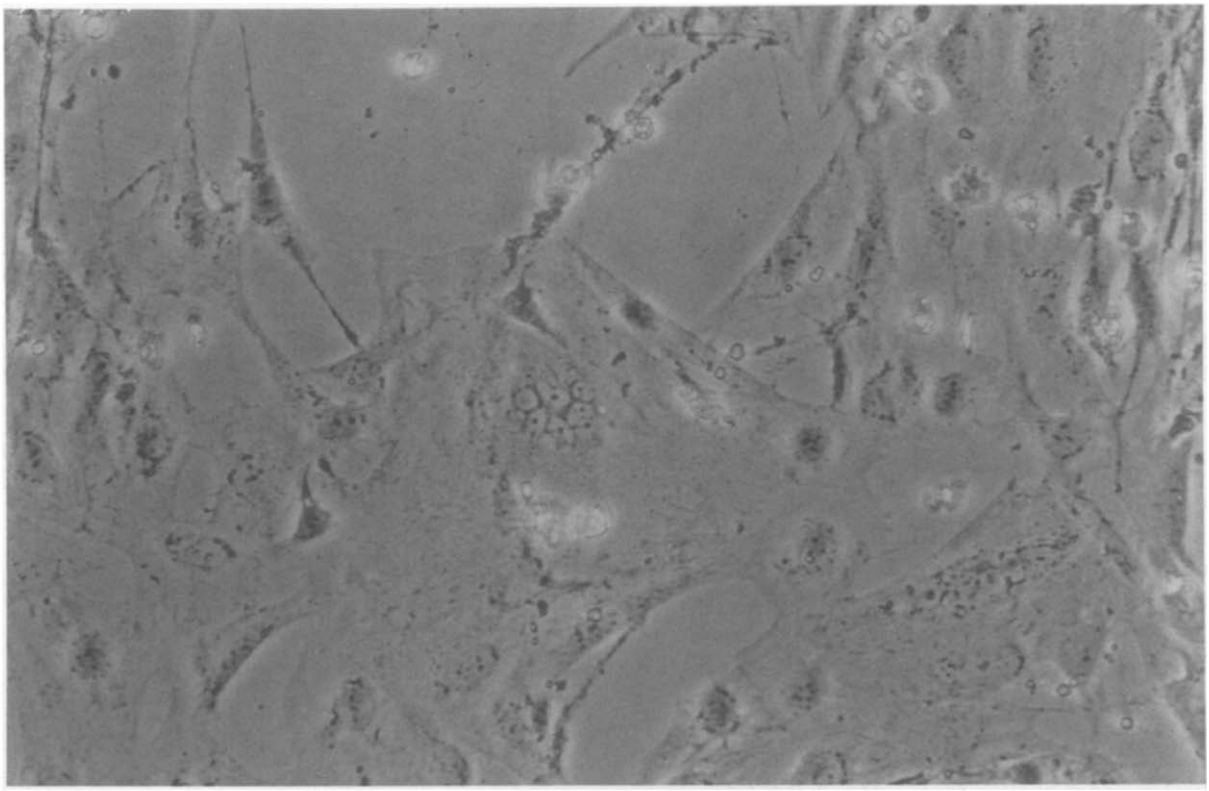

Fig. 1. BALB/c brain-derived endothelial cells, 1 day after infection with MHV-4 at an MOI of 0.1. This photomicrograph shows a large multi-nucleated giant cell (syncytium). Phase microscopy, 1200 ×.

effects (multi-nucleated giant cells) were observed 2–4 days after infection of the BALB/c (Fig. 1), SJL, (BALB/c × SJL) F1 and B10.S brain endothelial cells. Giant cells were present only during the earliest stages of infection, and then lifted off the plate and disintegrated. MHV antigens and granular cytoplasm were noted in virtually all of the cells remaining adherent to the dish, indicating infection of these endothelial cells at 4 days after infection (Fig. 2*a* and *b*). Granular cytoplasm was not observed following exposure to ultraviolet (UV)-inactivated virus

In the analysis of MHC antigen expression by flow cytometry, no differences in findings were observed between cells prepared by physical or enzymatic methods. The MHV-susceptible BALB/c strain showed differential modulation of class I antigen expression on both brain- and fat pad-derived endothelial cells (Table 1). We observed downregulation of the number of H2-K^d-positive cells. Fluorescence histograms of H-2K^d modulation on BALB/c-derived brain endothelial cells is shown in Fig. 3.

