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# Selective Vulnerability of Neural Cells and Age-Related Susceptibility to OC43 Virus in Mice

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With 4 Figures

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## Summary

Suckling CD1 mice infected intracerebrally or extraneurally with OC43 virus developed a lethal neurotropic infection with high titres of virus in the brain. Examination of infected brain by routine H & E staining revealed no necrosis even in extensively infected tissue. Resistance to infection developed with increasing age, and by 20 days of age mice were completely insusceptible to i. c. inoculation. Virus replication was also demonstrable by FA staining, in spinal cord, dorsal root ganglia and retina. All other tissues were insusceptible and in particular, macrophages from both susceptible and resistant mice were found to be resistant to infection both *in vivo* and *in vitro*. Immunosuppression rendered 15 day old mice more susceptible to infection but adult mice remained insusceptible. The transfer of immune or non immune spleen cells from resistant mice did not confer resistance to newborn mice. Treatment of resistant mice with anti interferon globulin (AIG) did not render them more susceptible. These results indicate that the immune response is partially responsible for the development of resistance to OC43 infection but that it is only partially protective and other factors must also be required. The basis for the unique susceptibility of neural tissues in suckling mice is being investigated.

## Introduction

An age-related resistance to infection has been described for various viruses in mice including members of the coronavirus family (5, 7, 11). Age-related resistance to coronaviruses has been attributed to:

i) Age-related changes in the ability of macrophages either to support infection or their ability to limit virus spread (4, 7, 9).

ii) Maturation of cell mediated immunity with increasing age (4).

iii) Age-related changes in interferon production (10).

iv) A combination of these mechanisms.

Macrophages have been shown to play an important role in the resistance of older mice, peritoneal macrophages taken from 4 week old (resistant) C3H mice infected *in vitro* with mouse hepatitis virus strains produced 1/10 as much virus as macrophages from 1 week old (susceptible) mice (9). The same workers showed that peritoneal cells transferred from 4 week old mice to one week old mice rendered these mice resistant to subsequent infection. Also young SJL mice can be protected against infection with the JHM strain of MHV by transferring adherent peritoneal cells (macrophages) from older resistant mice (7) or by transferring spleen cells from immune adults (5). The transfer of resistance to MHV 3 requires T lymphocytes together with adherent cells and older mice have been rendered susceptible to infection by treatment with immunosuppresive drugs (4).

Interferon also appears to play a role in age-related resistance to MHV3. Strain A/J mice are normally resistant to MHV3 when older than 15 days but mice up to 6 weeks old can be rendered susceptible when treated with anti-interferon globulin one hour prior to inoculation of virus (9, 10). Resistant 4 week old C3H mice infected with MHV-S generate 4—8 times as much serum interferon as susceptible 1 week old mice (9).

In different experiments therefore, age-related resistance of mice to infection with various types of MHV appears to be related to macrophages, with or without T cells, and also to interferon production.

All the above reports concern mouse coronavirus. No studies on the pathogenesis or neurotropism of OC43 or other human coronaviruses, which are of conceivable importance in diseases such as multiple sclerosis, (1) have been reported. Although these viruses are assumed to cause harmless upper respiratory tract infections the ability of other coronaviruses to invade the CNS make them worthy of study.

## Materials and Methods

### *Virus*

OC43 virus was received as the 8th passage of infected suckling mouse brain suspension and was obtained from Dr. D. A. J. Tyrrell. Clinical Research Centre, Northwick Park, Middlesex. Stock virus was an infected suckling mouse brain suspension which had a titre of $10^9$ SM ic LD50/ml.

### *Animals*

All CD1 outbred and C57 Bl6 mice were specific pathogen free, obtained from the Spaceway unit, Guy's Hospital Medical School. SJL/J inbred mice were received as a gift from Jay Wallace, Chester Beatty Institute, London.

### *Mouse Embryo Brain Cultures*

Mouse embryo brain (MEB) cells were derived from 16 day old embryos of CD1 mice and were plated on to plastic tissue culture flasks (Falcon) or "ring" cultures (2) on microscope slides.

### *Peritoneal Macrophage Cultures*

Resident peritoneal cells were obtained from mice in Eagles Minimal Essential Medium (MEM) containing 10 U Heparin/ml plus 15 per cent heat inactivated foetal calf serum (FCS). They were added directly into "ring" cultures, allowed to settle for 4 hours, washed vigorously to remove non-adherent cells, and heparin-free medium replaced.

