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Modulation of Cellular Macromolecular Synthesis by Coronavirus: Implication for Pathogenesis

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Infection with the murine coronavirus strain JHM decreases cell surface expression of major histocompatibility complex class I antigens. Northern blots showed that JHM virus infection rapidly reduced the level of actin mRNA, whereas the levels of major histocompatibility complex class I and tubulin mRNAs were reduced only slightly. By contrast, the mRNA levels of interleukin 1β, colony-stimulating factor 1 receptor, and tumor necrosis factor alpha increased following infection.

JHM virus (JHMV), a neurotropic strain of murine coronavirus consists of three glycoproteins designated the spike (S), membrane (M), and hemagglutinin-esterase (HE) proteins and a nucleocapsid (N) protein (16). Although wild-type JHMV induces fatal encephalomyelitis and primary demyelination in mice, a small percentage of survivors show paralytic disease with chronic demyelination (15). This system provides a valuable animal model of demyelinating diseases in the central nervous system (CNS) such as multiple sclerosis. In addition, isolation of monoclonal antibody (MAb) neutralization-resistant viral variants, which induce demyelinating disease without fatal encephalitis, have extended the usefulness of this model (5, 8, 40). A number of laboratories, including our own, have been pursuing the immunopathology of this model (15). These studies suggest that virus clearance from the CNS is mediated by $CD8^+$ cytotoxic T lymphocytes (CTL) (31, 32, 35, 41, 42). However, $CD4^+$ T cells are also required, perhaps as helper T cells for the induction of virus-specific CTL or as effector cells themselves (15, 35). The $CD8^+$ T-cell population might be involved not only in the clearance of virus but also in the development of demyelination following JHMV infection (9, 39, 42). $CD8^+$ CTL recognize viral antigen in the context of major histocompatibility complex (MHC) class I molecules. Accumulating evidence suggests that octapeptides or nonapeptides with allele-specific motifs bind in the groove of the MHC class I molecules within the endoplasmic reticulum (26, 38). These complexes are transported to the cell surface for presentation to $CD8^+$ CTL with appropriate T-cell receptors (37). Since the $CD8^+$ CTL-mediated immune response is essential in JHMV infection, the expression of MHC class I antigens during JHMV infection is an important consideration in pathogenesis.

Murine coronavirus infection inhibits cellular macromolecular synthesis in L-2 cells (13), DBT cells (36), and J774.1 cells (data not shown). By contrast, the expression of MHC class I antigen on astrocytes, oligodendroglia (17, 34), and brain endothelial cells (14) increases after murine coronavirus infection. Therefore, the effect of JHMV infection on cell surface expression of MHC class I molecules on J774.1 cells, a target cell line used for analysis of JHMV-specific CTL (31, 32, 43), was examined by radioimmunoassay. J774.1 cells grown in 96-well plates were infected with the DL variant of JHMV at a multiplicity of infection of 4 and incubated at 37°C for 10 h. Cells were washed with chilled phosphate-buffered saline (PBS) and incubated with 50 μl of MAb (1 μg/ml) per well at 4°C for 4 h. Cells were then washed five times with chilled PBS and incubated with 50 μl of ^{125}I-labeled protein A (approximately 10^5 cpm) at 4°C overnight. Unbound radioactivity was removed with five washes of chilled PBS, and the bound fraction was quantitated with a gamma counter. Table 1 shows decreased expression of MHC class I and increased expression of the JHMV S glycoprotein 10 h postinfection (p.i.). No expression of $I\text{-}A^d$ was detected. In vivo treatment with anti-N MAb protects mice from lethal coronavirus infection, perhaps by antibody-dependent cellular cytotoxicity response (18, 23, 24). Consistent with the binding of anti-N antibody to the surface of infected DBT cells (24), a determinant defined by MAb J.3.3 (7) was detected on JHMV-infected J774.1 cells, while binding of another anti-N MAb (J.3.5) was not detected (Table 1). We have recently mapped the binding domains for a panel of anti-N MAbs, including J.3.3 and J.3.5 (30). J.3.3 binds to the acidic carboxy terminus of the N protein, while J.3.5 binds to a more basic internal domain (amino acids 249 to 277) (30). The detailed mechanism of J.3.3 binding is not clear; however, the rapid cleavage of the carboxy-terminal portion of the N protein during infection (unpublished data) and the expression of this epitope on the surface of infected cells provide a possible explanation for the protection of mice from coronavirus infection mediated by anti-N MAb (18, 23, 24).

