The Biological Relationship
of Mouse Hepatitis Virus (MHV) Strains and Interferon:
In vitro Induction and Sensitivities

By

L. E. GARLINGHOUSE, JR.[1, 2], A. L. SMITH[1, 2], and T. HOLFORD[2]

[1] Section of Comparative Medicine and
[2] Department of Epidemiology and Public Health, School of Medicine,
Yale University, New Haven, Connecticut, U.S.A.

With 1 Figure

Summary

Five prototype strains of mouse hepatitis virus (MHV) -1,-3, -S,- A 59 and -JHM were analyzed for their ability to induce interferon (IFN) in seven cell lines of rodent origin. Induction of IFN by all of the prototype MHV strains was infrequent and unpredictable, while IFN was produced consistently by five cell lines treated with known inducers. Priming and/or aging of cells did not enhance IFN induction by the MHV strains except in the case of MHV-A59 which consistently induced moderate levels of IFN on L-cells which were both primed and aged. Kinetic studies of MHV-A59-induced IFN on primed and aged L-cells demonstrated that detectable levels of IFN were not produced until 24 hours post-inoculation (p.i.). Peak levels were attained at 30 hours p.i. with no additional IFN produced through 48 hours p.i. MHV-induced IFN was similar in compostion and properties to Newcastle disease virus-induced IFN.

The sensitivities of the five MHV strains to eight concentrations of pre-formed L-cell IFN were also assessed. All strains except MHV-S fit a linear model with MHV-3, MHV-A59 and MHV-JHM having similar slopes. At most concentrations MHV-3 was less sensitive than MHV-1, -A59 or -JHM to IFN. The response curve for MHV-S was non-linear. This strain was more sensitive to the antiviral effects of the pre-formed IFN except at the highest concentrations of IFN used.

Introduction

The mouse hepatitis viruses (MHV) are a group of enveloped, single-stranded RNA viruses whose genome is nonsegmented and infectious (23). The prototype strains include mouse hepatitis virus (MHV)-1, MHV-2, MHV-3, MHV-S, MHV-A 59 and MHV-JHM.

Different tropisms and disease producing potentials have been ascribed to the prototype strains of MHV (18, 30). These differences are dependent not only on virus strain, but also on host genotype (11, 14), age (16, 28) and the route of inoculation (6). Resistance or susceptibility has, in general, been attributed to the ability or inability of the virus to replicate in macrophages (2, 15) for the non-neurotropic strains or in neurons for the neurotropic strains (5, 11).

Despite a significant amount of recent information on MHV replicative strategies and natural history (18, 23), little has been published on the interferon (IFN) inducing characteristics of the MHV strains or on the relative sensitivities of the prototype strains to pre-formed IFN. IFN induction is a property common to most RNA viruses (25), although the ease with which this can be demonstrated may depend on several factors, including host cell type and age, prior treatment with IFN (priming) and inducing virus strain (3). The *in vitro* characteristics of IFN induction by MHV strains were of interest to us because this was an aspect of MHV biology that had not been thoroughly investigated. The three reports on the subject indicate that MHV-JHM does not induce IFN production in cultured neuronal cells (7, 26, 27). It was also of interest to determine if there was a differential sensitivity to IFN among the prototype strains of MHV.

Materials and Methods

Viruses

Mouse hepatitis virus strains and Sendai virus were obtained from the American Type Culture Collection (ATTC, Rockville, MD), Newcastle disease virus (Hickman strain), Vesicular Stomatitis virus (Indiana strain) and Sindbis virus (EgAr 339) were obtained from the Yale Arbovirus Research Unit (New Haven, CT). Mouse encephalomyelitis virus (GDV II) was isolated by the Section of Comparative Medicine (Yale University, New Haven, CT) from the brain of a naturally infected mouse. Propagation, quantification and storage were by standard methods (22).

