Mouse hepatitis virus (MHV-4, JHM) blocks γ-interferon-induced major histocompatibility complex class II antigen expression on murine cerebral endothelial cells

Jeymohan Joseph [1], Robert L. Knobler [1], Fred D. Lublin [1] and M.N. Hart [2]

[1] *Division of Neuroimmunology, Department of Neurology, Jefferson Medical College, Philadelphia, PA 19107, U.S.A.,*
and [2] *Department of Pathology (Neuropathology), University of Iowa College of Medicine, Iowa City, IA 52242, U.S.A.*

Summary

The regulation of γ-interferon-induced major histocompatibility complex (MHC) class II antigen expression on mouse cerebral endothelial cells by the neurotropic mouse hepatitis virus (MHV-4, JHM) was studied in vitro. The results presented demonstrate that MHV-4 can selectively block γ-interferon-induced class II antigen expression on cerebral endothelial cells. The blocking effect of class II expression occurs in a strain-dependent manner, and is limited to virus-susceptible mouse strains. Virus replication is not required to obtain the blocking effect since UV-inactivated MHV-4 produces the same result. MHV-4 blocking of γ-interferon-induced class II antigen expression is observed at both the cell surface (flow cytometry) and transcriptional level (Northern analysis).

Introduction

Murine cerebral vascular endothelial cells are integral components of the blood–brain barrier, and are believed to be an important site which can restrict infection of the central nervous system (CNS) by viruses and other pathogens (John-son, 1974, 1982; Wiley et al., 1986; Zurbriggen and Fujinami, 1988; Dropulic et al., 1990). A potential role of cerebral vascular endothelial cells in resisting CNS infection relates to their expression of major histocompatibility complex (MHC) molecules and its impact on viral antigen presentation and generation of anti-viral cytotoxic T cell activity (Hirschberg, 1981; Collins et al., 1984; McCarron et al., 1985, 1986; Pober et al., 1985, 1986). MHV-4 (mouse hepatitis virus, JHM strain) is a member of the coronavirus family that has been studied in models of virus infections of the CNS (Bailey et al., 1949). We previously reported the modulation of MHC class I

Address for correspondence: Dr. Jeymohan Joseph, Room 505, College Building, Department of Neurology, Jefferson Medical College, 1025 Walnut Street, Philadelphia, PA 19107, U.S.A.

antigens and the lack of effect on class II antigen expression, following MHV-4 infection of mouse cerebral vascular endothelial cells from selected mouse strains (Joseph et al., 1989). Other investigators have also shown class I modulation on mouse glial cells by a related coronavirus, MHV-A59 (Suzumura et al., 1986). The results presented in this report show a novel regulatory effect of MHV-4 on MHC expression, i.e., the ability to block γ-interferon-induced class II induction on cerebral vascular endothelial cells. This occurs in a strain-dependent manner. Virus replication is not required to obtain the blocking effect since comparable results were obtained when cells were exposed to UV-inactivated MHV-4. The relevance of these findings in understanding immunopathologic events in the CNS following MHV-4 infection is discussed.

Materials and methods

Cerebral microvascular endothelial cell cultures

Cerebral microvascular endothelial cells were isolated from the brains of MHV-susceptible BALB/c, (BALB/c × SJL)F1, B10.S and MHV-resistant SJL strains of mice by using the method described by Rupnick et al. (1988). Briefly, cerebral microvessels were isolated by digestion of cerebral white matter in 0.5% collagenase (Sigma, St. Louis, MO, U.S.A.) and density centrifugation of homogenized material in 15% dextran. The vascular pellet was further purified by gradient centrifugation on Percoll (45%). Capillaries were plated onto 0.1% gelatin-coated plates, and endothelial cells grew out in about 10 days. Endothelial cell lines were established from these initial outgrowths and were cultured in Medium 199 (Gibco, Grand Island, NY, U.S.A.) supplemented with 20% fetal bovine serum, 2 mM L-glutamine, 90 μg/ml heparin (porcine, Sigma, St. Louis, MO, U.S.A.) and 20 μg/ml endothelial cell growth factor (ECGF, Collaborative Research, Cambridge, MA, U.S.A.).

Endothelial cell identity was established by the uptake of DiI-Ac-LDL (acetylated low density lipoprotein, Biomedical Technologies, Stoughton, MA, U.S.A.) and specific binding of *Bandeiraea simplicifolia* BSI-B$_4$ (Voyta et al., 1984; Sahagun

et al., 1989). The endothelial cell lines used in these studies were between passages 12 and 16.

Infection of cultures

Endothelial cells grown in T-25 flasks (1×10^6 cells/flask) were infected with MHV-4 or UV-inactivated MHV-4 for 1 h at 37°C using a multiplicity of infection (MOI) of 0.1. This MOI reflects the working titer obtainable without extensive cytolytic effects using MHV-4 (JHM) based on previous studies (Joseph et al., 1989). MHV-4 was grown to a stock containing 5×10^5 plaque-forming units (PFU)/ml as previously described (Knobler et al., 1981). UV-inactivation of virus was carried out for 30 min (1250 μW/cm^2) under a Spectroline UV-illuminator (Fisher Scientific, Springfield, NJ, U.S.A.). Virus infectivity was tested by plaque assays on L-2 cells to determine if the inactivation was complete following UV treatment.