*Assay of Tissues for Infectious or Viral Antigen*

Homogenised tissues were obtained and dilutions added to ring cultures of primary MEB cells allowed to absorb for 1 hour and then refed with MEM containing 2 per cent heat inactivated FCS. Three days later the cell monolayers were fixed and stained by the fluorescent antibody (FA) technique (see below). Infected rings were then scored and the virus titre calculated as tissue culture infectious dose (TCID 50)/ml. On occasions the virus titre was determined as the suckling mouse i.c. lethal dose 50 (SMic LD 50).

*Immunofluorescence*

The indirect method was used for FA staining of both cryostat sections of tissues and cell cultures. Antiserum to OC43 virus was raised in mice (a 1 in 2560 dilution of AS was capable of neutralizing 100 sm LD 50 as detected by ic inoculation into 1 day old mice) and FITC conjugated goat anti-mouse immunoglobulin was purchased from Nordic, Maidenhead, Berks. stained slides mounted in neutral glycerol-PBS were examined with a Leitz Orthoplan microscope equipped for UV observation, using water immersion objectives.

*Spleen Cells*

Spleens from each group of mice were pooled and a single cell suspension was made by passing the spleens through a metal sieve in ice cold Hanks medium supplemented with 5 per cent FCS. The cells were washed and resuspended to give $3 \times 10^8$ cells/ml and 0.05 ml ($15 \times 10^6$ cells) was inoculated i.p. into suckling mice. 80 per cent of cells were viable as indicated by trypan blue exclusion. The spleens and cell suspensions were maintained at 4° C to minimize the loss of adherent cells.

## Results

*Infection of Mice by OC43 Virus*

Infection of suckling CD1 mice with OC43 virus by either intraperitoneal or intracerebral routes results in a lethal neurotropic infection and the mice die after 3—7 days. 1 day old mice were infected either i.p. or i.c. and taken when sick 2—4 days post infection. The brains of these mice contained $10^{10}$ SMic $LD_{50}$/G but neither viral antigen (FA) nor infectious virus could be detected in the heart, liver, spleen, lungs, kidney, intestine, muscle, thymus or adrenals. Immunofluorescent examination of brains revealed occasional foci of infection at 16 hours post infection. By 48 hours there was extensive and generalised inolvement of the cortical grey matter (Fig. 1) with striking infection of large bands of hippocampal neurones. Basal ganglia and thalamus were sparsely infected. Infection of the cerebellum, however, was restricted to Purkinje cells, white matter and granule cells did not contain viral antigen (Fig. 2). Infected cells were seen in spinal cord, dorsal root ganglia (Fig. 3) and in the retina (Fig. 4). The experiment was repeated with 5 day old mice with identical results. Examination of infected brains by H & E did not reveal cell damage even in tissue taken from extensively infected brains.

*Features of Age Dependent Susceptibility*

CD1 mice infected with OC43 virus display an age-related susceptibility becoming resistant to i.c. infection after 15 days old and resistant to i.p. infection after 10 days. The dose of virus required to cause death increases with the age of the animal (Table 1). Sub-lethal doses cause no detectable disease. Mice older than

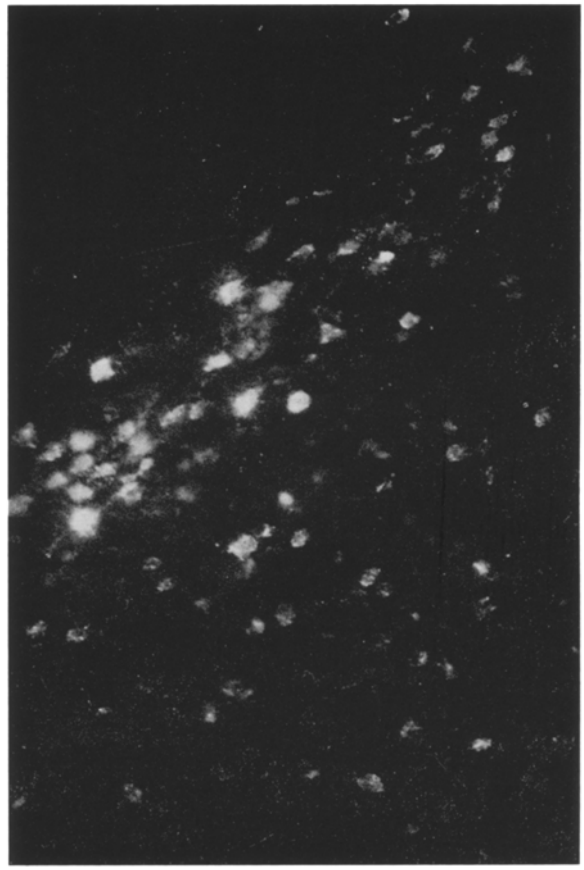

Fig. 1. Extensive infection of cerebral cortex. Photographs of fluorescent antibody stained mouse tissue 48 hours after i.c. infection with 100 $LD_{50}$ OC43. All photographs $\times 300$ magnification