Cultured Cells

L-cells (clone 929), BHK-21 (clone 13) and NCTC 1469 cells were obtained from ATTC. The history and maintenance of mouse neuroblastoma cells (clone N 18) and rat glioma cells (clone C 6) have been described previously (24). Wira cells are a rat "fibroblast-like" line and were obtained from Dr. Samuel Baron, University of Texas-Medical Branch, Galvaston, Texas. Primary mouse embryo (PME) cells were cultured by the method of HSIUNG (8). All cells were propagated and maintained by standard methods (22).

Interferon (IFN) Assay and Standard

All IFN assays were performed by the cytopathic effect (CPE) reduction assay of RUBENSTEIN *et al.* (19) in 96 well cluster dishes containing confluent cell monolayers. Vesicular stomatitis virus (VSV) was the indicator of antiviral activity. Putative IFN preparations derived from mouse cell lines were assayed on mouse L-cells, those from rat cell lines on Wira cells and those from hamster cells on BHK-21 cells. All samples were assayed in triplicate and compared to our laboratory standard.

The laboratory standard (LS) used in these studies was prepared by inoculating confluent monolayers of L-cells in 75 cm^2 culture vessels with 10^9 median egg infectious doses of Newcastle disease virus (NDV). The culture medium was removed after 24 hours, acidified to pH2 with 1N HCl, held at 4° C for seven days, neutralized with 1N and 0.2N NaOH and clarified by centrifugation at 80,000 × g for 60 minutes in a Beckman SW 28 rotor. Residual infectivity was not detected in the LS which was repeatedly compared to an NAIAD, WHO international reference standard (G002-904-511). One IU/ml of our LS was equivalent to 1 IU/ml of the reference standard. The specific activity of our LS was 8 × 10^3 IU/mg of protein.

The rat IFN standard (RLS) was prepared in the same manner as the LS except that Wira cells were used for production. In the absence of a reference standard, the RLS was assigned an arbitrary activity in units which was equal to the reciprocal of the highest dilution at which antiviral activity was detected.

IFN Induction

Induction of IFN was performed in 24-well cluster dishes by inoculating 90—100 percent confluent cell monolayers with 0.3 ml of one of the viruses listed above. Multiplicities of infection (MOI) for the murine coronaviruses were approximately 0.3. Multiple MOI for NDV and Sendai virus were used ranging from 100 to 0.0001. After a 90 minute absorption period at 37° C with continuous rocking, the inocula were removed and replaced with 1.0 ml of the appropriate maintenance media. Following an additional 30—36 hour incubation period at 37° C, the cell monolayers were examined microscopically for extent and type of CPE. The tissue culture fluid was removed and treated as described for the LS. The IFN induction potential of each virus on each cell type was assessed at least in triplicate.

Replication potential of the prototype MHV strains in each of the cell lines was assessed by quantifying infectious virus in culture supernates prior to acidification. One hundred μl of serial ten-fold dilutions of culture fluid were added to confluent monolayers of NCTC 1469 cells in 96-well cluster dishes. That syncytia formation was virus-specific was confirmed for selected samples by indirect immunofluorescence staining of cell smears. Titers were calculated by the method of REED and MUENCH (17).

Induction attempts with poly I-C were performed either by treating the cell monolayers with 20 μg/ml of poly I-C (Miles Laboratories, Elkhart, ID) as described for virus induction or by treating the cells with 400 μg/ml of DEAE-dextran (Pharmacia, Piscataway, NJ) for four hours at 37° C and then adding the poly I-C as described.

Priming and Aging

Priming was accomplished by pretreating L-cell monolayers with 100 IU/ml of L-cell IFN for two hours at 37° C prior to addition of virus (1). Aged L-cell monolayers were 8 to 10 day-old cultures maintained on medium containing a reduced serum concentration (9).

IFN Characterization

The LS IFN and representative samples from the various cell line-virus interactions which resulted in antiviral activity were characterized as IFN based upon their sensi-

tivity to 1 percent trypsin at 37° C for one hour, partial sensitivity to heat (56° C for 30 minutes) and stability at pH 2.0. Further characterization included measurement of activity before and after 36 hours of dialysis (500 volumes phosphate buffered saline pH 7.2), antiviral activity against more than one virus (the GDVII strain of mouse encephalomyelitis virus), decreased activity on cells of heterologous species, and retention of activity after ultra-centrifugation (LS only).