After a 1 h treatment of the endothelial cells with MHV-4 or UV-inactivated MHV-4 the cultures were washed 3 times with phosphate-buffered saline (PBS). After washes the cultures received 200 IU/ml of recombinant rat γ-interferon (Amgen Biologicals, Thousand Oaks, CA, U.S.A.). Control cultures were fed with endothelial cell culture media after virus infection and washes.

Antibody labeling and flow cytometry

At various times (days 1–4) after virus and interferon treatments endothelial cells were removed from the flasks using 0.25% trypsin and 0.1% EDTA for 1 min at 37°C. This treatment did not affect the level of MHC class I or II expression measured on the cells when compared to cells removed by scraping from the plates. Endothelial cells were labeled with antibody to either I-A^d (a mouse monoclonal antibody of the IgG$_{2a}$ isotype, Becton Dickinson, Mountain View, CA, U.S.A.), I-A^s (a rat monoclonal antibody obtained from Dr. Larry Steinman, Stanford University, Stanford, CA, U.S.A.) or H-2K^d (a mouse monoclonal antibody of the IgG$_{2a}$ isotype, Pharmingen, San Diego, CA, U.S.A.). Fluorescein-conjugated sheep anti-mouse or anti-rat IgG F(ab')$_2$ fragments (Organon-Teknika Cappel, Cochranville, PA, U.S.A.) were used as secondary

reagents. The percentage of positive cells and mean fluorescence intensities (MFI) were determined by duplicate sample analysis on a flow cytometer (EPICS C, Coulter Diagnostics, Hialeah, FL, U.S.A.), equipped with an argon laser tuned to 488 nm. Gate windows on the flow cytometer were chosen to exclude dead cells and cellular debris. Ethidium bromide was used as an exclusion method for dead cells (Crissman and Steinkamp, 1982). Variability between duplicate samples was less than 10%. All percent positive cell numbers presented here were obtained by subtracting the reading measured on cells labeled with the secondary reagent alone.

Specificity of the reagents was determined by their ability to react with spleen cells of the appropriate haplotype and lack of reactivity with cells of the irrelevant haplotype (data not shown). Isotype controls of the primary antibodies were also included to exclude non-specific binding.

RNA isolation and Northern analysis

24 h after exposure of endothelial cells to MHV-4, UV-MHV-4 or a combination of virus with γ-interferon, total cellular RNA was isolated by immediate solubilization of cells in guanidine hydrochloride as previously described (Maniatis et al., 1982). The solution was sonicated and centrifuged to remove cellular debris. The RNA was ethanol precipitated, phenol extracted and then reprecipitated with ethanol. The RNA was quantified by absorbance at 260 nm and by visual estimation of ribosomal RNA content by ethidium bromide staining following electrophoresis in denaturing formaldehyde gels. RNA was transferred to nylon membranes (Hybond-N, Amersham, Arlington Heights, IL, U.S.A.) and fixed by UV-irradiation. The RNA was prehybridized in 1 M NaCl, 0.1% sodium dodecyl sulfate (SDS), 1.5 mg/ml herring sperm DNA, and 10% dextran for 3 h at 65°C. The RNA was then hybridized overnight at 65°C with a radiolabeled I-A$_\alpha$b genomic probe (obtained from Dr. Laurie Glimcher, Department of Cancer Biology, Harvard School Of Public Health, Cambridge, MA, U.S.A.). This cDNA probe is a 1.3 kb HindIII fragment of the 3' end of a genomic clone (Ben-Nun et al., 1985). The probe was radiolabeled (^{32}P) in low melting agarose by random hexamer priming (Maniatis et al., 1982). The blots were washed under high stringency conditions (0.1 × SSC (1 × SSC = 0.15 M NaCl, 15 mM trisodium citrate), 0.1% SDS, 1 mM EDTA (pH 8), 10 mM sodium phosphate (pH 6.8) at 68°C) and analyzed by autoradiography with intensifying screens (Dupont, Hoffman Estates, IL, U.S.A.). Densitometry of the autoradiographs was carried out using a Macbeth densitometer (model TD-932, Macbeth Process Measurements, Newburg, NY, U.S.A.).