The downregulation of the number of H2-K^d-positive cells was not likely due to shutdown of host cell protein synthesis, since an increase in the number of BALB/c-derived brain and fat pad endothelial cells expressing H2-D^d antigens (Fig. 4) was observed at the same time (Table 1), when infection of all cells had been established. The decline in H-2K^d was likely due to fewer cells expressing MHC molecules, rather than fewer molecules being expressed per cell, since there was no difference in the mean fluorescence intensity of H-2K^d expression on uninfected and infected cells (data not shown). This downregulation pattern of H-2K^d response was also observed following infection of BALB/c-derived brain endothelial cells with the related coronavirus MHV-A59 (Table 1).

The observed differential response of class I antigens on BALB/c brain endothelial cells in MHV-4 infection did not reflect defective regu-

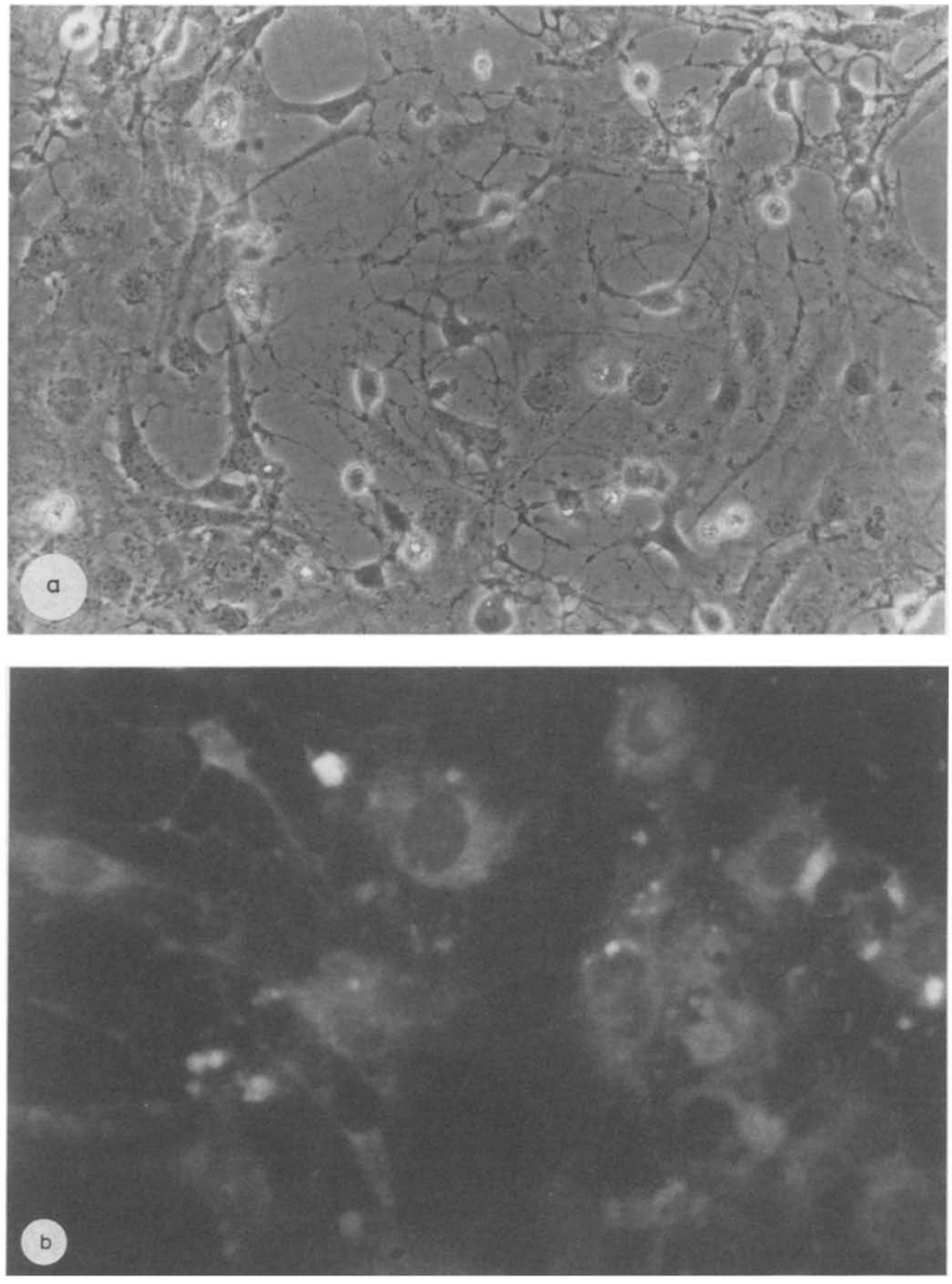

Fig. 2. (*a*) BALB/c brain-derived endothelial cells, 4 days after infection with MHV-4 at an MOI of 0.1. This photomicrograph shows granular cytoplasm of infected endothelial cells. Phase microscopy, 600 ×. (*b*) BALB/c brain-derived endothelial cells, 4 days after infection with MHV-4 at an MOI of 0.1. This immunofluorescence photomicrograph shows labeling of the E1 glycoprotein of MHV in the cytoplasm of infected endothelial cells. Immunofluorescence microscopy, 1200 ×.

lation of the H2-K^d antigen in these cells, since exposure to gamma interferon led to a parallel increase in the number of cells expressing all of the class I (H2-K^d and H2-D^d) and class II (I-A^d) antigens measured (Table 1), as would be expected. Furthermore, downregulation of H-2K^d

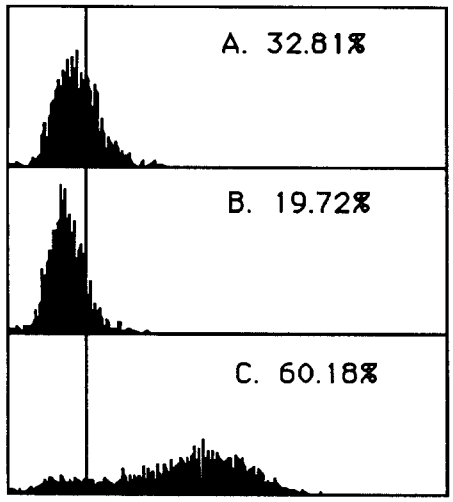