20 days were not susceptible to intracerebral infection with up to $10^7$ SMic $LD_{50}$, and infected cells could not be detected in the brain on thorough FA examination. Wistar rats and $C_{57}$ Bl6, Balb C, $C_3H$ and SJL/J mice showed a very similar pattern of age-related resistance to the virus (Data not shown).

### *Mechanisms of Age-Dependent Resistance*

#### Immunosuppression

Immunosuppression of adult mice (12 weeks old) with cyclophosphamide (12) (200 mg/Kg 3 and 7 days p.i.) did not render them susceptible to very large amounts of OC43 virus inoculated i.c. (Table 2). All the treated mice remained well throughout the experiment and on FA examination at 7dpi all brains were negative for viral antigen. 40 per cent of the 15 day old mice, however, became susceptible to $10^2$ SMic $LD_{50}$ following cyclophosphamide treatment and died within 7 days. The brains of 6 of these mice were examined by FA staining and

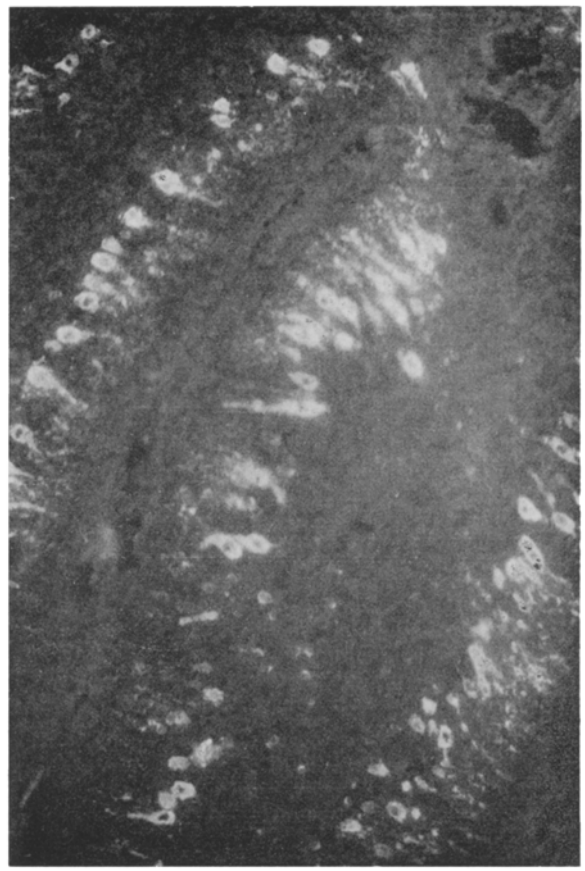

Fig. 2. Cerebellum showing infection restricted to Purkinje cells

found to contain viral antigen, the pattern of infection being the same as that for mice given a lethal dose of OC43 without immunosuppression.

## Spleen Cell Transfer

Immune and non-immune spleen cells from 12 week old CD1 mice were transferred to 3 day old and also to 9 day old suckling mice by i.p. inoculation. The mice were challenged i.p. or i.c. with a lethal dose of virus 24 hours after transfer. Neither immune nor non immune spleen cells were capable of protecting the suckling mice (Table 3) in spite of the fact that large numbers of spleen cells were transferred and when as few as 2 lethal doses of virus were given.

## Macrophages

Cultures of peritoneal macrophages from mice of different ages were established and infected *in vitro*. Peritoneal macrophages were also removed from infected animals and examined for the presence of viral antigen.