The LS and IFN-containing test samples were treated with mouse anti-β IFN globulin to determine the proportion of β IFN. The anti-β IFN globulin was obtained from LEE BioMolecular (San Diego, CA). The globulin was reported to be 99 percent pure anti-β IFN and to have an IFN neutralizing activity of 1×10^4 IU/ml. In our studies, 1×10^3 IU/ml or less of IFN were treated with 1000 IU/ml of anti-β IFN globulin at 37° C for two hours. Rat IFN was not antigenically characterized.

Sensitivity of MCV to IFN

L-cell monolayers in 96-well cluster dishes were pre-treated at 37° C with serial 0.5 $\log_{10}$ dilutions of L-cell IFN. After 18 hours, the IFN was decanted and the cells were rinsed with Hanks' balanced salt solution (HBSS). Serial ten-fold dilutions of the MHV strain or VSV were then added to each pre-treated set of L-cells (0.1 ml per well). After a 90 minute absorption period, the virus was removed and replaced with 0.1 ml of minimal essential medium (Earle's salts) containing 2 percent fetal bovine serum. The cluster dishes were incubated at 37° C in a 5 percent CO_2 atmosphere for 24 hours, fixed and stained with a 5 percent glutaraldehyde — 1 percent crystal violet solution and evaluated macroscopically (VSV) or microscopically (MHV) for virus-induced CPE. Virus titers at each IFN dilution were calculated by the method of REED and MUENCH (17). Results are expressed as $\log_{10}$ reduction in virus titer compared to the virus titer on diluent pre-treated L-cells. Each determination was based on three experiments with three replicates per dilution. Straight line models were generated for each virus by linear regression using the least squares method. The goodness of fit for the linear model was determined by analysis of variance. The individual slopes for the viruses where a linear model was appropriate were compared using an F test for parallelism. If the slopes did not differ significantly, intercepts were compared by analysis of covariance. The calculations were performed using PROC GLM in the statistical package, SAS (20).

Results

Interferon Induction by MHV Strains

Five prototype strains of MHV were evaluated for their ability to induce IFN production in cells of mouse, rat or hamster origin (Table 1). Measurable IFN was not produced by any of the cells, despite evidence of viral replication. All of the MHV strains replicated well in L929 and N18 cells (titers of 4.0 to ≥6.0 $\log_{10}$ TCID$_{50}$ per ml). Replication was intermediate in wira and PME cells (2.2 to 4.5 $\log_{10}$ TCID$_{50}$ per ml, except for ≥6.0 $\log_{10}$ TICD$_{50}$ per ml for MHV-A59 in PME cells). Replication in C6 and BHK21 cells was poor or undetectable (<1.0 to 2.5 $\log_{10}$ TCID$_{50}$ per ml). When NDV was used as the inducing agent, measurable quantities of IFN were detected in all cell lines except N18 and BHK-21.

Table 1. *Interferon induction by MHV strains and known inducers in cultured cells*[a]

Inducer	L Cells	NCTC 1469	N 18	BHK	C 6	WIRA	PME
MHV-1	—	—	—	—	—	—	—
MHV-3	—	—	—	—	—	—	—
MHV-S	—	—	—	—	—	—	—
A 59	—	—	—	—	—	—	—
JHM	—	—	—	—	—	—	—
NDV	12,000	120	—	—	42	100	4,200
Sendai	12,000	420	—	—	100	540	ND
Sindbis	4,200	—	—	—	25	760	ND
Poly I-C	±4	—	—	—	—	300	ND
Poly I-C + Dextran	42,000	40	250	—	10	300	ND

[a] Results are given as geometric mean IFN concentration (IU per ml) based on triplicate cultures and assays performed three times; — is less than 4 IU per ml; ND is not done

Interferon Production in Response to Known Inducers

To determine if the cells used were responsive only to NDV, i.e., essentially poor producers, or whether the MHV strains are poor inducers, the cells were pre-treated with two other known viral inducers of IFN, the synthetic polynucleotide poly I—C, or DEAE-dextran plus poly I—C to induce IFN production. All of the cell lines tested except BHK-21 were capable of producing IFN in response to one or more of the inducers (Table 1). Three of six cell lines (L-cells, C 6 and Wira) produced IFN in response to at least four known inducers. Two cell lines (N 18 and BHK) were poor producers. NCTC 1469 cells were intermediate.