Fig. 3. Fluorescence histograms of H-2K^d expression on BALB/c brain endothelial cells. Flow cytometry profile; *X*-axis: fluorescence intensity, *Y*-axis: cell number. Gate window for green fluorescence lay between channels 0 and 255 (*x*-axis). Gate window for red fluorescence was set for exclusion of non-viable cells stained with ethidium bromide. *A*: Untreated cells. *B*: Cells 96 h after infection with MHV-4 at an MOI of 0.1. *C*: Cells treated with gamma interferon (200 IU/ml, Amgen) for 72 h.

expression following MHV-4 infection, thus far, appears to be a property of the endothelial cells and not a property of the H-2^d haplotype. The number of BALB/c astrocytes expressing H-2K^d

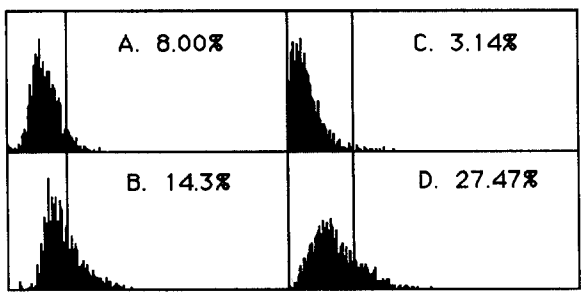

Fig. 4. Fluorescence histograms of H-2D^d expression on BALB/c brain endothelial cells (*A*, *B*) and epididymal fat pad endothelial cells (*C*, *D*). Flow cytometry profile; *X*-axis: fluorescence intensity, *Y*-axis: cell number. Gate window for green fluorescence lay between channels 0 and 255 (*x*-axis). Gate window for red fluorescence was set for exclusion of non-viable cells stained with ethidium bromide. *A*: Untreated brain endothelial cells. *B*: Brain endothelial cells 96 h after infection with MHV-4 at an MOI of 0.1. *C*: Untreated epididymal fat pad endothelial cells. *D*: Epididymal fat pad cells 96 h after infection with MHV-4 at an MOI of 0.1.

TABLE 2

PARALLEL MODULATION OF CLASS I ANTIGENS ON SJL BRAIN ENDOTHELIAL CELLS FOLLOWING MHV-4 INFECTION

	Class I		Class II
	H-2K^s	H-2D^s	I-A^s
Experiment 1 [a]			
Untreated	4.64 [b]	0.56	0.72
MHV-4	18.14	3.75	0.73
Gamma interferon [c]	79.1	11.21	12.3
Experiment 2			
Untreated	10.11	3.83	0.32
MHV-4	22.95	5.29	0.05
Experiment 3			
Untreated	4.00	3.03	0.32
MHV-4	22.98	4.45	1.69

[a] Each data point represents at least duplicate sample analysis for any given measurement.

