REGULATION OF MHC CLASS I AND II ANTIGENS ON CEREBRAL ENDOTHELIAL CELLS AND ASTROCYTES FOLLOWING MHV-4 INFECTION

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The direct induction of major histocompatibility complex coded molecules following virus infection of cerebral endothelial cells and astrocytes may play an important role in the pathogenesis of organ specific CNS disease. Mouse hepatitis virus type 4 (MHV-4)(JHM strain), a member of the coronavirus family, causes a spectrum of disease ranging from fatal acute encephalomyelitis to demyelination in susceptible murine hosts (Bailey et al., 1949). 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. In contrast in the C57BL/6 mouse, Suzumura et al., (1986) showed that a related coronavirus, MHV-A59, induced class I but not class II antigens on mouse astrocytes and oligodendrocytes.

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 occuring in the CNS following MHV-4 infection. We assessed the expression of class I and II MHC antigens following MHV-4 infection, in brain endothelial cells derived from strains of mice that differed in their susceptibility to MHV-4 induced disease. The response of astrocytes to MHV-4 infection was also studied in order to compare them with endothelial cells.

Our studies were performed using cultured brain endothelial cell lines derived from MHV-susceptible (Balb/c, B10.S and (Balb/c x SJL) F1) and MHV-resistant (SJL) strains of mice (Stohlman and Frelinger, 1978; Knobler et al., 1981). Astrocytes were isolated and cultured from Balb/c, CXJ-8, B10.S and SJL strains of mice. Cytopathic effects and the degree of expression of class I and II MHC antigens were examined after MHV-4 infection of cerebral endothelial cells and astrocytes. Our findings demonstrate that following MHV-4 infection, there was a differential modulation of the H-2K and

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H-2D class I molecules on endothelial cells derived from Balb/c, but not SJL, B10.S and (Balb/c x SJL)F1 mice in which parallel modulation occurred. Parallel modulation of class I molecules was also seen in astrocytes derived from Balb/c, SJL, B10.S and CXJ-8 strains of mice. In contrast class II molecules did not fluctuate following MHV-4 infection of either endothelial cells or astrocytes. MHV-4 infection, however, selectively down regulated gamma interferon induced class II antigen expression in both endothelial cells and astrocytes. Gamma interferon induced class I antigen expression appears unaffected by MHV-4 infection.

MATERIALS AND METHODS

Endothelial cell culture

Cerebral endothelial cells were isolated from the brains of Balb/c, SJL, B10.S, (Balb/c x SJL)F1 strains of mice using a modification of the method described by DeBault et al., (1981). Endothelial cell lines obtained were maintained in Medium 199 with 20% fetal bovine serum and additional supplements that included (BME) Basal medium Eagle amino acids, BME vitamins, glutamine, Bacto-peptone and penicillin-streptomycin. Endothelial identity was established by studying the uptake of DiI-Ac-LDL (Biomedical Technologies, Stoughton, MA, USA) and specific binding of Bandeirea simplicifolia BSI-B_4 (Voyta et al., 1984; Schelper et al., 1985).

Astrocyte cultures Astrocyte cultures were established from Balb/c, SJL, B10.S and CXJ-8 strains of mice. Brain cortices derived from 14 day mouse embryos were mechanically dissociated through Nitex mesh bags (210 microns). Astrocytes were cultured in Hams F-10 medium containing 10% fetal bovine serum. Astrocytic identity of these cells was established by their positive reactivity to an antibody directed against glial fibrillary acidic protein.

Virus infection and interferon treatment of cultures
Endothelial cells or astrocytes grown in T25 flasks (1 x 10^6/flask) were infected for 1 hour at an MOI of 0.1 with MHV-4(JHM strain). After three washes the infected cultures were refed with either Med 199 (with added supplements) or 200U/ml recombinant rat gamma interferon (Amgen Biologicals, Thousand Oaks, CA). Control uninfected cultures treated with gamma interferon were also set up.