Sendai virus and NDV were used to induce IFN at MOI ranging from 100 to 0.0001. Maximum IFN concentrations were induced at MOI of 1 through 100 (12,000—18,000 IU/ml). An approximate 20-fold decrease in IFN concentration was observed at an MOI of 0.1 (600—1000 IU/ml), a 100-fold decrease at an MOI of 0.01 (100—500 IU/ml) and no IFN production was observed at MOI of 0.001 or 0.0001 (<4 IU/ml).

Interferon Induction by MHV Strains on Primed and/or Aged Cells

Priming or aging or both had no consistent effect on the ability of MHV-1, MHV-3, MHV-S or MHV-JHM to induce IFN production in L-cells (Table 2). In contrast, MHV-A 59 induced IFN production in six of six cultures which were both primed and aged. The levels of IFN induced by MHV-A 59 were modest (27—143 IU/ml), but induction was consistent. The other MHV strains, excluding MHV-3, induced IFN production in one or more of the 24 cultures. Induction was not associated with any particular manipulation and was unpredictable.

Table 2. *Effect of L cell priming and aging on interferon induction by MHV strains*[a]

Virus	Untreated	Primed	Aged	Primed and aged	Total positive (%)
MHV-1	—	—	14 [1]	—	1/24 (4)
MHV-3	—	—	—	—	0/24 (0)
MHV-S	—	40 [1]	9 [1]	—	2/24 (8)
A 59	—	57 [1]	73 [1]	89 (27—143) [6]	8/24 (33)
JHM	15 (7—30) [2]	—	4,200 [1]	95 [1]	4/24 (17)
NDV	5,012 (4,200—12,000) [6]	26,915 (12,000—120,000) [6]	38,905 (12,000—81,000) [6]	144,514 (42,000—270,000) [6]	24/24 (100)

[a] Results are given as geometric mean IFN concentration in IU per ml (range) [number of positive samples used to calculate mean]; — is less than 4 IU per ml; three replicate determinations per assay

NDV consistently induced IFN production regardless of monolayer age or pre-treatment status. Cells primed or aged produced substantially more IFN than those that were untreated prior to induction. Combined priming and aging further increased the amount of IFN produced in response to NDV. This effect appeared to be more than additive since there was a four-fold increase in IFN production in primed and aged cells compared to production by cells which were either primed or aged.

Characteristics of IFN and Lab Standard

The characteristics and properties of IFN induced by the MHV strains and other agents fit the basic criteria for IFN. MHV-induced IFN did not differ significantly in the proportion of β IFN compared to IFN produced by L-cells induced by NDV. NCTC cells induced by NDV appeared to produce about 10-fold more β IFN than did L-cells. Differences of 10—20 percent are difficult to evaluate using our methods due to small variations in the IFN assay. In all cases however, β IFN accounted for 70 percent or more of the IFN produced in these studies. Mouse encephalomyelitis virus seemed to be somewhat more sensitive to MHV-induced IFN than was VSV although this, too, may reflect assay variation.

Kinetics of IFN Induction by MHV-A 59

The kinetics of MHV IFN induction were studied using MHV-A59 as the inducing agent on primed and aged L-cells. Attempts at using other MHV prototypes as the inducing agents were unsuccessful due to the inconsistent nature of IFN induction by these strains of MHV. The induction scheme was that described in Methods with the modification that putative

IFN samples were removed and assayed after time intervals of 0—48 hours after virus inoculation. IFN was not produced during the first 18 hours after virus inoculation. Measurable IFN was evident at 24 hours post-inoculation (42 IU/ml$\pm$16 IU/ml). In a second study, these results were confirmed and peak IFN concentrations were achieved by 30 hours post-inoculation (120 IU/ml). Additional IFN production could not be detected between 30 and 48 hours.