[b] This number signifies the percent number of positive cells as analyzed by the flow cytometer.

[c] This data point was obtained after 72 h of exposure to 200 IU of recombinant rat gamma interferon (Amgen).

rose following MHV-4 infection (Joseph et al., in preparation). Finally, these responses represent a direct effect of virus replication, since exposure to UV-inactivated MHV-4 did not produce any change in either class I or class II antigens.

In contrast to the findings for the BALB/c strain, brain endothelial cells derived from the SJL strain (MHV resistant) showed an increased number of cells expressing both H2-K^s and H2-D^s antigens following infection with MHV-4 (Table 2). However, this finding was not unique to the resistant phenotype, since B10.S-derived brain endothelial cells (MHV susceptible, but with an H-2^s MHC haplotype) also had an increase in the number of cells expressing both the H2-K^s and H2-D^s antigens following infection with MHV-4 (Table 3). Fluorescence histograms of class I modulation on SJL- and B10.S-derived cerebral endothelial cells are shown in Figs. 5 and 6, respectively.

Additional studies were done with the MHV-4-susceptible (BALB/c × SJL) F1 cerebral endothelial cell line. In this particular cell line, the H-2^d haplotype is preferentially expressed compared to the H-2^s haplotype (Table 4). However, the pattern of modulation of the H-2K^d molecule

TABLE 3

PARALLEL MODULATION OF CLASS I ANTIGENS ON B10.S BRAIN ENDOTHELIAL CELLS FOLLOWING MHV-4 INFECTION

	Class I		Class II
	H-2K^s	H-2D^s	I-A^s
Experiment 1 [a]			
Untreated	8.15 [b]	0.68	−0.8
MHV-4	11.41	4.31	−0.15
Gamma interferon [c]	40.27	1.04	13.83
Experiment 2			
Untreated	3.4	0.41	0.54
MHV-4	10.52	2.46	−0.24

[a] Each data point represents at least duplicate sample analysis for any given measurement.

[b] This number signifies the percent number of positive cells as analyzed by the flow cytometer.

[c] This data point was obtained after 72 h of exposure to 200 IU of recombinant rat gamma interferon (Amgen).

following MHV-4 infection is different from that observed following infection of BALB/c-derived brain endothelial cells with this virus. There is a

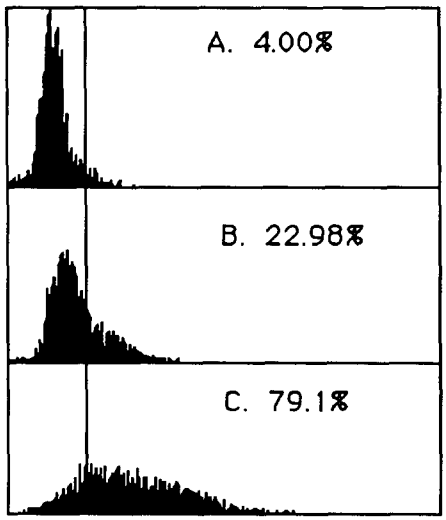

Fig. 5. Fluorescence histograms of H-2K^s expression on SJL brain endothelial cells. Flow cytometry profile; *X*-axis: fluorescence intensity, *Y*-axis: cell number. Gate window for green fluorescence lay between channels 0 and 255 (*x*-axis). Gate window for red fluorescence was set for exclusion of non-viable cells stained with ethidium bromide. *A*: Untreated cells. *B*: Cells 96 h after infection with MHV-4 at an MOI of 0.1. *C*: Cells treated with gamma interferon (200 IU/ml, Amgen) for 72 h.

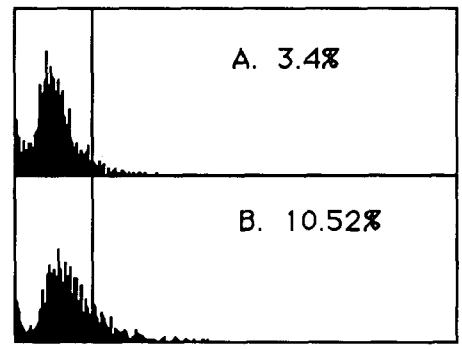

Fig. 6. Fluorescence histograms of H-2K^s expression on B10.S brain endothelial cells. Flow cytometry profile; *X*-axis: fluorescence intensity, *Y*-axis: cell number. Gate window for green fluorescence lay between channels 0 and 255 (*x*-axis). Gate window for red fluorescence was set for exclusion of non-viable cells stained with ethidium bromide. *A*: Untreated cells. *B*: Cells 96 h after infection with MHV-4 at an MOI of 0.1.

parallel increase in the number of cells expressing H-2K^d and H-2D^d following infection with MHV-4. Although H-2D^s was upregulated in one experiment, this was not consistently found. These data are included in Table 4, and a fluorescence histogram demonstrating upregulation of H-2K^d on these (BALB/c × SJL) F1 cells is shown in Fig. 7.