Results

Effect of MHV-4 on γ-interferon-induced class II antigen expression

Our initial experiments were carried out using cerebral endothelial cell lines derived from MHV-susceptible strains of mice (BALB/c, (BALB/c × SJL)F1). Infection of these cells with MHV-4 resulted in a failure of induction of I-A^d by γ-interferon (Fig. 1, compare panel B vs. C, F vs. G and J vs. K). The percentage of positive cells and mean fluorescence intensities were de-
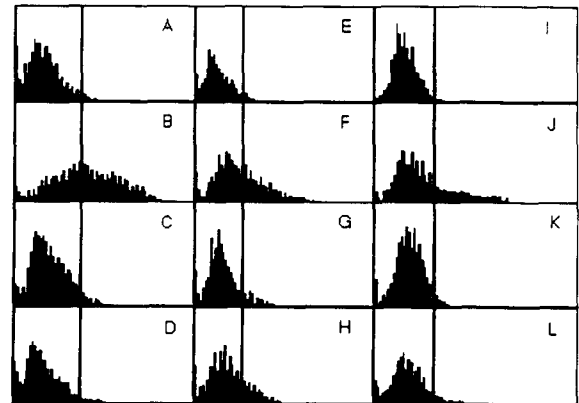

Fig. 1. Fluorescence histograms of I-A^d expression on cerebral endothelial cells. Flow cytometry profile: x-axis fluorescence intensity; y-axis cell number. Gate windows for green fluorescence lay between channels 0 and 255. Panels A–D: BALB/c endothelial cell line I. A: untreated; B: γ-interferon; C: MHV-4 + γ-interferon; D: UV-MHV-4 + γ-interferon. Panels E-H: (BALB/c×SJL)F1 endothelial cell. E: untreated; F: γ-interferon; G: MHV-4 + γ-interferon; H: UV-MHV-4 + γ-interferon. Panels I–L: BALB/c endothelial cell line II. I: untreated; J: γ-interferon; K: MHV-4 + γ-interferon; L: UV-MHV-4 + γ-interferon.

184

termined on day 4 post-infection by flow cytometry (Table 1). The 4-day time point was chosen because peak induction of I-A^d by γ-interferon was obtained between 72 and 96 h (data not shown).

Comparison of the effect of MHV-4 vs. UV-inactivated MHV-4 on γ-interferon-induced class II expression

In order to determine if virus replication is required for blocking of γ-interferon-induced class II expression, UV-inactivated virus was tested under identical conditions. As shown in Fig. 1, UV-inactivated MHV-4 was as effective as MHV-4 in blocking γ-interferon-induced class II antigen expression (compare panels *B* vs. *D*, *F* vs. *H*, *J* vs. *L*). The percent positive cells and mean fluorescence intensities are shown in Table 1.

Time course of MHV-4 and UV-inactivated MHV-4 effect on γ-interferon-induced class II antigen expression

Fig. 2 summarizes the results obtained with studies of I-A^d expression measured on days 1–3 after various treatments of BALB/c endothelial cells. The treatments and results shown in Fig. 2 include (a) untreated (row 1), (b) γ-interferon (row 2), (c) MHV-4 (row 3), (d) UV-inactivated MHV-4 (row 4), (e) γ-interferon + MHV-4 (row 5), (e) γ-interferon + UV-MHV-4 (row 6).

The ability of the virus to block γ-interferon-induced class II expression was observed as early as day 1 (rows 5 and 6). This suggests that the
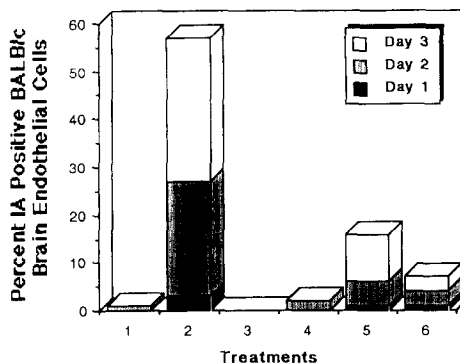

Fig. 2. Time course of MHC class II expression on BALB/c endothelial cells. The percentage of I-A^d-positive cells was determined on days 1–3 after infection by flow cytometry. Treatments were as follows (rows 1–6): (1) untreated; (2) γ-interferon; (3) MHV-4; (4) UV-MHV-4; (5) MHV-4 + γ-interferon; (6) UV-MHV-4 + γ-interferon.

virus begins to exert its effects on early events of the γ-interferon-triggered class II induction pathway.

Time course of MHV-4 and UV-inactivated MHV-4 effect on γ-interferon-induced class I antigen induction

In order to examine if MHV-4 and UV-inactivated MHV-4 block class II antigen expression selectively, the effect of virus on γ-interferon-induced class I antigen expression was studied. Fig. 3 summarizes the results obtained with MHV-4 and UV-inactivated MHV-4 on γ-interferon-induced H-2K^d expression on (BALB/c × SJL)F1 cerebral endothelial cells. Table 2 shows the percent positive cells and mean fluorescence

TABLE 1

REGULATION OF γ-INTERFERON-INDUCED CLASS II (I-A^d) ANTIGEN EXPRESSION ON SUSCEPTIBLE STRAIN-DERIVED CEREBRAL ENDOTHELIAL CELLS BY MHV-4 AND UV-MHV-4

The data presented are obtained from the histograms shown in Fig. 1.

	BALB/c I		(BALB/c × SJL)F1		BALB/c II	
	% POS [a]	MFI [b]	% POS	MFI	% POS	MFI
Untreated	0	29	1	27	0	31
γ-Interferon	54	75	31	49	23	53
MHV-4 + γ-interferon	3	40	3	34	0	34
UV-MHV-4 + γ-interferon	2	35	3	37	8	41

[a] Percent positive cells.
[b] Mean fluorescence intensity.