To examine the down regulation of MHC class I during the 6- to 10-h incubation used for CTL assays (31, 32, 43), the kinetics of down regulation of MHC class I was compared with the increased expression of the JHMV S glycoprotein on the cell surface. There was no detectable loss of MHC expression for the first 6 h p.i. (Fig. 1). However, expression of all three $H\text{-}2^d$ MHC class I molecules was decreased by 8 h p.i. In contrast to decreased MHC class I expression, the expression of the JHMV S protein on the cell surface of infected J774.1 cells was clearly detectable at 8 h p.i. and increased with time following JHMV infection. These data contrast with results showing up regulation of MHC class I expression on primary astrocytes, oligodendroglia, and brain endothelial cells (14, 17, 34). Differences among cell types might account for this

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TABLE 1. Cell surface expression of MHC class I, class II and viral proteins on JHMV-infected J774.1 cells

MAb[a]	Specificity	^{125}I-protein A bound (mean cpm ± SD)[b]	
		Uninfected	JHMV-infected[c]
SF1-1.1.10	K^d	27,394 ± 1,040	13,412 ± 512
34-5-8S	D^d	23,011 ± 203	10,335 ± 208
28-14-8S	L^d	20,724 ± 534	10,706 ± 355
MK-D6	A^d	295 ± 15	168 ± 32
J.2.5	JHMV S	819 ± 180	5,175 ± 821
J.3.5	JHMV N	403 ± 345	335 ± 24
J.3.3	JHMV N	677 ± 21	31,660 ± 1,151

[a] Antibodies were purified from serum-free culture supernatants by protein A column chromatography. The following hybridomas were obtained from American Type Culture Collection: SF1-1.1.10 (specific for K^d; IgG2a), 34-5-8S (specific for α2 domain of D^d; IgG2a), 28-14-8S (specific for α3 domain of L^d; IgG2a), and AF6-88.5.3 (specific for K^b; IgG2a). MAb J.2.5 (specific for JHMV S protein; IgG2a), J.3.3 (specific for JHMV N protein; IgG2a), and J.3.5 (specific for JHMV N protein; IgG2b) were produced in our laboratory (7).

[b] Each value is the mean ± standard deviation of the values from three wells.

[c] 10 h p.i.

discrepancy, suggesting that regulation of MHC class I expression may be cell type dependent. In addition, different strains of MHV have different abilities to down regulate MHC class I (4). Differential regulation of MHC class I by MHV strains and among different cell types may partially explain the absence of detectable CTL responses in some haplotypes (21).

Control of viral and cellular gene expression, either at the transcriptional or posttranscriptional levels, is an important consequence of viral infection. Most viruses modify host cell macromolecular machinery for viral replication, and shutoff is a common property of lytic infections, although the underlying mechanisms vary among viruses (27). RNA was prepared from J774.1 cells at various times p.i., and MHC class I and actin mRNA levels were compared with the stability of MHC class I mRNA following JHMV infection. J774.1 cells were infected with JHMV at a multiplicity of infection of 2 for 1 h at 37°C. At various times p.i., the cells were lysed by the addition of 2.5 ml of guanidine thiocyanate homogenization buffer and the DNA was sheared. RNA was isolated by centrifugation over 2.5 ml of 5.7 M CsCl for 18 h at 100,000 × *g*, resuspended in sterile distilled water, and quantitated by UV absorbance. RNA samples were stored in 70% ethanol at −70°C until analysis. For Northern analysis, RNA samples (30 μg) were suspended in sample buffer consisting of 50% formamide, 2.2 M formaldehyde, 1× MOPS buffer (0.02 M morpholinopropanesulfonic acid, 5 mM sodium acetate, 1 mM EDTA; pH 7.0), and 0.005% bromophenol blue and separated by electrophoresis in 1.5% agarose gels containing MOPS buffer and formaldehyde (0.66 M) at 15 V for 18 to 24 h. RNA was transferred to Hybond-N^+ membranes (Amersham) with 10× SSC (1× SSC is 150 mM NaCl and 15 mM sodium citrate) and fixed with 0.05 M NaOH for 5 min, and then the membranes were washed for 1 min with 2× SSC.