Sensitivity of the MCV to Pre-formed IFN

Fig. 1 illustrates the relative sensitivities of five MHV and VSV to pre-formed L-cell IFN. All of the viruses fit a linear model with the exception of MHV-S [goodness of fit F (5, 16)=6.18, p>.05]. Tests for lack of fit for the other virus models were not significant at the 5 percent level. Tests for parallelism, an assessment of sensitivity pattern, established a lack of parallelism between MHV-1 and the remaining four viruses [F(4,109)=2.76
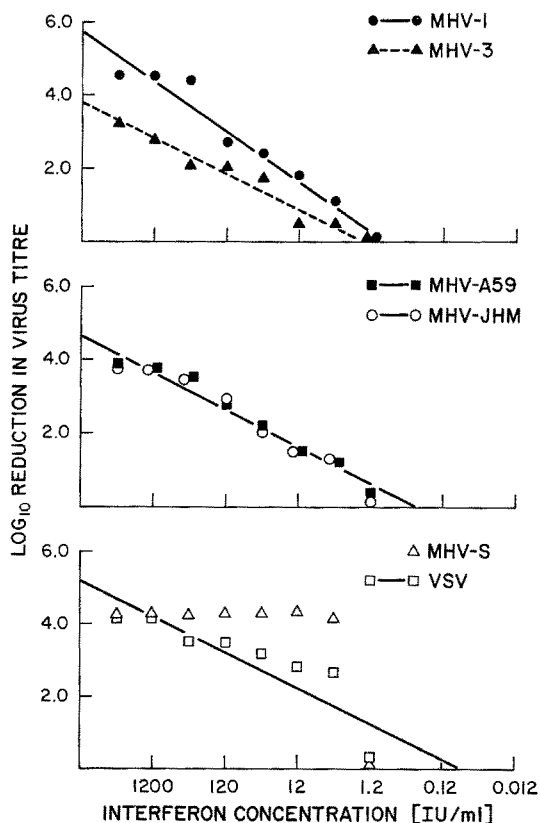

Fig. 1. Sensitivities of five prototype strains of mouse hepatitis virus and vesicular stomatitis virus to pre-formed L-cell interferon. Mean $\log_{10}$ reductions in virus titers at each interferon concentration and regression lines from least squares analysis are plotted. The response of MHV-S to pre-formed interferon was non-linear

p>.05] with MHV-1 having a significantly higher slope. MHV-3, MHV-A59, MHV-JHM and VSV all had slopes that were not significantly different from each other [F(3,89)=0.28, p>0.05]. Analysis of the differences in Y-intercepts among these four viruses (a test of quantitative differences in sensitivity) indicated significant differences overall [F(3,90)=21.38, p<0.0001]; however, there were no significant differences between MHV-A59 and MHV-JHM. MHV-3 had a Y-intercept that was significantly lower than those for each of the other viruses, while that for VSV was significantly higher, implying that MHV-3 was less sensitive and VSV more sensitive to the antiviral effects of IFN. MHV-S was more sensitive to IFN than were the other strains at most of the IFN concentrations used. MHV-JHM and MHV-A59 were equivalent in IFN sensitivity and were intermediate to MHV-S and MHV-3.

Discussion

There have been few reports regarding IFN induction by MHV strains. MHV-JHM did not induce IFN in cultured neuronal cells (27) or in the spleens of mice injected intraperitoneally or intracranially (29). However, MHV-3 given to mice intraperitoneally did induce detectable IFN in the spleen (21, 29). Because of the difficulty associated with interpreting results of studies in which different host systems or routes of inoculation were used, we have done a systematic study of the *in vitro* capacities of several MHV strains to induce IFN production in several host types. Our results strongly suggest that MHV strains are poor IFN inducers in otherwise untreated cells, regardless of the host cell used. At least one of the known chemical or viral IFN inducers used in these studies resulted in IFN production, except with BHK cells which are known to be poor IFN producers (10).