Another question explored was whether the direction of MHC modulation observed following MHV-4 infection was related to the background

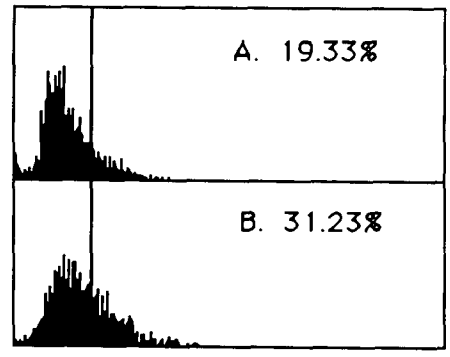

Fig. 7. Fluorescence histograms of H-2K^d expression on (BALB/c × SJL) F1 brain endothelial cells. Flow cytometry profile; *X*-axis: fluorescence intensity, *Y*-axis: cell number. Gate window for green fluorescence lay between channels 0 and 255 (*x*-axis). Gate window for red fluorescence was set for exclusion of non-viable cells stained with ethidium bromide. *A*: Untreated cells. *B*: Cells 96 h after infection with MHV-4 at an MOI of 0.1.

TABLE 4

PARALLEL MODULATION OF CLASS I ANTIGENS ON (BALB/c × SJL) F1 BRAIN ENDOTHELIAL CELLS FOLLOWING MHV-4 INFECTION

	Class I				Class II	
	H-2K^d	H-2D^d	H-2K^s	H-2D^s	I-A^d	I-A^s
Experiment 1 [a]						
Untreated	12.8 [b]	1.2	−0.27	0.34	−0.28	−0.44
MHV-4	17.96	7.7	0.17	5.83	−0.87	−12.2
Experiment 2						
Untreated	19.3	6.53	1.43	1.23	ND [c]	ND
MHV-4	31.23	10.00	0.00	0.00	ND	ND
Experiment 3						
Untreated	8.93	0.47	0.33	0.33	−0.03	0.67
Gamma interferon [d]	73.94	0.21	0.16	0.18	5.08	0.75

[a] Each data point represents at least duplicate sample analysis for any given measurement.
[b] This number signifies the percent number of positive cells as analyzed by the flow cytometer.
[c] ND, not done.
[d] This data point was obtained after 72 h of exposure to 200 IU of recombinant rat gamma interferon (Amgen).

level of expression of these markers. H-2K^d is ordinarily expressed at relatively high levels on BALB/c endothelial cells (Table 1), when compared to H-2K^s on SJL and B10.S brain endothelial cells (Tables 2 and 3). Therefore, we addressed this issue by upregulating H-2K^s on SJL and B10.S brain endothelial cells, by exposing them to gamma interferon, and then determining their response to MHV-4 infection (Table 5). Our results indicate that virus infection is followed by an increase in H-2K^s expression, albeit slight, suggesting that the direction of modulation of this MHC marker is not linked to its background level of expression.

TABLE 5

EFFECT OF UPREGULATION OF CLASS I ANTIGENS BY GAMMA INTERFERON ON VIRUS-INDUCED ANTIGEN MODULATION

	Percent H-2K^s-positive cells
SJL endothelial cells pretreated with 200 IU/ml gamma interferon for 72 h	48.43
SJL endothelial cells pretreated with 200 IU/ml gamma interferon for 72 h followed by MHV-4 for 96 h	49.5
B10.S endothelial cells pretreated with 200 IU/ml gamma interferon for 72 h	40.27
B10.S endothelial cells pretreated with 200 IU/ml gamma interferon for 72 h followed by MHV-4 for 96 h	43.97