Monoclonal antibody labeling and flow cytometry
Four days after infection cells were removed from plates by trypsin-EDTA treatment. 5×10^5 cells were labeled with an antibody to H-$2k^d$, H-$2D^d$ (Organon Teknika-Cappel, Malvern, PA), H-$2K^s$, H-$2D^s$ (Dr. Chella David, Mayo Clinic, MN), I-A^d (Becton Dickinson, Mountain View, CA) or I-A^s (Dr. Larry Steinman, Stanford Univ.). Fluorescein conjugated F(ab)'$_2$ fragments of sheep anti-mouse or anti rat IgG were used as a secondary reagent at a 1:40 dilution. Percent positive cells were determined by analysis on the flow cytometer (EPICS C, Coulter diagnostics, Hialeah, FL) equipped with an argon laser tuned to 488nm. Only live cells were analyzed using ethidium bromide as an exclusion method for non viable cells. All values in the Result's section are presented by subtracting the reading measured on cells labeled with 2nd antibody alone.

RESULTS

MHV-4 infection of cerebral endothelial cells

The cell populations were identified as brain endothelial cells by their uptake of DiI-Ac-LDL (Voyta et al., 1984), and labeling of the lectin Bandeiraea simplicifolia BSI-B_4 (Schelper et al., 1985). Characteristic MHV-4 induced cytopathic effects (multinucleate giant cells) were observed 2-4 days after infection of BALB/c (Fig.1), SJL, (BALB/c x SJL)F1 and B10.S brain endothelial cells. Similar results were observed with MHV-4 infected astrocytes

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). The down regulation of the number of H-$2K^d$ positive cells was not likely due to shut down of host cell protein synthesis, since an increase in the number of BALB/c derived brain and fat pad endothelial cells expressing H-$2D^d$ antigens was observed at the same time (Table I). The observed differential response of class I antigens on BALB/c derived brain endothelial cells in MHV-4 infection did not reflect defective regulation of the H-$2K^d$ 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 (H-$2K^d$ and H-$2D^d$) and class II (I-A^d) antigens measured (Table I).

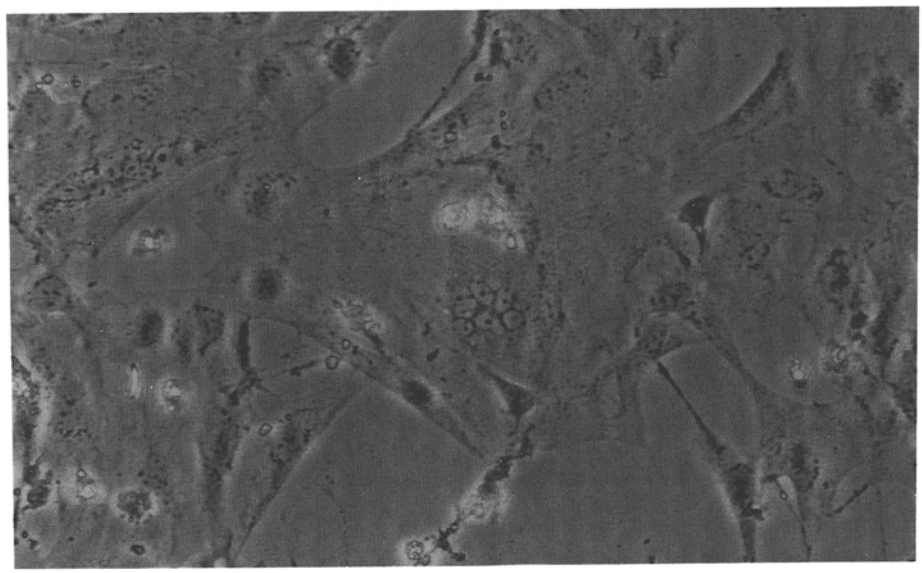

Figure 1. Photomicrograph of Balb/c cerebral endothelial cells one day after infection with MHV-4 (JHM strain) at an MOI of 0.1. Multinucleate giant cell formation is evident.