Northern blots were analyzed with the following DNA probes. A 0.4-kb cDNA encoding a conserved sequence of the murine MHC class I gene was excised from pH-2IIa (29) with

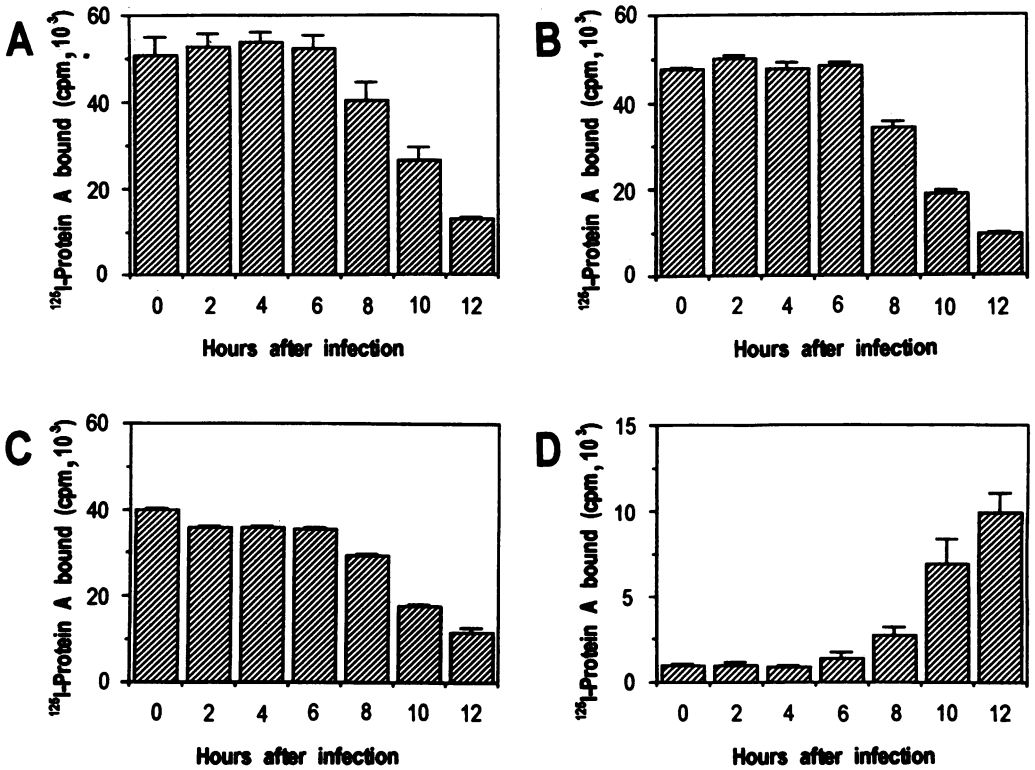

FIG. 1. Expression of MHC class I molecules and JHMV S protein on J774.1 cells during JHMV infection. J774.1 cells were infected with JHMV and incubated at 37°C for the times indicated. Cells were incubated with MAbs specific for K^d (A), D^d (B), L^d (C) and JHMV S protein (D) and further incubated with ^{125}I-labeled protein A as described in footnote *a* of Table 1. Results represent mean counts per minute of ^{125}I-labeled protein A bound ± standard deviation of three values.