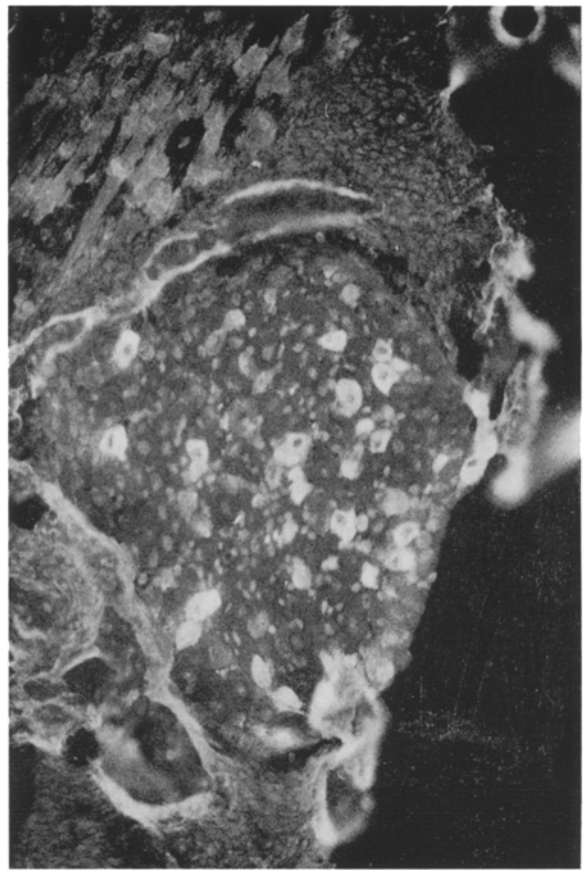

Fig. 3. Dorsal root ganglia displaying infected neurones

Table 1. *Age susceptibility of CD1 mice to OC43 virus*

| Age of animal (days) | $LD_{50}$[b] to give 100% mortality | |
|---|---|---|
| | i.c. inoculation | i.p. inoculation |
| 1 | — | $10^4$ |
| 5 | $10^1$ | $10^6$ |
| 10 | $2 \times 10^1$ | $10^7$ |
| 15 | $2 \times 10^2$ | Non lethal[a] |
| 20 | Non lethal[a] | Non lethal[a] |

[a] No deaths when $> 2 \times 10^7$ SMic $LD_{50}$ inoculated.
[b] SMic $LD_{50}$ calculated for 1 day old CD1 mice inoculated intracerebrally.

Peritoneal macrophages from 2 day old and from 12 week old CD1 mice were infected with $10^5$ $TCID_{50}$ of OC43 virus per ring culture. At various times after infection the cultures were fixed and stained by FA to demonstrate viral antigen. All macrophages were negative for viral antigen at 24, 48 and 72 hours and 7 days

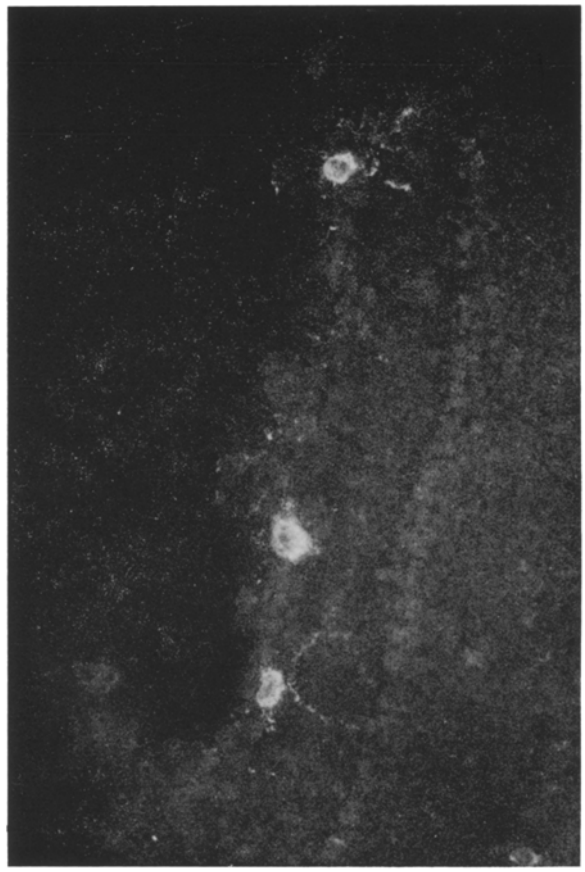

Fig. 4. Infected cells in retina

Table 2. *Effect of cyclophosphamide on the susceptibility of CD1 mice to OC43 virus*

| Age of Animal | 15 days | | 12 weeks | |
|---|---|---|---|---|
| Cyclophosphamide | + | − | + | − |
| Dose of virus[a] | $10^2$ | $10^2$ | $10^7$ | $10^7$ |
| Mortality | 13/32 | 0/11 | 0/10 | 0/10 |

[a] Dose of virus administered i.c. and calculated as SMic $LD_{50}$.

after infection *in vitro*. It would therefore appear that macrophages from both 2 day old and adult mice cannot be infected *in vitro*.