TABLE I
Differential Modulation of Class I Antigens on BALB/c Endothelial Cells Following MHV-4 Infection

	$H\text{-}2K^d$	$H\text{-}2D^d$	$I\text{-}A^d$
Cerebral Endothelial Cells			
Expt.1			
Untreated	54.26*	11.5	-0.13
MHV-4	25.72	20.77	-0.2
Expt.2			
Untreated	68.26	8.0	-0.94
MHV-4	45.75	14.3	-4.77
Expt.3			
Untreated	49.77	0.43	ND
MHV-4	17.54	11.03	ND
Expt.4			
Untreated	32.9	2.1	-2.3
Gamma IFN*	60.18	7.6	42.63
Expt.5			
Untreated	32.81	1.79	0.04
MHV-4	19.72	5.5	1.23
UV-Inact MHV-4	32.23	1.54	0.08
Expt.6 Fat pad endothelial cells			
Untreated	67.17	3.14	-1.23
MHV-4	61.91	27.47	1.01

All numbers represent percent positive cells and are averages of duplicate sample analysis on the flow cytometer.
* Cells were treated with 200U/ml of recombinant rat gamma interferon (Amgen,Thousand Oaks, CA) for 72 hrs.

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 $H\text{-}2K^s$ and $H\text{-}2D^s$ antigens following infection with MHV-4 (Table II). However this finding was not unique to the resistant phenotype, since B10.S derived brain endothelial cells (MHV susceptible, but with an $H\text{-}2^s$ MHC haplotype) also had an increase in the number of cells expressing both the $H\text{-}2K^s$ and $H\text{-}2D^s$ antigens following infection with MHV-4 (Table III).

TABLE II

Parallel Modulation of Class I Antigens on SJL Cerebral Endothelial Cells

	$H\text{-}2K^s$	$H\text{-}2D^s$	$I\text{-}A^s$
Expt.1			
Untreated	4.64	0.56	0.72
MHV-4	18.14	3.75	0.73
Gamma IFN*	79.1	11.21	12.3
Expt.2			
Untreated	10.11	3.83	0.32
MHV-4	22.95	5.29	0.05
Expt.3			
Untreated	4.00	3.03	0.32
MHV-4	22.98	4.45	1.69

All numbers represent percent positive cells and are averages of duplicate sample analysis on the flow cytometer.

* Cells were treated with 200U/ml of recombinant rat gamma IFN (Amgen, Thousand Oaks, CA) for 72 hrs.

TABLE III

Parallel Modulation of Class I Antigens on B10.S Brain Endothelial Cells

	$H\text{-}2K^s$	$H\text{-}2D^s$	$I\text{-}A^s$
Expt.1			
Untreated	8.15	0.68	-0.8
MHV-4	11.41	4.31	-0.15
Gamma IFN*	40.27	1.04	13.83
Expt.2			
Untreated	3.4	0.41	0.54
MHV-4	10.52	2.46	-0.24

All numbers represent percent positive cells and are averages of duplicate sample analyses on the flow cytometer.

Additional studies were done with the MHV-4 susceptible (BALB/c x SJL)F1 cerebral endothelial cell line. In this particular cell line the $H\text{-}2^d$ haplotype is preferentially expressed compared to the $H\text{-}2^s$ haplotype (Table IV). However the pattern of modulation of the $H\text{-}2K^d$ molecule following MHV-4 infection is different from that observed following infection of BALB/c derived brain endothelial cells with this virus. There is a parallel increase in the number of cells expressing $H\text{-}2K^d$ and $H\text{-}2D^d$ following infection with MHV-4.

MHV-4 infection of astrocytes

Unlike results obtained with endothelial cells, a parallel modulation of H-2K and H-2D antigens was observed following MHV-4 infection of astrocytes derived from susceptible (BALB/c, CXJ-8, B10.S) and resistant (SJL) strains of mice (Table V). The results obtained with BALB/c astrocytes are of particular interest since they contrasted with the differential modulation of class I antigens observed in brain and fat pad derived endothelial cells following infection with MHV-4.

TABLE IV

Parallel Modulation of Class I Antigens on (BALB/c X SJL) F1 Cerebral Endothelial Cells.