TABLE 2

TIME COURSE OF REGULATION OF γ-INTERFERON-INDUCED MHC CLASS I (H-2K^d) EXPRESSION ON (BALB/c × SJL)F1 CEREBRAL ENDOTHELIAL CELLS BY MHV-4 AND UV-MHV-4

Data shown are obtained from the histograms in Fig. 3.

	Day 1		Day 2		Day 3	
	% POS [a]	MFI [b]	% POS	MFI	% POS	MFI
Untreated	22	32	31	32	20	30
γ-Interferon	71	53	89	81	89	87
MHV-4	29	34	30	32	36	37
UV-MHV-4	29	34	34	32	34	37
MHV-4 + γ-interferon	83	61	92	81	89	87
UV-MHV-4 + γ-interferon	80	57	91	82	89	91

[a] Percent positive cells.
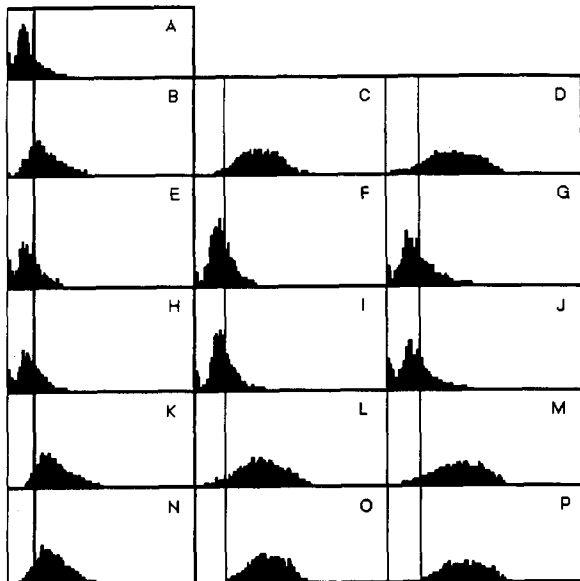[b] Mean fluorescence intensity.

Fig. 3. Fluorescence histograms of the time course of the effect of MHV-4 treatment on γ-interferon-induced class I (H-2K^d) expression on (BALB/c × SJL)F1 endothelial cells. Flow cytometry profile: x-axis fluorescence intensity; y-axis cell number. Gate windows for green fluorescence lay between channels 0 and 255 (x-axis). Class I antigen expression was measured using a monoclonal anti-mouse H-2K^d (Pharmingen, San Diego, CA, U.S.A.) as a primary reagent. Panel A: untreated; B: γ-interferon day 1; C: γ-interferon day 2; D: γ-interferon day 3; E: MHV-4 day 1; F: MHV-4 day 2; G: MHV-4 day 3; H: UV-MHV-4 day 1; I: UV-MHV-4 day 2; J: UV-MHV-4 day 3; K: MHV-4 + γ-interferon day 1; L: MHV-4 + γ-interferon day 2; M: MHV-4 + γ-interferon day 3; N: UV-MHV-4 + γ-interferon day 1; O: UV-MHV-4 + γ-interferon day 2; P: UV-MHV-4 + γ-interferon day 3.

intensities obtained from the histograms in Fig. 3. Unlike results obtained with I-A^d expression, class I antigen (H-2K^d) expression was not blocked in the same manner. This suggests that MHV-4 or UV-inactivated MHV-4 was not blocking the ability of γ-interferon to interact with its receptor since class I antigen induction by this lymphokine was minimally affected. A class II-specific inhibition pathway appears to be activated by MHV-4 and UV-MHV-4.

TABLE 3

EFFECT OF PRETREATMENT OF γ-INTERFERON-TREATED CEREBRAL ENDOTHELIAL CELLS WITH MHV-4 OR UV-MHV-4 ON MEASUREMENT OF CLASS II (I-A^d) ANTIGEN LEVELS BY FLOW CYTOMETRY

Treatment	Percent I-A^d-positive cells [a]
Untreated	2.34
γ-Interferon	32.97
γ-Interferon + MHV-4 [b]	31.5
γ-Interferon + UV-MHV-4 [b]	28.0

[a] The percent I-A^d-positive cells was determined by flow cytometry.
[b] BALB/c endothelial cells were treated with γ-interferon for 72 h. Prior to antibody labeling and flow cytometry the cells were treated with 5×10^4 PFU of active or UV-inactivated MHV-4 for 1 h at 37°C. This quantity of virus was chosen because on day 3 after virus infection in vitro 5×10^4 PFU is within the range of virus yields obtained after MHV-4 replication in BALB/c-derived endothelial cells (data not shown).