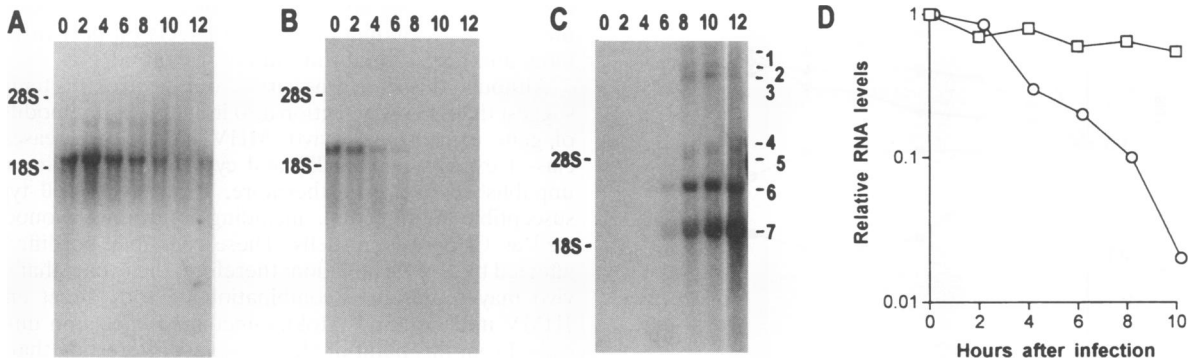

FIG. 2. MHC class I, actin and viral mRNAs in J774.1 cells following JHMV infection. J774.1 cells were infected with JHMV and incubated at 37°C, and RNA was extracted at the times (in hours) indicated. MHC class I (A), actin (B), and JHMV (C) RNA levels were analyzed by Northern blot hybridization. The positions of 28S and 18S rRNA are indicated at the left, and the numbers of JHMV-specific mRNAs are indicated at the right (C). Autoradiographs were scanned by densitometry, and relative levels of actin (○) and MHC class I (□) RNA are presented (D).

restriction enzymes *Hha*I and *Sac*I. A 0.85-kb cDNA encoding a conserved sequence of the murine MHC class II gene was excised by digestion with *Eco*RI from pABk (6). A 1.4-kb cDNA encoding the murine interleukin 1β (IL-1β) sequence was excised by digestion of pMuIL-1β with *Eco*RI, kindly supplied by P. Gray, Genentech (11). A 0.6-kb insert encoding the IL-6 sequence was excised by digestion of pIL-6, with *Hin*dIII and *Eco*RI kindly supplied by K. Uyemura, University of California at Los Angeles (44). A 1.1-kb insert encoding the tumor necrosis factor alpha (TNF-α) sequence was excised by digestion of pGEM-3-Cach, with *Eco*RI and *Pst*I, kindly supplied by L. Schook, University of Illinois (22). Colony-stimulating factor 1 (CSF-1) receptor mRNA was quantitated by using the 1.0-kb insert encoding the v-*fms* oncogene derived by digestion of pSM7C (ATCC 41016) with *Xho*I. A 1.4-kb cDNA encoding the human γ actin gene was excised from plasmid ActBlu by digestion with *Pst*I (2). A 0.5-kb insert encoding the murine tubulin gene was excised by digestion of plasmid Ma1 with *Eco*RI and *Hin*dIII kindly supplied by N. Cowan, New York University (19). A cDNA encoding the JHMV N gene was excised by digestion with *Hin*dIII and *Eco*RI from pT7-4 (2), kindly supplied by M. Lai. The cDNA fragments were isolated by electrophoresis in low-melting-point agarose (FMC, Rockland, Maine), labeled with [α-^{32}P]dCTP (300 Ci/mmol) using a random-primed DNA labeling kit (Boehringer Mannheim, Indianapolis, Ind.), followed by desalting with a Sephadex G-50 Quick Spin column (Boehringer Mannheim). Membranes were prehybridized for 15 to 45 min in Hybrisol 1 (Oncor, Gaithersburg, Md.) at 42°C. Denatured probe was added (approximately 2 × 10^6 cpm/ml) and incubated for 18 to 72 h at 42°C. Membranes were washed four times in 2× SSC containing 0.1% sodium dodecyl sulfate at room temperature and then washed twice in 2× SSC at 65°C. Membranes were air dried and exposed to XAR-5 film (Kodak).

MHC class I mRNA levels gradually decreased after JHMV infection (Fig. 2A). In contrast, actin mRNA levels rapidly decreased after infection (Fig. 2B), as previously observed following MHV-A59 infection of L2 cells (13). These changes in host cell mRNA levels occurred when viral mRNA synthesis increased (Fig. 2C). Comparison of the relative mRNA levels of actin and MHC class I following infection (Fig. 2D) clearly show that the amount of actin mRNA decreases more rapidly than the levels of MHC class I mRNA following JHMV infection.

Since degradation of cellular mRNA is involved in the shutoff phenomenon in other virus infections (27), the effect of JHMV infection on mRNA levels in the absence of transcription was examined by using actinomycin D treatment. The mRNA levels were examined by preparing RNA from JHMV-infected cells, uninfected actinomycin D-treated cells, and JHMV-infected cells treated with actinomycin D. The levels of MHC class I and actin mRNA in JHMV-infected cells were clearly reduced more rapidly than in actinomycin D-treated uninfected cells (Fig. 3). The MHC class I and actin mRNA levels in JHMV-infected actinomycin D-treated cells at 4, 6, and 8 h p.i. were similar to those in untreated JHMV-infected cells. These results indicate that JHMV infection results in degradation of these cellular mRNAs, although the effect varies among mRNA species (see Fig. 4). It is not clear whether this degradation is due to a direct effect of a viral component or a virus-induced effect mediated via interruption of a host component regulating mRNA levels. Although the effect of actinomycin D on the levels of JHMV mRNA was examined, there was no obvious changes in either the levels or kinetics (data not shown), consistent with the growth of virus in enucleated and/or actinomycin D-treated cells (16).