Susceptible 2 day old mice were inoculated i.p. with a lethal dose ($10^6$ SMic $LD_{50}$) of OC43 and sacrificed 24, 48 and 72 hours post infection. Peritoneal cells were removed into ring cultures and immediately fixed and stained by FA. The cells were negative for viral antigen at all times. Adult mice were infected i.p. with $10^7$ $SMLD_{50}$ and peritoneal cells removed at 24, 48 and 72 hours were also negative for OC43 antigen.

Table 3. *Effect of adult spleen cell transfer on outcome of infection of suckling mice with OC43 virus*

| Age of animal | No. | Spleen cells transferred | No. spleen cells | Virus dose (SMic $LD_{50}$) | Route | Mortality |
|---|---|---|---|---|---|---|
| 3 days | 30 | Non immune | $15 \times 10^6$ | $1 \times 10^6$ ($100 \times$ LD)[a] | i.p. | 30/30 |
| 3 days | 26 | Immune | $15 \times 10^6$ | $1 \times 10^6$ ($100 \times$ LD) | i.p. | 26/26 |
| 3 days | 7 | — | 0 | $1 \times 10^6$ ($100 \times$ LD) | i.p. | 7/7 |
| 9 days | 10 | Non immune | $15 \times 10^6$ | $2 \times 10^7$ ($2 \times$ LD) | i.p. | 10/10 |
| 9 days | 10 | Immune | $15 \times 10^6$ | $2 \times 10^7$ ($2 \times$ LD) | i.p. | 10/10 |
| 9 days | 5 | — | 0 | $2 \times 10^7$ ($2 \times$ LD) | i.p. | 5/5 |
| 9 days | 10 | Non immune | $15 \times 10^6$ | $2 \times 10^3$ ($80 \times$ LD) | i.p. | 10/10 |
| 9 days | 10 | Immune | $15 \times 10^6$ | $2 \times 10^3$ ($80 \times$ LD) | i.p. | 10/10 |
| 9 days | 5 | — | 0 | $2 \times 10^3$ ($80 \times$ LD) | i.c. | 5/5 |

[a] The figure in brackets represents the virus dose in relation to the lethal dose for that route of inoculation in that age of mice.

### Effect of Interferon

Twelve 6 week old mice were treated with 0.1 ml anti-interferon globulin (10) (AIG) (a gift from Ion Gresser-Institute de Recherches Scientifiques sur le cancer, Villejuif) intravenously followed one hour later by i.c. inoculation of $10^6$ $SMLD_{50}$ OC43. At 3 days post infection the animals were given a second dose of AIG. 15 control mice were treated with virus only. 11 of the 12 mice treated with AIG remained well and FA examination of these brains at 7 days p.i. did not reveal any viral antigen. One mouse died 3 days p.i. and extensive infection of the brain was seen by FA staining. Of the 15 mice treated with virus only, none became sick and none demonstrated viral antigen in the brain.

## Discussion

When OC43 virus infects suckling mice it invariably causes death following acute infection of the nervous system. High titres ($10^{10}$ SMic $LD_{50}$/G) of virus can be recovered from the brain and extensive infection can be demonstrated by FA staining. Spinal cord, dorsal root ganglia and retina are also infected. There is, however, no growth of virus in non neural tissues as detectable either by the presence of infectious virus or viral antigen.

CD1 mice become resistant to i.c. or i.p. infection by 20 days of age and this resistance could reflect the inability of the virus to either reach or grow in its target organ. The failure to detect infected cells in the brain of adult mice following direct intracerebral injection of virus suggests that they are resistant because OC43 virus does not grow in their brain cells. The timecourse of maturation of the immune system (6) appears to parallel the development of resistance in mice. For this reason adult (resistant) mice were immunosuppressed with cyclophosphamide (12) prior to infection with OC43, but this did not render them susceptible to infection. Treatment of 15 day old mice with cyclophosphamide, however, rendered them susceptible to a previously sub-lethal dose. This indicates that the immune system, whilst being partially protective in 15 day old mice, was not the sole factor in this resistance. Suckling mice could not be protected from infection by the transfer