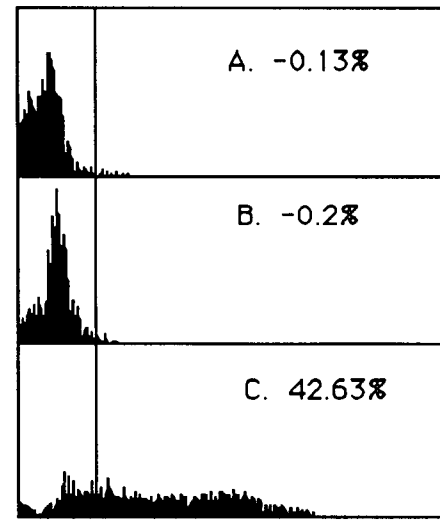

Fig. 8. Fluorescence histograms of I-A^d expression on BALB/c brain endothelial cells. Flow cytometry profile; *X*-axis: fluorescence intensity, *Y*-axis: cell number. Gate window for green fluorescence lay between channels 0 and 255 (*x*-axis). Gate window for red fluorescence was set for exclusion of non-viable cells stained with ethidium bromide. *A*: Untreated cells. *B*: Cells 96 h after infection with MHV-4 at an MOI of 0.1. *C*: Cells treated with gamma interferon (200 IU/ml, Amgen) for 72 h.

Unlike results reported for rat astrocytes, which demonstrated an increased in class II antigen expression following exposure to either live or inactivated MHV-4 (Massa et al., 1986, 1987a), there usually was no detectable rise in the number of brain endothelial cells expressing class II antigen from any of the mouse strains examined following infection with MHV-4 (Tables 1–4). However, the results of experiment 5 in Table 1 demonstrate a small but measurable increase in the number of cells (1%) expressing class II antigens following MHV-4 infection, although this is negligible compared to the substantial rise to 43% of the cells expressing class II antigens at 72 h after exposure to 200 IU of gamma interferon (experiment 4, Table 1). A fluorescence histogram of class II (I-A^d) modulation on BALB/c-derived brain endothelial cells by gamma interferon, but not MHV-4, is shown in Fig. 8.

In all of our flow cytometric analyses of infected cells at 4 days after infection, only single cells were analyzed. Multinucleated giant cells were not usually found at this time point, but if present, they did not survive cell preparation for FACS analysis. Fig. 9 shows a 90° vs. forward angle light scatter profile of uninfected (panel *A*) and infected (panel *B*) cells to indicate the cell populations analyzed.

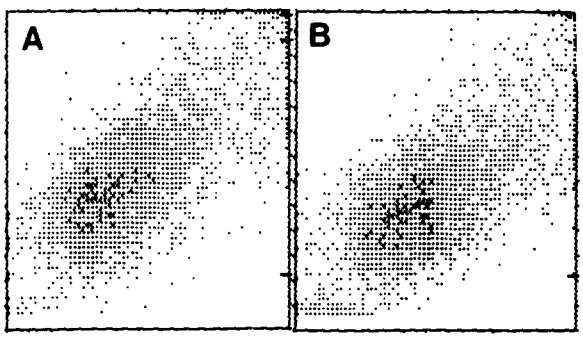

Fig. 9. Forward angle vs. 90° light scattering profile of uninfected (panel *A*) and MHV-4-infected (panel *B*) BALB/c endothelial cells. *X*-Axis is 90° light scatter, *Y*-axis is forward angle light scatter. Cells were analyzed on day 4 after infection. Windows were set to gate out dead cells and cellular debris.

Discussion

Cerebral endothelial cells are a major structural component of the blood brain barrier. These cells can play an important role in regulating virus entry into the brain (Johnson, 1974, 1982; Wiley et al., 1986), and in modulating immune-mediated events occurring within the CNS (McCarron et al., 1985, 1986). Our studies address these issues in vitro, by analyzing changes in endothelial cell class I and class II MHC antigen expression following infection with the neurotropic coronavirus, MHV-4. We have examined the effect of the neurotropic coronavirus, MHV-4 (JHM) on cerebral endothelial cell class I and class II expression because of the potential role of these molecules on these cells in viral antigen presentation and antiviral cytotoxic T cell responses (Zinkernagel and Doherty, 1975, 1979; McCarron et al., 1985, 1986; Paabo et al., 1986; Burgert et al., 1987; Yamaguchi et al., 1988).

We demonstrate a differential modulation of the number of cells expressing class I MHC molecules, following infection with MHV-4, and this has been confined, thus far, to BALB/c-derived brain and fat pad endothelial cells (Table 1). The percentage of H2-K^d-positive cells drops following infection with MHV-4, while the number of cells expressing H2-D^d increases. This pattern of differential modulation of class I expression has also been observed following infection with the related coronavirus MHV-A59 (Table 1). There is no change in the percentage of class II (I-A^d)-positive BALB/c brain endothelial cells after either MHV-4 or MHV-A59 infection.