The decrease in MHC class I mRNA relative to actin mRNA suggested that not all mRNAs were degraded with the same kinetics following JHMV infection. We took advantage of the monocyte-macrophage nature of J774.1 cells to examine the

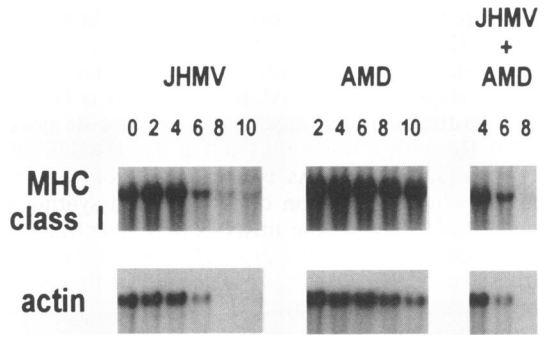

FIG. 3. Stability of MHC class I and actin RNA in uninfected and JHMV-infected J774.1 cells. J774.1 cells either untreated or treated with 2 μg of actinomycin D (AMD) per ml were infected with JHMV and incubated at 37°C for the times (in hours) indicated. MHC class I and actin mRNA levels were analyzed by Northern blot hybridization.

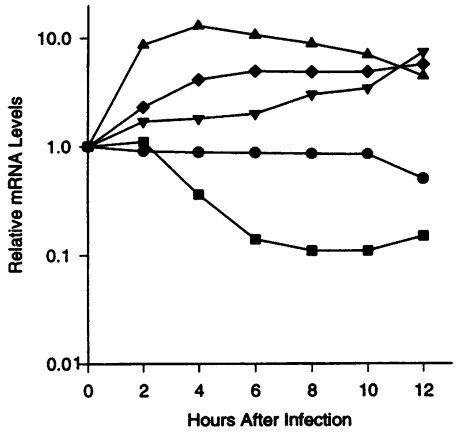

FIG. 4. Relative host cell mRNA levels in J774.1 cells following JHMV infection. J774.1 cells were infected with JHMV and incubated at 37°C for the times indicated. RNA was isolated and analyzed by Northern blot hybridization. RNA levels of tubulin (■), CSF-1 receptor (▲), TNF-α (◆), IL-1β (▼), and 28S rRNA (●) relative to those found in uninfected cells are presented.

stability of a number of other mRNAs, including factors involved in immune regulation. Figure 4 shows the level of 28S rRNA was relatively unchanged for the first 10 h p.i. and decreased by 50% at the last time point examined (12 h p.i.). Tubulin mRNA was degraded with kinetics similar to actin mRNA (Fig. 2) but to a lesser extent. No mRNAs specific for MHC class II or IL-6 were detected in either uninfected or infected J774.1 cells (data not shown). The inability to detect MHC class II mRNA is consistent with the radioimmunoassay data (Table 1). By contrast, the levels of mRNAs encoding the CSF-1 receptor, TNF-α, and IL-1β increased rapidly following JHMV infection and were sensitive to actinomycin D (data not shown). This rapid transcriptional activation appears to be a direct result of virus replication, since infection with an identical UV-inactivated pool of virus did not activate transcription (data not shown). Interestingly, the level of CSF-1 receptor mRNA increased early following infection but appeared to be susceptible to the same degradation mechanism affecting actin and tubulin mRNAs by 4 h p.i. By contrast, both TNF-α and IL-1β mRNA appeared to be resistant to JHMV-induced degradation. A striking feature is that these two cytokine mRNAs have normal half-lives of 30 min or less. This instability is due to the presence of AUUUA motifs in their 3′-untranslated regions which promote mRNA instability and turnover. A 32-kDa protein (AUBF) binds to this region and is believed to stabilize the mRNA from degradation by protection of this sequence motif. AUBF activation is believed to require a posttranslational mechanism and not de novo synthesis (20). Recently it was reported that the destabilization of AUUUA-containing mRNAs is a cotranslational event (1); thus, JHM-induced inhibition of host protein synthesis may stabilize AUUUA-containing mRNAs by preventing association with ribosomes and subsequent translation. Alternatively an increase in AUBF activity or a decline in the AUUUA-specific nuclease activity during JHMV infection could be responsible for the increased steady-state levels of these mRNAs. In addition to RNA degradation, JHMV also uses a mechanism for preferential translation of viral proteins (36), i.e., mRNAs containing JHMV leader RNA sequences are more efficiently translated than mRNA which do not contain the sequence. Taken together, coronavirus-induced shutoff of host cell synthesis appears to involve a number of different mechanisms including differential effects on host mRNA stability and preferential translation of viral mRNA.