of immune or non-immune spleen cells, in spite of the fact that large numbers of cells were transferred and care was taken to include adherent cells. The failure of transferred spleen cells to protect against infection could be due to their inability to localise in the site of viral growth i.e. the CNS as OC43 is exclusively neurotropic. It has been demonstrated in $MHV_3$ virus infections (3, 7) that the macrophages of (susceptible) suckling mice support viral replication whilst those of adult (resistant) mice do not, and that this accounts for the differences in susceptibility. In the above experiments the macrophages of CD1 mice of all ages were uniformly insusceptible to OC43 virus infection, both *in vitro* and *in vivo*. The age-related resistance to OC43 virus infection therefore does not depend on the inability of virus to grow in macrophages. This does not rule out a contribution of adult macrophages to control the infection by controlling virus replication in other cells (8).

Treatment of adult (resistant) mice with AIG did not cause a significant increase in mortality following infection. This indicates that interferon is not involved in the resistance of adult mice to OC43 virus, in contrast to the results reported for MHV3 infection (10). This difference in response to AIG could be due to the action of interferon on the extraneural growth of MHV3 whereas it was not capable of limiting the rapid growth of OC43 in the CNS. Resistance to i.p. inoculated virus appears earlier in life than resistance to i.c. inoculated virus (Table 1). It is possible that the rate of clearance of virus from the blood is more efficient in older mice following i.p. infection, and this prevents virus from reaching the brain. Experiments to monitor the rate of clearance from the blood did indicate that inoculated virus could be detected in the blood of suckling mice at higher concentrations for slightly longer periods of time (data not shown). This could contribute to the development of resistance to i.p. infection.

It is concluded that age-related resistance of mice to OC43 virus is likely to depend on changes in the ability of neural cells to support virus growth, possibly because of the absence of virus receptors. Experiments with cultured neural cells are being carried out to substantiate this.

## Acknowledgments

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## References

1. Burks, J. S., de Vald, B. L., Jankovsky, L. D., Gerdes, J. C.: Two coronaviruses isolated from central nervous system tissue of two multiple sclerosis patients. Science **209**, 933—934 (1980).
2. Cairns, J.: The initiation of vaccinia virus infection. Virology **11**, 603—623 (1960).
3. Gailly, R., Warwick, A., Bang, F. B.: Ontogeny of macrophage resistance to mouse hepatitis *in vivo* and *in vitro*. J. Exp. Med. **125**, 537—547 (1976).
4. Levy Leblond, E., Dupuy, J. M.: Neonatal susceptibility to $MHV_3$ infection in mice. I. Transfer of resistance. J. Immunol. **118**, 1219—1222 (1977).
5. Pickel, K., Müller, M. A., ter Meulen, V.: Analysis of age-dependent resistance to murine coronavirus JHM infection in mice. Infect. Immun. **34**, 648—654 (1981).
6. Spear, P. G., Edelman, G. M.: Maturation of the humoral immune response in mice. J. Exp. Med. **139**, 249—263 (1974).

7. Stohlman, S. A., Frelinger, J. A., Weiner, L. P.: Resistance to fatal central nervous system disease by mouse hepatitis virus strain JHM. J. Immunol. **124**, 1733—1739 (1980).
8. Stohlman, S. A., Woodward, J. G., Frelinger, J. A.: Macrophage antiviral activity: Extrinsic versus instrinsic activity. Infect. Immun. **36**, 672—677 (1982).
9. Taguchi, F., Yamada, A., Fujiwara, K.: Factors involved in the age-dependent resistance of mice infected with low virulence mouse hepatitis virus. Arch. Virol. **62**, 333—340 (1979).
10. Virelizier, J.-L., Gresser, I.: Role of interferon in the pathogenesis of viral diseases of mice as demonstrated by the use of anti-interferon serum. V. Protective role in mouse hepatitis virus type 3 infection of susceptible and resistant strains of mice. J. Immunol. **120**, 1616—1619 (1978).
11. Wege, H., Siddell, S., ter Meulen, V.: The biology and pathogenesis of coronaviruses. Curr. Top, Microbiol. Immunol. **99**, 165—200 (1982).
12. Willenbourg, D. O., Shah, K. V., Bang, F. B.: Effect of cyclophosphamide on the genetic resistance of $C_3H$ mice to mouse hepatitis virus. Proc. Soc. Exp. Biol. & Med. **142**, 762—766 (1973).

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