The decline in the number of cells expressing H2-K^d molecules is not due to either defective expression or defective regulation of H2-K^d on BALB/c-derived brain endothelial cells, since the cells expressing both class I (H2-K^d and H2-D^d) and class II antigens exhibit a characteristic increase in number following exposure to gamma interferon (Table 1). Infection with live virus is required, since UV-inactivated MHV-4 did not lead to changes in either class I or class II expression (Table 1).

Differential modulation of class I MHC expression, in BALB/c brain endothelial cells, is in contrast to the parallel rise in expression of both

the H2-K and H2-D molecules observed following MHV-4 infection of other BALB/c cell types or brain endothelial cells derived from other strains of mice. Infection of BALB/c astrocytes with MHV-4 results in an increase in the number of cells expressing both H2-K^d and H2-D^d (Joseph et al., in preparation). Infection of resistant SJL or susceptible H-2 congenic B10.S-derived brain endothelial cells also leads to an increase in the number of cells expressing both H2-K^s and H2-D^s molecules (Tables 2 and 3), as does infection of (BALB/c × SJL) F1 (Table 4). Finally, differential modulation of class I molecules does not appear to be a function of the background level of expression of these MHC markers.

Several conclusions can be drawn from this data. Differential modulation of the MHC class I H-2K molecule by MHV-4 infection appears to be strain dependent, suggesting genetic regulation. Since B10.S mice are susceptible to MHV replication (Stohlman and Frelinger, 1978; Knobler et al., 1981), like BALB/c mice, perhaps a separate gene from the chromosome 7 locus (Knobler et al., 1984), encoding a virus receptor (Knobler et al., 1987) and determining susceptibility to MHV replication is involved. This suggestion is also supported by the observation of upregulation of H-2K on (BALB/c × SJL) F1 brain endothelial cells, which thus follows the SJL pattern of MHC modulation, but the BALB/c pattern of MHV susceptibility (Knobler et al., 1981). This suggests the possibility that an SJL background gene(s) may have an effect on MHC class I regulation.

A second gene regulating susceptibility to MHV-4-induced fatal encephalomyelitis had previously been proposed following studies comparing B10.S and SJL mice and their progeny (Stohlman and Frelinger, 1978; Stohlman et al., 1985). Recombinant inbred mice between BALB/c and SJL, the CXJ series (Knobler et al., 1985), can be a useful tool to search for, sort and map the location of this gene through characterization of the brain-derived endothelial cell class I MHC responses to MHV infection. We are also generating a panel of brain endothelial cells from other MHV-susceptible mice in order to better understand the genetic regulation of susceptibility to MHV-4.

Although Fc receptors may be induced following certain viral infections (Cines et al., 1982), and Fc-bound molecules could affect reactivity with a variety of antibodies, it is unlikely that this accounts for the differential modulation observed in the present experiments. This is because antibodies to three separate determinants (H-2K^d, H-2D^d and I-A^d) were used and produced divergent rather than identical results on cells from BALB/c mice. Identical results, demonstrating a uniform increase of all three determinants, would have been expected on these cells if our findings were due to non-specific binding to increased numbers of Fc receptors present.

The effects of MHV-4 on differential modulation of MHC class I expression do not appear to be due to production of a soluble factor induced by infection, as suggested in the studies of Suzumura et al. (1986, 1988), using astrocytes. Although this possibility has not yet been completely ruled out, it is considered less likely in the present experiments. This is because the response was confined to BALB/c brain and fat pad endothelial cells, and was not found with MHV-4-infected SJL, (BALB/c × SJL) F1 and B10.S brain endothelial cells, or MHV-4-infected BALB/c, B10.S or SJL astrocytes (Joseph et al., in preparation). Lavi et al. (1988) demonstrated that tumor necrosis factor will induce class I antigens on mouse astrocytes. Further experiments testing the supernatants of MHV-4-infected BALB/c endothelial cells, as compared to the supernatant of endothelial cells from other strains, and tumor necrosis factor are currently in progress.