Although this study was carried out in vitro, it is tempting to suggest that JHMV infection also induces similar modifications of gene expression in vivo. MHV infection increases MHC class I expression (10, 25) and cytokine mRNA in vivo (25; unpublished data). Furthermore, a variety of cell types are susceptible to infection, including infiltrating monocytes as well as CNS resident cells. These cells may be differentially affected by JHMV infection; therefore, the events that occur in vivo may represent a combination of both direct effects of JHMV infection and cytokine-mediated effects on uninfected cells. From this point of view, it is very interesting that JHMV infection increased TNF-α and IL-1β mRNA levels in infected cells. Both of these cytokines have been implicated in CNS disease (3) and TNF-α has been implicated in a variety of demyelinating diseases (3, 12, 28, 33). However, since coronavirus-infected cells preferentially translate viral mRNA (36), increased mRNA levels may not correlate with increased cytokine secretion; therefore, cytokine synthesis following JHMV infection must be measured to clarify their role in development of JHMV-induced demyelination.

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REFERENCES

1. **Aharon, T., and R. J. Schneider.** 1993. Selective destabilization of short-lived mRNAs with the granulocyte-macrophage colony-stimulating factor AU-rich 3′ noncoding region is mediated by a cotranslational mechanism. Mol. Cell. Biol. **13**:1971–1980.
2. **Baric, R. S., G. W. Nelson, J. O. Fleming, R. J. Deans, J. G. Keck, N. Casteel, and S. A. Stohlman.** 1988. Interactions between the coronavirus nucleocapsid protein and viral RNAs: implications for viral transcription. J. Virol. **62**:4280–4287.
3. **Benvenisti, E. N.** 1992. Inflammatory cytokines within the central nervous system: sources, function and mechanism of action. Am. J. Physiol. **263** (Cell Physiol. 32):C1–C16.
4. **Bergmann, C., M. McMillan, and S. Stohlman.** 1993. Characterization of the L^d-restricted cytotoxic T-lymphocyte epitope in the mouse hepatitis virus nucleocapsid protein. J. Virol. **67**:7041–7049.
5. **Dalziel, R. G., P. W. Lampert, P. J. Talbot, and M. J. Buchmeier.** 1986. Site-specific alteration of murine hepatitis virus type 4 peplomer glycoprotein E2 results in reduced neurovirulence. J. Virol. **59**:463–471.
6. **Estess, P., A. B. Begovich, M. Koo, P. P. Jones, and H. O. McDevitt.** 1986. Sequence analysis and structure-function correlation of murine q,k,u,s and f haplotype I-A beta cDNA clones. Proc. Natl. Acad. Sci. USA **83**:3594–3598.
7. **Fleming, J. O., S. A. Stohlman, R. C. Harmon, M. M. C. Lai, J. A. Frelinger, and L. P. Weiner.** 1983. Antigenic relationship of murine coronaviruses: analysis using monoclonal antibodies to JHM (MHV-4) virus. Virology **131**:296–307.
8. **Fleming, J. O., M. D. Trousdale, F. A. K. El-Zaatari, S. A. Stohlman, and L. P. Weiner.** 1986. Pathogenicity of antigenic variants of murine coronavirus JHM selected with monoclonal antibodies. J. Virol. **58**:869–875.
9. **Fleming, J. O., F.-I. Wang, M. D. Trousdale, D. R. Hinton, and S. A. Stohlman.** 1993. Interaction of immune and central nervous system: contribution of anti-viral Thy-1^+ cells to demyelination induced by coronavirus JHM. Reg. Immunol. **5**:37–43.
10. **Gombold, J. L., and S. R. Weiss.** 1992. Mouse hepatitis virus A59 increases steady-state levels of MHC mRNAs in primary glial cell cultures and in the murine central nervous system. Microb. Pathog. **13**:493–505.