Effect of MHV-4 and UV-inactivated MHV-4 on binding of anti-class II antibody to cerebral endothelial cells

Another possible mechanism for the apparent reduction of class II antigens measured following exposure to MHV-4 or UV-inactivated MHV-4 would be the interaction of the virus with class II antigens on the cell surface. This would impair the binding of anti-class II antibody and thus mask detectable levels of MHC class II antigens. This possibility was ruled out when γ-interferon-

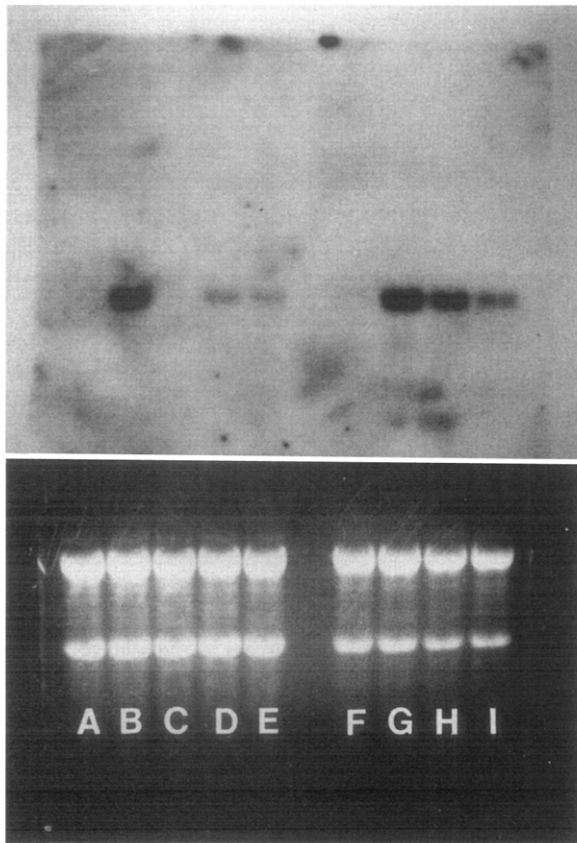Fig. 4. Northern blot analysis of γ-interferon-induced IA mRNA regulation by MHV-4. Lanes *A – E* show RNA derived from (BALB/c×SJL)F1 cerebral endothelial cells. Lanes *F – I* show RNA derived from SJL cerebral endothelial cells. Cells were treated for 24 h as follows. Lane *A*: untreated; *B*: γ-interferon; *C*: MHV-4; *D*: MHV-4 + γ-interferon; *E*: UV-MHV-4 + γ-interferon; *F*: untreated; *G*: γ-interferon; *H*: MHV-4 + γ-interferon; *I*: UV-MHV-4 + γ-interferon. The top panel shows the autoradiograph and the lower panel is the ethidium bromide profile to demonstrate equivalent loading of RNA (5 μg of RNA loaded per lane).

TABLE 4

DENSITOMETRIC QUANTITATION OF THE AUTORADIOGRAPH FROM A NORTHERN BLOT DETECTING IA mRNA

Treatment	Optical density units [a]
(BALB/c×SJL)F1 endothelial cells	
Untreated	0.30 [a]
γ-Interferon	0.76
MHV-4	0.27
MHV-4 + γ-interferon	0.30
UV-MHV-4 + γ-interferon	0.29
SJL endothelial cells	
Untreated	0.27
γ-Interferon	0.81
MHV-4 + γ-interferon	0.63
UV-MHV-4 + γ-interferon	0.41

[a] Determined by using a Macbeth densitometer (model TD-932, Macbeth Process Measurements, Newburg, NY, U.S.A.).

treated endothelial cells (treated for 72 h) were exposed to either MHV-4 or UV-inactivated MHV-4 for 1 h prior to anti-class II antibody labeling and measurement of MHC class II antigen expression by flow cytometry. As shown in Table 3, there was no significant reduction in measurable class II antigen expression observed, suggesting that the binding of the virus to IA antigens was not a potential mechanism for the observed blocking effect.

Effect of MHV-4 and UV-inactivated MHV-4 on transcription of γ-interferon-induced IA mRNA

Further studies to understand the mechanism of blocking of γ-interferon-induced class II expression were carried out at the mRNA level. By using Northern analysis it was shown that both MHV-4 and UV-inactivated MHV-4 can block the induction of IA mRNA. Fig. 4 shows inhibition of γ-interferon-induced steady-state levels of IA mRNA by both MHV-4 and UV-inactivated MHV-4 in (BALB/c × SJL)F1 cerebral endothelial cells (lanes A–E, top panel). Table 4 shows densitometric quantitation of the autoradiograph from Fig. 4. These results suggest that virus-induced regulation of class II antigen expression has an impact at the transcriptional level.
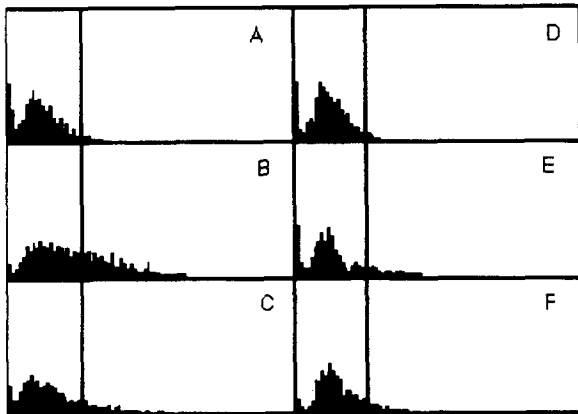
Fig. 5. Fluorescence histograms of I-A^s expression on B10.S ($A-C$) and SJL-derived ($D-F$) cerebral endothelial cells. Flow cytometry profile: x-axis fluorescence intensity; y-axis cell number. Gate windows for green fluorescence lay between channels 0 and 255. Panel A: untreated; B: γ-interferon; C: MHV-4+γ-interferon; D: untreated; E: γ-interferon; F: MHV-4+γ-interferon.