Differential modulation of MHC class I antigens has been reported in several other systems. For example, there is a differential response to gamma interferon by the murine leukemia virus-induced AKR SL3 tumor cell line. A large increase in H-2D^k, and no change or a slight decrease in H-2K^k expression following exposure to gamma interferon occurs (Green and Phillips, 1986). Additional examples of altered regulation of MHC expression are based upon a series of observations following the infection of cells with murine retroviruses (Flyer et al., 1985). Fibroblasts infected with Moloney murine leukemia virus (M-MuLV) show increased class I MHC expression, which correlates with increased susceptibility to lysis by specific cytotoxic lympho-

cytes (CTL). In contrast, failure to express MHC antigens may lead to failure of CTL in class I-restricted killing (Zinkernagel and Doherty, 1975, 1979), or failure of immune surveillance (Tanaka et al., 1985).

An important extension of our studies will also be to examine the role of endothelial cell class I modulation in regulating cytotoxic T cell activity against MHV-4. It has been difficult to demonstrate cytotoxic T cells against MHV-4, until the recent development of CTL clones against MHV-4 (Yamaguchi et al., 1988). We will determine if MHV-4-infected endothelial cells can serve as targets for an MHV-specific CTL response. CTL activity against virus-infected cells may have damaging effects on the host. For instance, if CTLs lyse virus-infected BBB endothelial cells, the resulting damage may more readily permit virus entry into the brain. Alternatively, CTLs may aid in the rapid clearing of the virus, thus preventing further infection and spread.

The mechanism of differential modulation of class I MHC expression, as observed in the decline of H-2K^d expression on BALB/c-derived brain endothelial cells, is presently unknown. Recent studies of the adenovirus type 2 system demonstrate one potential mechanism in that the E3/19K protein of this virus prevents class I MHC expression (Paabo et al., 1986; Burgert et al., 1987). This is due to direct binding of the viral protein to the class I molecules, preventing their terminal glycosylation and inhibiting cell surface expression. As a result, reduced cellular immune recognition and reduced target cell lysis occur (Paabo et al., 1986; Burgert et al., 1987). This may provide a mechanism for escape from immune surveillance and persistent viral infection.

Another possible mechanism of downregulation to consider is the potential role of the MHC antigen as a receptor for virus binding (Helenius et al., 1978; Dasgupta and Yunis, 1987; Grundy et al., 1987). This would suggest ligand-mediated receptor downregulation as a result of virus binding. The difference in response of brain endothelial cells (Table 1) and astrocytes (Joseph et al., in preparation) from BALB/c mice, sharing identical MHC molecules, provides evidence against this hypothesis.

There is no change in the low level of class II expression on brain endothelial cells from all three strains studied following infection with MHV-4, regardless of their susceptibility or resistance to virus or immune-mediated demyelinating diseases. This is in contrast to the results reported for Lewis rat astrocytes, which demonstrated an increase in class II antigen expression following exposure to either live or inactivated MHV-4 (Massa et al., 1986, 1987a). It has been suggested that the late immune-mediated demyelinating disease in the rat following MHV-4 infection, in part at least, is correlated to the observed induction of class II antigens on astrocytes by MHV-4 (Massa et al., 1986, 1987a).

Class II expression on brain endothelial cells also correlates with the demyelinating diseases produced in the mouse experimental allergic encephalomyelitis model (McCarron et al., 1985, 1986; Traugott et al., 1985), and the Theiler's murine encephalomyelitis virus model (Rodriguez et al., 1987) in SJL mice. Therefore, the absence of a late immune-mediated demyelinating disease in the mouse following MHV-4 infection may in part reflect the failure of induction of class II MHC antigens on brain endothelial cells, and astrocytes (Joseph et al., in preparation) after exposure to this virus. It is presently unclear whether exposure to gamma interferon can enhance the expression of MHC class II antigens following MHV-4 infection of mouse brain endothelial cells, in a fashion similar to the enhancement of class II antigen expression on rat astrocytes exposed to measles virus and tumor necrosis factor (Massa et al., 1987b). This is presently being investigated.

In summary, we have demonstrated differential modulation of MHC class I antigen in BALB/c brain and fat pad endothelial cells in vitro, which may influence virus clearance and immune responsiveness. We have also demonstrated that no change occurs in class II expression on these mouse cells following MHV-4 infection, although class II expression on these cells is regulated by gamma interferon. In all other strains examined, i.e., B10.S, SJL and (BALB/c × SJL) F1, we observed an increase in the number of cells expressing both H-2K and H-2D antigens. The role of MHC antigen modulation in the immune response to MHV-4

is currently being investigated by examining virus-specific CTL activity using endothelial cells as targets.

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