11. **Gray, P. W., D. Glaister, E. Chen, D. V. Goeddel, and D. Pennica.** 1986. Two interleukin 1 genes in the mouse: cloning and expression of the cDNA for murine interleukin 1β. J. Immunol. **137:** 3644–3648.
12. **Hauser, S. L., T. H. Doolittle, R. Lincoln, R. H. Brown, and C. A. Dinarello.** 1990. Cytokine accumulation in CSF of multiple sclerosis patients: frequent detection of interleukin-1 and tumor necrosis factor but not interleukin-6. Neurology **40:**1735–1739.
13. **Hilton, A., L. Mizzen, G. Macintyre, S. Cheley, and R. Anderson.** 1986. Translational control in murine hepatitis virus infection. J. Gen. Virol. **67:**923–932.
14. **Joseph, J., R. L. Knobler, F. D. Lubin, and M. N. Hart.** 1989. Differential modulation of MHC class I antigen expression on mouse brain endothelial cells by MHV-4 infection. J. Neuroimmunol. **22:**241–253.
15. **Kyuwa, S., and S. A. Stohlman.** 1990. Pathogenesis of a neurotropic murine coronavirus, strain JHM in the central nervous system of mice. Semin. Virol. **1:**273–280.
16. **Lai, M. M. C.** 1990. Coronavirus: organization, replication and expression of genome. Annu. Rev. Microbiol. **44:**303–333.
17. **Lavi, E., A. Suzumura, E. M. Murray, D. H. Silberberg, and S. R. Weiss.** 1989. Induction of MHC class I antigens on glial cells is dependent on persistent mouse hepatitis virus infection. J. Neuroimmunol. **22:**107–111.
18. **Lecomte, J., V. Cainelli-Gebara, G. Mercier, S. Mansour, P. J. Talbot, G. Lussier, and D. Oth.** 1987. Protection from mouse hepatitis virus type 3-induced acute disease by an anti-nucleoprotein monoclonal antibody. Arch. Virol. **97:**123–130.
19. **Lewis, S. A., M. G. Lee, and N. J. Cowan.** 1985. Five mouse tubulin isotypes and their regulated expression during development. J. Cell Biol. **101:**852–861.
20. **Malter, J. S., and Y. Hong.** 1991. A redox switch and phosphorylation are involved in the post-translational up-regulation of the adenosine-uridine binding factor by phorbol ester and ionophore. J. Biol. Chem. **266:**3167–3171.
21. **Mobley, J., G. Evans, M. O. Dailey, and S. Perlman.** 1992. Immune response to a murine coronavirus: identification of a homing receptor-negative $CD4^+$ T cell subset that responds to viral glycoproteins. Virology **187:**443–452.
22. **Myers, M. J., J. F. Pullen, N. Ghildyal, E. Eustis-Turf, and L. B. Schook.** 1989. Regulation of IL-1 and TNF-α expression during the differentiation of bone marrow derived macrophage. J. Immunol. **142:**153–160.
23. **Nakanaga, K., K. Yamanouchi, and K. Fujiwara.** 1986. Protective effect of monoclonal antibodies on lethal mouse hepatitis virus infection in mice. J. Virol. **59:**168–171.
24. **Nakanaga, K., K. Yamanouchi, and K. Fujiwara.** 1987. Protective effect of the $F(ab')_2$ fragments of monoclonal antibodies to mouse hepatitis virus. Adv. Exp. Med. Biol. **218:**365–371.
25. **Pearce, B. D., M. V. Hobbs, and M. J. Buckmeier.** 1994. Cytokine induction during T-cell-mediated clearance of mouse hepatitis virus from neurons in vivo. J. Virol. **68:**5483–5495.
26. **Rotzschke, O., K. Falk, K. Deres, H. Schild, M. Norda, J. Metzger, G. Jung, and H.-G. Rammensee.** 1990. Isolation and analysis of naturally processed viral peptides as recognized by cytotoxic T cells. Nature (London) **348:**252–254.
27. **Schneider, R. I., and T. Shenk.** 1987. Impact of virus infection on host cell protein synthesis. Annu. Rev. Biochem. **56:**317–332.