	$H\text{-}2K^d$	$H\text{-}2D^d$	$I\text{-}A^d$	$H\text{-}2K^s$	$H\text{-}2D^s$	$I\text{-}A^s$
Expt.1						
Untreated	12.8	1.2	-0.28	-0.27	0.34	-0.44
MHV-4	17.96	7.7	-0.87	0.17	5.83	-12.2
Expt.2						
Untreated	19.3	6.53	ND	1.43	1.23	ND
MHV-4	31.23	10.00	ND	0.0	0.0	ND
Expt.3						
Untreated	8.93	0.47	-0.03	0.33	0.33	0.67
Gamma IFN*	73.94	0.21	5.08	0.16	0.18	0.75

All numbers represent percent positive cells and are averages of duplicate sample analyses on the flow cytometer.

* Cells were treated with 200U/ml of recombinant rat gamma interferon (Amgen,Thousand Oaks, CA) for 72hrs.

Effect of MHV-4 infection on gamma interferon induced class I and II antigens in endothelial cells and astrocytes

When endothelial cells were simultaneously treated with MHV-4 and gamma interferon, there is a reduced level of class II antigen expression compared to cells treated with gamma interferon alone (Table VI). These results were obtained with both $I\text{-}A^s$ and $I\text{-}A^d$ expressing cells. Similar observations were made with astrocytes that were simultaneously treated with MHV-4 and gamma interferon (Table VII). In contrast, the induction of class I antigens by interferon was not blocked by MHV-4 infection of either endothelial cells or astrocytes (Table VIII).

TABLE V

Parallel Modulation of Class I Antigens on Astrocytes Following MHV-4 Infection

	$H\text{-}2K^d$	$H\text{-}2D^d$	$I\text{-}A^d$
BALB/C			
Untreated	24.82	7.07	2.65
MHV-4	54.69	8.15	0.87
CXJ-8			
Untreated	5.32	0.17	0.00
MHV-4	23.33	0.87	0.48
	$H\text{-}2K^s$	$H\text{-}2D^s$	$I\text{-}A^s$
SJL			
Untreated	10.6	-0.1	-2.44
MHV-4	33.36	6.35	-1.74
B10.S			
Untreated	34.93	3.62	1.53
MHV-4	55.92*	4.82	-3.61

* This number signifies the percent number of positive cells as analyzed by the flow cytometer.

Table VI

Effect of MHV-4 Infection on Gamma Interferon Induced Class II Expression on Cerebral Endothelial Cells

	BALB/c	B10.S	(BALB/c x SJL) F1
	$I\text{-}A^d$	$I\text{-}A^s$	$I\text{-}A^d$
Untreated	0.78	0.56	0.33
Gamma IFN	*63.36	25.73	91.66
MHV-4	1.85	1.95	-1.0
MHV-4 + Gamma IFN	12.02	8.13	70.5

* This number signifies the percent number of positive cells as analyzed by the flow cytometer.

Table VII

Effect of MHV-4 Infection on Gamma Interferon Induced Class II Antigen Expression on Astrocytes

	BALB/c	SJL	B10.S
	$I\text{-}A^d$	$I\text{-}A^s$	$I\text{-}A^s$
Untreated	0.3	5.57	0.2
Gamma IFN	61.47	36.47	36.95
MHV-4	0.68	-1.57	-0.19
MHV-4 + Gamma IFN	23.73	14.47	14.43*

* This number signifies the percentage of positive cells as analyzed by the flow cytometer.

Table VIII

Effect of MHV-4 Infection on Gamma Interferon Induced Class I Antigen Expression on Endothelial Cells and Astrocytes

	Balb/c astrocyte $H\text{-}2K^d$	B10.S astrocyte $H\text{-}2K^s$	(Balb/c x SJL)F1 endothelium $H\text{-}2K^d$
Untreated	12.89	14.3	5.27
Gamma IFN	60.73	52.66	52.3
MHV-4	34.53	28.87	16.9
MHV-4 + Gamma IFN	77.09	71.83	54.86*

* This number signifies the percent number of positive cells as analyzed by the flow cytometer.