Strain-dependent blocking of γ-interferon-induced class II antigen expression by MHV-4 and UV-inactivated MHV-4

Fig. 4 and Table 4 also show that the magnitude of blocking of IA-specific mRNA was much lower in SJL (MHV-resistant, lanes F–I, top panel) than in MHV-4-susceptible (BALB/c × SJL)F1-derived endothelial cells. SJL-derived cells lack a virus-binding receptor for MHV (Boyle et al., 1987). It is therefore possible that a specific virus–receptor interaction is required for MHV-4 to influence the regulation of MHC class II expression at the transcriptional level.

TABLE 5

REGULATION OF γ-INTERFERON-INDUCED CLASS II (I-A^s) EXPRESSION ON SUSCEPTIBLE (B10.S) AND RESISTANT (SJL) STRAIN-DERIVED CEREBRAL ENDOTHELIAL CELLS BY MHV-4 AND UV-MHV-4

The data presented are obtained from the histograms in Fig. 5.

	B10.S		SJL	
	% POS [a]	MFI [b]	% POS	MFI
Untreated	0	32	0	27
γ-Interferon	26	57	14	38
MHV-4 + γ-interferon	8	41	13	39

[a] Percent positive cells.
[b] Mean fluorescence intensity.

The effect of MHV-4 on cell surface I-A^s expression on SJL-derived cerebral endothelial cells was also studied. Fig. 5 compares the effect of MHV-4 on I-A^s congeneic B10.S (MHV-4 susceptible, panels $A-C$) vs. SJL-derived endothelial cells (panels $D-F$). Table 5 shows the percent positive cells and mean fluorescence intensities obtained from the histograms in Fig. 5. Interestingly, the magnitude of blocking of γ-interferon-induced I-A^s expression by MHV-4 was much lower on the endothelial cells derived from the resistant SJL strain than on the susceptible B10.S-derived endothelial cells.

Discussion

The data presented in this report demonstrate a novel regulatory effect of the murine neurotropic coronavirus MHV-4 on MHC antigen expression by cerebral endothelial cells. MHV-4 is able to selectively block γ-interferon-induced class II but not class I antigen expression in a strain-dependent manner. The actual mechanism by which MHV-4 blocks class II antigen expression induced by γ-interferon is not yet clear. Similar findings have been described with the Kirsten murine sarcoma virus (Ki-MSV), which carries the v-Ki-ras oncogene. Ki-MSV prevents class II MHC antigen (H-2A) induction by γ-interferon on C3H10T1/2 fibroblasts (Maudsley and Morris, 1989a, b). In the Ki-MSV system it has been suggested that interferon response sequences identified in the virus genome might act in *trans* to down-regulate MHC expression. The human hepatitis B virus (HBV, double-stranded DNA virus) has also been shown to have down-regulatory effects on α-interferon activity. For instance, it has been shown to reduce the sensitivity of hepatocytes to interferon. It is believed that the HBV genome integrates into the host interferon consensus sequence, thereby altering the host cellular response to interferon (Thomas et al., 1986). Both these mechanisms are unlikely to play a role in the blocking of γ-interferon-induced class II antigen expression by MHV-4. MHV-4 is a positive polarity RNA virus that completes its replication cycle entirely within the cytoplasm (Wilhelmsen et al., 1981). Therefore, it

is difficult to envision a direct effect by this RNA virus at the DNA level to block the induction of class II-specific genes.

It is unlikely that the MHV-4 is blocking the ability of γ-interferon to bind to the γ-interferon receptor. This is because class I antigen induction by γ-interferon appears to be minimally affected by MHV-4 or UV-inactivated MHV-4. If binding of γ-interferon to its receptor was impaired a global blocking of its effects would be expected. However, formal proof of γ-interferon receptor blockade by MHV-4 requires competitive binding studies using radioiodinated (^{125}I) γ-interferon. Such studies are currently in progress in our laboratory.

The possibility that MHV-4 is blocking the binding of anti-class II antibody was also ruled out in experiments where γ-interferon-treated cerebral endothelial cells were exposed to virus for 1 h immediately before antibody labeling and flow cytometry (Table 3). No significant drop in the percentage of class II-positive cells was observed in endothelial cells pretreated with MHV-4 or UV-inactivated MHV-4.