28. **Selmaj, K., C. S. Raine, and A. H. Cross.** 1991. Anti-tumor necrosis factor therapy abrogates autoimmune demyelination. Ann. Neurol. **30:**694–700.
29. **Steinmetz, M., J. G. Frelinger, D. Fisher, T. Hunkapiller, D. Pereira, S. M. Weissman, H. Uehara, S. Nathanson, and L. Hood.** 1981. Three cDNA clones encoding mouse transplantation antigens: homology to immunoglobulin genes. Cell **24:**125–134.
30. **Stohlman, S. A., C. B. Bergmann, D. Cua, H. Wege, and R. van der Veen.** 1994. Location of antibody epitopes within the mouse hepatitis virus nucleocapsid protein. Virology **202:**146–153.
31. **Stohlman, S. A., S. Kyuwa, M. Cohen, C. Bergmann, J. M. Polo, J. Yeh, R. Anthony, and J. G. Keck.** 1992. Mouse hepatitis virus nucleocapsid protein-specific cytotoxic T lymphocytes are L^d restricted and specific for the carboxy terminus. Virology **189:**217–224.
32. **Stohlman, S. A., S. Kyuwa, J. M. Polo, D. Brady, M. M. C. Lai, and C. C. Bergmann.** 1993. Characterization of mouse hepatitis virus-specific cytotoxic T cells derived from the central nervous system of mice infected with the JHM strain. J. Virol. **67:**7050–7059.
33. **Stoll, G., S. Jung, S. Jander, P. van der Meide, and H. P. Hartung.** 1993. Tumor necrosis factor-α in immune-mediated demyelination and Wallerian degeneration. J. Neuroimmunol. **45:**175–182.
34. **Suzumura, A., E. Lavi, S. R. Weiss, and D. H. Silberberg.** 1986. Coronavirus infection induces H-2 antigen expression on oligodendrocytes and astrocytes. Science **232:**991–993.
35. **Sussman, M. A., J. O. Fleming, H. Allen, and S. A. Stohlman.** 1989. T-cell-mediated clearance of mouse hepatitis virus strain JHM from the central nervous system. J. Virol. **63:**3051–3056.
36. **Tahara, S. M., T. A. Dietlin, C. C. Bergmann, G. W. Nelson, S. Kyuwa, R. P. Anthony, and S. A. Stohlman.** 1994. Coronavirus translational regulation: leader affects mRNA efficiency. Virology **202:**621–630.
37. **Townsend, A., and H. Bodmer.** 1989. Antigen recognition by class I-restricted T lymphocytes. Annu. Rev. Immunol. **7:**601–624.
38. **Van Bleek, G. M., and S. G. Nathenson.** 1990. Isolation of an endogenously processed immunodominant viral peptide from the class I $H\text{-}2k^d$ molecule. Nature (London) **348:**213–216.
39. **Wang, F., S. A. Stohlman, and J. O. Fleming.** 1990. Demyelination induced by murine hepatitis virus JHM strain (MHV-4) is immunologically mediated. J. Neuroimmunol. **30:**31–41.
40. **Wege, H., J. Winter, and R. Meyermann.** 1988. The peplomer protein E2 of coronavirus JHM as a determinant of neurovirulence: definition of critical epitopes variant analysis. J. Gen. Virol. **69:**87–98.
41. **Williamson, J. S. P., and S. A. Stohlman.** 1990. Effective clearance of mouse hepatitis virus from the central nervous system requires both $CD4^+$ and $CD8^+$ T cells. J. Virol. **64:**4589–4592.
42. **Yamaguchi, K., N. Goto, S. Kyuwa, M. Hayami, and Y. Toyoda.** 1991. Protection of mice from a lethal coronavirus infection in the central nervous system by adoptive transfer of virus-specific T cell clones. J. Neuroimmunol. **32:**1–9.
43. **Yamaguchi, K., K. Kyuwa, K. Nakanaga, and M. Hayami.** 1988. Establishment of cytotoxic T-cell clones specific for cells infected with mouse hepatitis virus. J. Virol. **62:**2505–2507.
44. **Yamamura, M., X.-H. Wang, J. D. Ohmen, K. Uyemura, T. H. Rea, B. R. Bloom, and R. L. Modlin.** 1992. Cytokine patterns of immunologically mediated tissue damage. J. Immunol. **149:**1470–1475.