Manipulation of L cells by priming or aging had no consistent effect on IFN induction by the MHV strains, but increased the IFN yield in response to NDV by 5- and 8-fold, respectively. Combined priming and aging resulted in consistent low-level IFN induction only by MHV-A59. The same treatment increased NDV-associated IFN production by almost 30-fold over control values. There was no indication during these studies that IFN production by any of the cell lines in response to MHV strains was directly correlated with the relative abilities of the cells to support virus replication.

The kinetics of IFN induction were studied using MHV-A59-infected primed and aged L cells. IFN production began and peaked later than reported (4, 13) and found in these studies (data not shown) for NDV-induced IFN. One potential explanation for this finding is a requirement for a threshold concentration of virus, achieved by virus replication and below which IFN induction does not occur. However, since MHV-3, -S, -A59 and -JHM all achieved titers of ≥ 5.5 $\log_{10}$ TCID$_{50}$ per ml within 24 hours after infection of L cells, this explanation may be simplistic.

The sensitivities of the MHV strains to pre-formed IFN were highly variable. MHV-S was most sensitive and displayed a non-linear response pattern. In contrast, the sensitivity patterns of the other four MHV strains fit a linear model. The lines generated for MHV-A59 and MHV-JHM were indistinguishable. The line generated for MHV-3 had a slope identical to that for MHV-A59 and MHV-JHM, but higher IFN concentrations were required to inhibit replication of MHV-3. MHV-1 was more sensitive to the anti-viral effects of high concentrations of IFN than were the other MHV strains.

Mouse hepatitis virus strains are common contaminants of laboratory mice. After inoculation by a natural route, MHV infection results in a spectrum of clinical manifestations ranging from no visible disease to death. The outcome of infection is dependent on several factors which include host age and genotype and strain of infecting virus (12). In an effort to extend the findings reported here and to learn more about the basic biology of MHV, current studies are directed toward evaluating the relative role of IFN in determining the outcome of infection.

Acknowledgments

References

1. ABREU, S. L., BANCROFT, F. C., STEWART II, W. E.: Interferon Priming effects on interferon messenger RNA. J. Biol. Chem. **254**, 4114—4118 (1978).
2. BANG, F. B., WARWICK, A.: Mouse macrophages as host cells for the mouse hepatitis virus and the genetic base of their susceptibility. Proc. Nat. Acad. Sci. U.S.A. **46**, 1065—1075 (1960).
3. BARON, S., DIANZANI, F., STANTON, G. J.: The interferon system: A review to 1982—Part 1. Texas Rept. on Biol. Med. **41**, 64—98 (1982).
4. CANTELL, K., PAUKER, K.: Quantitative studies on viral interference in suspended L-cells. IV. Production and assay of interferon. Virology **21**, 11—21 (1963).
5. DUBOIS-DALCQ, M. E., DOLLER, E. W., HASPEL, M. V., HOLMES, K. V.: Cell tropism and expression of mouse hepatitis viruses (MHV) in mouse spinal cord cultures. Virology **119**, 317—331 (1982).
6. GOTO, N., HIRANO, N., AIVCHI, M., HAYASHI, T., FUJIWARA, K.: Nasoencephalopathy of mice infected intranasally with a mouse hepatitis virus, JHM strain. Jpn. J. Exp. Med. **47**, 59—64 (1977).
7. HIRANO, N., GOTO, N., MAKINO, S., FUJIWARA, K.: Persistent infection with mouse hepatitis virus, JHM strain in DBT cell culture. Adv. Exp. Med. Biol. **142**, 301—308 (1981).
8. HSIUNG, G. D., FONG, C. K. Y.: Diagnostic Virology, 245—251. New Haven: Yale Press 1982.