One possible mechanism of blocking of γ-interferon-induced class II antigens could be through cytokines released by endothelial cells following virus treatment. There is an extensive literature on cytokine release by endothelial cells by a variety of stimuli (Mantovani and Dejana, 1989). MHV-4 and UV-inactivated MHV-4 could stimulate endothelial cells to release cytokines like interferon-α/β (IFN-α/β), tumor necrosis factor (TNF) and interleukin-1 (IL-1). There is evidence suggesting that these cytokines (IFN-α/β, TNF, IL-1) can block γ-interferon-induced class II expression in a variety of cell lines (Joseph et al., 1988; Leeuwenberg et al., 1988; Johnson et al., 1989). The role of virus-induced cytokines in blocking γ-interferon-induced class II expression is currently being examined in our laboratory.

Another mechanism for γ-interferon-induced class II blocking by MHV-4 could be through viral activation of endothelial second messenger systems. There is recent evidence obtained using rat astrocytes which suggests that protein kinase C activation and elevation of intracellular cyclic AMP selectively suppresses γ-interferon-induced Ia antigen expression (Sasaki et al., 1990). Fur-

ther evidence needs to be obtained on the activation of these second messenger systems by MHV-4 treatment of endothelial cells.

The strain-dependent pattern of blocking of γ-interferon-induced class II expression is particularly interesting. It suggests that a specific interaction of MHV-4 with its receptor on the cell surface may be important to obtain the blocking effect. There is evidence for the presence of MHV receptor on BALB/c-derived cells while it has not been detected on SJL-derived cells (Boyle et al., 1987). This correlates with the observation in the present report of the reduced magnitude of blocking of γ-interferon-induced class II antigens by MHV-4 in SJL-derived endothelial cells.

Blocking of γ-interferon-induced class II antigen expression in MHV-4-susceptible strains may play an important role in evading the immune-mediated events occurring at the level of the blood–brain barrier. MHC class II-dependent viral antigen presentation and subsequent generation of anti-viral response may be blocked, thus allowing the virus to evade host immunity and successfully enter the brain.

The lack of a perivascular mononuclear cell infiltrate in MHV-4 infection of mouse brain may be related to virus-mediated blocking of class II antigen expression on the endothelial cell surface (Knobler et al., 1982). The blocking of class II antigen expression may also explain the absence of late immune-mediated demyelinating disease in mice infected with MHV-4 (Knobler et al., 1985). In contrast, in the Lewis strain of rat, MHV-4 has been demonstrated to induce class II antigens on astrocytes and is associated with a late-onset immune-mediated demyelinating disease (Massa et al., 1986, 1987). The mechanism by which these divergent effects on MHC class II induction occur, following exposure to MHV-4 in the rat as compared to the mouse, is unknown at the present time and is an area of further investigation.

References

Bailey, O.T., Pappenheimer, A.M. and Cheever, F.S. (1949) A murine virus (JHM) causing extensive destruction of myelin. Part 2: Pathology. J. Exp. Med. 90, 195–212.

Ben-Nun, A., Choi, E., McIntyre, K.R., Leeman, S.A., Mc-Kean, D.J., Seidman, J.G. and Glimcher, L.H. (1985) DNA-mediated transfer of major histocompatibility class II I-A^b and I-A^{bm12} genes into B lymphoma cells: molecular and functional analysis of introduced antigens. J. Immunol. 135, 1456–1464.

Boyle, J.F., Weissmiller, D.G. and Holmes, K.V. (1987) Genetic resistance of mouse hepatitis virus correlates with absence of virus-binding activity on target tissues. J. Virol. 61, 185–189.

Collins, T., Krensky, A.M., Clayberger, C., Fiers, W., Gimbrone, M.A., Burakoff, S.J. and Pober, J.S. (1984) Human cytolytic T-lymphocyte interactions with vascular endothelium and fibroblasts: role of effector and target molecules. J. Immunol. 133, 1878–1884.

Crissman, H.A. and Steinkamp, J.A. (1982) Rapid one step staining procedures for analysis of cellular DNA and protein by single and dual laser flow cytometry. Cytometry 3, 84–90.

Dropulic, B. and Masters, C.L. (1990) Entry of neurotropic arboviruses into the central nervous system: an in vitro study using mouse brain endothelium. J. Infect. Dis. 161, 685–691.

Hirschberg, H. (1981) Presentation of viral antigens by human vascular endothelial cells in vitro. Hum. Immunol. 2, 235–241.

Johnson, R.T. (1974) Pathophysiology and epidemiology of acute viral infections of the nervous system. Adv. Neurol. 6, 27–40.

Johnson, R.T. (1982) Viral Infections of the Nervous System. Raven Press, New York, p. 49.

Johnson, W.J., Kelley, A., Connor, J.C., Dalton, B.J. and Meunier, P.C. (1989) Inhibition of interferon-γ-induced Ia antigen expression on synovial fibroblasts by IL-1. J. Immunol. 143, 1614–1618.

Joseph, J., Knobler, R.L., D'Imperio, C. and Lublin, F.D. (1988) Down-regulation of interferon-γ-induced class II expression on human glioma cells by recombinant interferon-β: effect of dosage treatment schedule. J. Neuroimmunol. 20, 39–44.

Joseph, J., Knobler, R.L., Lublin, F.D. and Hart, M.N. (1989) Differential modulation of MHC class I antigen expression on mouse brain endothelial cells by MHV-4 infection. J. Neuroimmunol. 22, 241–253.