DISCUSSION

Cerebral endothelial cells are a major structural component of the blood brain barrier (BBB). 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). We have examined the effect of the neurotropic coronavirus MHV-4 (JHM) infection on cerebral endothelial cell class I and II expression because of the potential role of these molecules in viral antigen presentation and anti-viral cytotoxic T cell activity (Zinkernagel and Doherty 1975, 1979; Paabo et al., 1986; Burgert et al., 1987; Yamaguchi et al., 1988; McCarron et al., 1985, 1986).

In the BALB/c derived brain and fat pad endothelial cells, we observe differential modulation of MHC class I molecules following infection with MHV-4. MHV-4 induced a decrease in the percentage of $H\text{-}2K^d$ positive cells and an increase of $H\text{-}2D^d$ expressing cells (Table I). This pattern of response to MHV-4 infection does not appear to be a unique characteristic of the BALB/c cerebral endothelial cell line since endothelial cells derived from another organ source (fat pad) show identical patterns of modulation. The BALB/c brain derived endothelial cells also responds in a predicted manner to treatment with gamma interferon. There is an increase in the percentage of class I ($H\text{-}2K^d$, $H\text{-}2D^d$) and class II ($I\text{-}A^d$) expressing cells as shown in Table II. This suggests that the regulation of MHC gene expression in this cell line is not defective. The differential modulation observed does not appear to be a property of the $H\text{-}2^d$ haplotype since BALB/c derived astrocytes respond with an increase in both $H\text{-}2K^d$ and $H\text{-}2D^d$ expression following infection with MHV-4.

Infection of endothelial cells derived from MHV-4 resistant (SJL) or susceptible (B10.S) strains of mice leads to an increase in percent of H-2K^s and H-2D^s expressing cells. The (BALB/c x SJL) F1 derived brain endothelial cells show patterns of modulation similar to B10.S and SJL derived cells. There is an increase in the number of cells expressing H-2K^d and H-2D^d antigen. Unlike BALB/c derived endothelial cells, no differential modulation is observed.

Several conclusions can be drawn from this data. Differential modulation appears to be strain dependent, suggesting genetic regulation. Since both B10.S and Balb/c mice are susceptible to MHV-4 replication (Stohlman and Frelinger 1978; Knobler et al., 1981), this may suggest the involvement of a separate gene from the chromosome 7 locus (Knobler et al., 1984) determining susceptibility to MHV replication. 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 strains, 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 brain derived endothelial cell class I MHC responses to MHV infection.

Our preliminary studies on MHC modulation using (BALB/c x SJL) F1 endothelial cells indicate that although the H-2^d haplotype is preferentially expressed in this cell line, the pattern of modulation is not like that seen with BALB/c derived endothelial cells. An SJL background gene(s) appears to have an effect on class I antigen modulation since we observe an increase in the expression of both H-2K^d and H-2D^d following infection of the (BALB/c X SJL)F1 cerebral endothelial cell line. We are currently involved in generating endothelial cell cultures from a panel of MHV susceptible mice in order to better understand the genetic regulation of susceptibilty to MHV-4.

An important extension of our studies will also be to examine the role of endothelial class I modulation in regulating cytotoxic T cell (CTL) activity against MHV-4. Anti-viral CTL's have been demonstrated to be class I restricted (Zinkernagel and Doherty, 1975,1979). It has been difficult until recently to demonstrate cytotoxic T cells directed against MHV-4. However, Yamaguchi et al., (1988) have recently developed cytotoxic T cell clones that are MHV-4 (JHM) specific. We will examine if MHV infected endothelial cells can act as targets in an MHV specific cytotoxic T cell response. CTL activity against virus infected endothelial cells may have damaging effects on the host. For instance, if CTL's lyse virus infected blood brain barrier endothelial cells there might be damage to the barrier permitting the virus to more readily enter the brain. Alternatively, CTL's may aid in rapid clearing of the virus preventing further spread and infection.

Differential modulation of MHC class I antigens has been

reported previously. In the murine leukemia virus-induced AKR SL3 tumor cell line, a large increase in H-2D and no change or a slight decrease in $H\text{-}2K^k$ is observed following exposure to gamma interferon (Green and Philips, 1986).