9. Kawade, Y., Yamamoto, Y.: Induction and production of L-cell interferon. Meth. Enzymol. **78**, 139—143 (1981).
10. Kawai, A., Matsumoto, S., Tanabe, K.: Characterization of rabies viruses recovered from persistently infected BHK cells. Virology **67**, 520—533 (1975).
11. Knobler, R. L., Haspel, M. V., Oldstone, M. B. A.: Mouse hepatitis virus type 4 (JHM strain)-induced fatal central nervous system disease. J. Exp. Med. **153**, 832—843 (1981).
12. Kraft, L. M.: Viral diseases of the digestive system. In: Foster, H. L., Small, J. D., Fox, J. G. (eds.), The Mouse in Biomedical Research, Vol. II: Diseases, 159—193. New York: Academic Press 1982.
13. Kronenberg, L. H., Friedman, T.: Relative quantitative assay on the biological activity of interferon messenger RNA. J. gen. Virol. **27**, 225—232 (1975).
14. MacNaughton, M. R., Patterson, S.: Mouse hepatitis virus strain 3 infection of C 57, A/Sn and A/J strain mice and their macrophages. Arch. Virol. **66**, 71—75 (1980).
15. Morgensen, S. C.: Role of macrophages in natural resistance to virus infections. Microbiol. Rev. **43**, 1—26 (1979).
16. Pickel, K., Muller, M. A., ter Meulen, V.: Analysis of age-dependent resistance to murine coronavirus JHM infection in mice. Infect. Immun. **34**, 648—654 (1981).
17. Reed, J., Muench, H.: A simple method of estimating fifty percent end points. Am. J. Hyg. **27**, 493—496 (1938).
18. Robb, J. A., Bond, C. W.: Coronaviridae. In: Fraenkel-Conrat, H., Wagner, R. R. (eds.), Comprehensive Virology, Vol. 14, 193—247. New York: Plenum Press 1979.
19. Rubinstein, S., Familletti, P. C., Pestka, S.: Convenient assay for interferons. J. Virol. **37**, 755—758 (1981).
20. SAS Institute Inc.: SAS User's Guide: Statistics. Cary, N.C.: SAS Institute Inc. 1982.
21. Schindler, L., Engler, H., Kirchner, H.: Activation of natural killer cells and induction of interferon after injection of mouse hepatitis virus type 3 in mice. Infect. Immun. **35**, 869—873 (1982).
22. Schmidt, N. J.: Cell culture techniques for diagnostic virology. In: Lennette, E. H., Schmidt, W. V. (eds.), Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections, 5th ed., 65—140. Washington, D.C.: American Public Health Association 1979.
23. Siddel, S., Wege, H., ter Meulen, V.: The biology of coronaviruses. J. gen. Virol. **64**, 761—776 (1983).
24. Smith, A. L., Tignor, G. H.: Host cell receptors for two strains of Sindbis virus. Arch. Virol. **66**, 11—26 (1980).
25. Stewart II, W. E., Gosser, L. B., Lockert, Jr., R. Z.: Distinguishing characteristics of the interferon responses of primary and continuous mouse cell cultures. J. gen. Virol. **13**, 35—50 (1971).
26. Stohlman, S. A., Weiner, L. P.: Stability of neurotropic mouse hepatitis virus (JHM strain) during chronic infection of neuroblastoma cells. Arch. Virol. **57**, 53—61 (1978a).
27. Stohlman, S. A., Sakaguchi, A. Y., Hiti, A.: Interferon production and activity in mouse neuroblastoma cells. Arch. Virol. **57**, 91—96 (1978b).
28. Stohlman, S. A., Frelinger, J. A., Weiner, L. P.: Resistance to fatal central nervous system disease by mouse hepatitis virus, strain JHM-II. Adherent cell-mediated protection. J. Immunol. **124**, 1733—1739 (1980).

29. STOHLMAN, S. A., BRAYTON, P. R., HARMON, R. C., STEVENSON, D., GANGES, R. G., MATSUSHIMA, G. K.: Natural killer cell activity during mouse hepatitis virus infection: Response in the absence of interferon. Intl. J. Cancer **31**, 309—314 (1983).
30. VIRELIZIER, J. L., DAYAN, A. D., ALLISON, A. C.: Neuropathological effects of persistent infection of mice by mouse hepatitis virus. Infect. Immun. **12**, 1127—1140 (1975).

Authors' address: Dr. L. E. GARLINGHOUSE JR., Section of Comparative Medicine, Yale University School of Medicine, 375 Congress Avenue, POB 3333, New Haven, CT 06510, U.S.A.