Knobler, R.L., Haspel, M.V. and Oldstone, M.B.A. (1981) Mouse hepatitis virus type 4 (JHM strain) induced fatal central nervous system disease. I. Genetic control and the murine neuron as the susceptible site of disease. J. Exp. Med. 153, 832–843.

Knobler, R.L., Tunison, L.A., Lampert, P.W. and Oldstone, M.B.A. (1982) Selected mutants of mouse hepatitis virus type 4 (JHM) induces different CNS diseases. Pathobiology of disease induced by wild type and mutants ts8 and ts15 in BALB/c and SJL mice. Am. J. Pathol. 109, 157–168.

Knobler, R.L., Linthicum, D.S. and Cohn, M. (1985) Host genetic regulation of acute MHV-4 viral encephalomyelitis and acute experimental autoimmune encephalomyelitis in (BALB/cKe×SJL/J) recombinant inbred mice. J. Neuroimmunol. 8, 15–28.

Leeuwenberg, J.F.M., Van Damme, J., Meager, T., Jeunhomme, T.M.A.A. and Buurman, W.A. (1988). Effect of tumor necrosis factor on the interferon-γ-induced major histocompatibility complex class II antigen expression by human endothelial cells. Eur. J. Immunol. 18, 1469–1472.

Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning, a Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Mantovani, A. and Dejana, E. (1989) Cytokines as communication signals between leukocytes and endothelial cells. Immunol. Today 10, 370–375.

Massa, P.T., Dorries, R. and ter Meulen, V. (1986) Viral particles induce Ia antigen expression on astrocytes. Nature 320, 543–546.

Massa, P.T., Brinkmann, R. and ter Meulen, V. (1987) Inducibility of Ia antigen on astrocytes is rat strain dependent. J. Exp. Med. 166, 259–264.

Maudsley, D.J. and Morris, A.G. (1989a) Regulation of interferon-γ-induced host cell MHC antigen expression by Kirsten MSV and MLV. I. Effects on class I antigen expression. Immunology 67, 21–25.

Maudsley, D.J. and Morris, A.G. (1989b) Regulation of interferon-γ-induced host cell MHC expression by Kirsten MSV and MLV. II. Effects on class II antigen expression. Immunology 67, 26–31.

McCarron, R.M., Kempski, O., Spatz, M. and McFarlin, D.E (1985) Presentation of myelin basic protein by murine cerebral vascular endothelial cells. J. Immunol. 134, 3100–3103.

McCarron, R.M., Spatz, M., Kempski, O., Hogan, R.N., Muehl, L. and McFarlin, D.E. (1986) Interaction between myelin basic protein-sensitized T lymphocytes and murine cerebral vascular endothelial cells. J. Immunol. 137, 3428–3435.

Pober, J.S., Collins, T., Gimbrone, M.A., Libby, P. and Reiss, C.S. (1986) Inducible expression of class II histocompatibility complex antigens and the immunogenicity of vascular endothelium. Transplantation 41, 141–146.

Rupnick, M.A., Carey, A. and Williams, S.K. (1988) Phenotypic diversity in cultured cerebral microvascular endothelial cells. In vitro Cell Dev. Biol. 24, 435–444.

Sahagun, G., Moore, S.A., Fabry, Z., Schelper, R.L. and Hart, M.N. (1989) Purification of murine endothelial cell cultures by flow cytometry using fluorescein-labeled *Griffonia simplicifolia* agglutinin. Am. J. Pathol. 134, 1227–1232.

Sasaki, A., Levinson, S.W. and Ting, J.P.Y. (1990) Differential suppression of interferon-induced Ia antigen expression on cultured rat astroglia and microglia by second messengers. J. Neuroimmunol. 29, 213–222.

Suzumura, A., Lavi, E., Weiss, S.R. and Silberberg, D.H. (1986) Coronavirus infection induces H-2 antigen expression on oligodendrocytes and astrocytes. Science 232, 991–993.

Thomas, H.C., Pignatelli, M. and Lever, A.M.L. (1986) Homology between HBV-DNA and a sequence regulating the interferon induced antiviral system: possible mechanism of persistent infection. J. Med. Virol. 19, 63–69.

Voyta, J.C., Netland, P.A., Via, D.P. and Zetter, B.R. (1984) Specific labeling of endothelial cells using fluorescent acetylated low density lipoproteins. J. Cell Biol. 99, 81A.

Wiley, C.A., Schrier, R.D., Nelson, J.A., Lampert, P.W. and Oldstone, M.B.A. (1986) Cellular localization of human immunodeficiency virus infection within the brains of acquired immune deficiency syndrome patients. Proc. Natl. Acad. Sci. U.S.A. 83, 7089–7093.

Wilhelmsen, K.C., Leibowitz, J.L., Bond, C.W. and Robb, J.A. (1981) The replication of murine coronavirus in enucleated cells. Virology 110, 225–230.

Zurbriggen, Z. and Fujinami, R.S. (1988) Theiler's virus infection in nude mice: viral RNA in vascular endothelial cells. J. Virol. 62, 3589–3596.