The mechanism of differential modulation of class I MHC expression as observed in the decline of $H\text{-}2K^d$ expression on BALB/c derived brain endothelial cells is presently unknown. In other viral systems like adenoviruses, the mechanism of regulation of MHC antigens is better understood. The E3/19K protein of this virus binds directly to class I molecules preventing their terminal glycosylation and inhibiting cell surface expression. This reduced level of class I antigen has been correlated with reduced cellular immune response and target cell lysis (Paabo et al., 1986; Burgert et al., 1987).

Soluble factors released by infected cells may also have a role in MHC class I modulation. Such a factor has recently been characterized from infected mouse astrocytes (Suzumura et al., 1988). This factor was found to be, nondialyzable, heat and trypsin sensitive, but resistant to treatment at pH 2.0. The authors speculate based on molecular weight determination and antibody blocking experiments, that the factor is not interferon, but most likely tumor necrosis factor. The potential role of tumor necrosis factor in class I antigen modulation of virus infected endothelial cells is currently being investigated in our laboratory.

There is no change in the low level of class II expression on brain endothelial cells following infection with MHV-4 in all strains examined, regardless of their susceptibility or resistance to virus or immune mediated demyelinating disease. This is in contrast to the results reported for Lewis rat astrocytes which demonstrated an increase in class II expression following exposure to either live or UV-inactivated virus (Massa et al., 1986). It has been suggested that the late immune mediated demyelinating disease in the rat is correlated with the observed induction of class II antigens on astrocytes (Massa et al., 1986, 1987). Our results are identical to that observed with mouse astrocytes and oligodendrocytes by Suzumura et al. (1986), where no change in class II antigens were observed following MHV-A59 infection. Therefore, the absence of late immune mediated demyelinating disease in the mouse may in fact reflect the failure of induction of MHC class II antigens on brain endothelial cells and astrocytes.

The selective blocking of gamma interferon induced class II antigen by MHV-4 infection may also contribute to the absence of late immune mediated demyelinating disease in the mouse. It would of interest to study the interaction of lymphokines and MHV-4 infection in the rat system. The mechanism of blocking of gamma interferon induced class II antigen by MHV-4 infection is unclear. The effect may be mediated indirectly by induction of cytokines like interferon alpha/beta or tumor necrosis factor, both of which have been demonstrated to downregulate interferon gamma induced class II antigens (Joseph et al., 1988; Leeuwenberg et al., 1988).

In summary, we have demonstrated differential modulation of MHC class I antigens on BALB/c derived endothelial cells in vitro. In all other strains examined [SJL, B10.S, (BALB/c X SJL) F1] we observed an increase in the percentage of H-2K and H-2D antigen expressing cells. MHV-4 infection has no effect on class II antigen expression in any of the strains examined. However, gamma interferon induced class II, but not class I antigen, is blocked by MHV-4 infection. The effects of endothelial cell MHC modulation, by MHV-4, on the host immune response to the virus is currently being investigated.

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REFERENCES

Bailey, O. T., Pappenheimer, A. M., and Cheever., F. S., 1949, J. Exp. Med., 90: 195.

Burgert, H. G., Marayanski, J. L., and Kvist, S., 1987, Proc. Natl. Acad. Sci. USA., 84:1356.

Crissman, H. A., and Steinkamp, J. A., 1982, Cytometry., 3:84.

Dasgupta, J. V., and Yunis, E. J., 1987, J. Immunol., 129: 672.

DeBault, L. E., Henriquez, E., Hart, M. N., and Cancilla, P. A., 1981, In Vitro., 17:480.

Flyer, D. C., Burakoff, S. J., and Faller, D. V., 1982, J. Immunol., 135: 2287.

Fontana, A., Fierz, W., and Wekerle, H., 1984, Nature., 307:273.

Green, W. R., and Phillips, J. D., 1986, J. Immunol., 137:814.

Grundy, J. E., McKeating, J. A., and Griffiths, P. D., 1987, In: Abstracts VII International Congress of Virology, National Research Council Canada, Ottawa, Ont., 125.

Helenius, A., Morein, B., Fries, E., Simons, K., et al., 1978, Proc. Natl. Acad. Sci. USA., 75: 3846.

Johnson, R. T., 1974, Adv. Neurol., 6:27.

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

Joseph, J., Knobler, R. L., D'Imperio, C., Lublin, F.D., 1988, J. Neuroimmunol., 20:39.

Knobler, R. L., Haspel, M. V., and Oldstone, M. B. A. 1981, J.Exp. Med ., 153:832.

Knobler, R. L., Taylor, B. A., Wooddell, M.K., Beamer, W. G., and Oldstone, M. B. A. 1984, Exp. Clin. Immunogenet ., 1:217.

Knobler, R. L., Linthicum, D. S., and Cohn, M., 1985, J. Neuroimmunol., 8:15.

Knobler, R. L., Weismiller, D. G., Williams, R. K., Cardellichio, C., and Holmes, K. V., 1987, In: Abstracts VII International Congress of Virology, National Research Council, Canada, Ottawa, Ont., 73.

Lavi, E., Suzumura, A., Murasko, D. M., Murray, E. M., Silberberg, D. H., and Weiss, S., 1988, J. Neuroimmunol., 18:245.

Leeuwenberg, J. F. M., Van Damme, J., Meager, T., Jeunhomme, T. M. A. A., and Buurman, W. A., 1988, Eur. J. Immunol., 18:1469.

Massa, P. T., Dorries, R., and ter Meulen, V, 1986, Nature., 320:543.
Massa, P. T., Brinkmann, R., and ter Meulen, V., 1987a, J.Exp.Med., 166:259.
Massa, P. T., Schimpl, A., Wecker, E., and ter Meulen, V., 1987b, Proc. Natl. Acad. Sci. USA., 84:7242.
McCarron, R.M., Kempski, O., Spatz, M., and McFarlin, D. E., 1985, J. Immunol., 134:3100.
McCarron, R. M., Spatz, M., Kempski, O., Hogan, R. N., Muehl, L., and McFarlin, D. E., 1986, J. Immunol., 137: 3428.
Paabo, S., Nilsson, T., and Peterson, P. A., 1986, Proc. Natl. Acad. Sci. USA., 83:9665.
Rodriguez, M., Pierce, M. L., and Howie, E. A., 1987, J. Immunol., 138:3438.
Schelper, R. L., Whitters, E., and Hart, M. N., 1985, Fed. Proc., 44:1261.
Stohlman, S. A., and Frelinger, J. A., 1978, Immunogenet., 6:227.
Stohlman, S. A., Knobler, R. L., and Frelinger, J. A., 1985, In: E. Skamene (Ed.), Genetic Control of Host Resistance to Infection and Malignancy, Alan R. Liss, New York, 125.
Suzumura, A., Lavi, E., Weiss, S. R., and Silberberg, D. H., 1986, Science., 232:991.
Suzumura, A., Lavi, E., Bhat, S., Murasko, D., Weiss, S. R. and Silberberg, D. H, 1988, J. Immunol., 140:2068.
Tanaka, K., Isselbacher, K. J., Khoury, G., and Jay, G., 1985, Science., 228:26.
Trauggot, U., Raine, C. S., and McFarlin, D. E., 1985, Cell. Immunol., 91:240.
Voyta, J. C., Netland, P. A., Via, D. P., and Zetter, B. R., 1984, J. Cell. Biol., 99:81A.
Wagner, R. C., and Mathews, M. A., 1975, Microvasc. Res., 10:287.
Watanabe, R., Wege, H., and ter Meulen, V., 1983, Nature., 305:150.
Wiley, C. A., Schrier, R. D., Nelson, J. A., Lampert, P. W., and Oldstone, M. B. A., 1986, Proc. Natl. Acad. Sci. USA., 83:7089.
Yamaguchi, K., Kyuwa, S., Nakanaga, K., and Hayami, M, 1988, J. Virol., 62:2505.
Zinkernagel, R. M., and Doherty, P. C., 1975, J. Exp. Med.,141:1427.