Title
Murine hepatitis virus-4 (strain JHM)-induced neurologic disease is modulated in vivo by monoclonal antibody.

Permalink
https://escholarship.org/uc/item/6km6t7pt

Journal
Virology, 132(2)

ISSN
0042-6822

Authors
Buchmeier, MJ
Lewicki, HA
Talbot, PJ
et al.

Publication Date
1984

DOI
10.1016/0042-6822(84)90033-3

Peer reviewed
Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Murine Hepatitis Virus-4 (Strain JHM)-Induced Neurologic Disease Is Modulated in Vivo by Monoclonal Antibody

MICHAEL J. BUCHMEIER,1 HANNA A. LEWICKI, PIERRE J. TALBOT, AND ROBERT L. KNOBLER

Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, California 92037

Received May 20, 1983; accepted October 22, 1983

Monoclonal hybridoma antibodies directed against the polypeptides of murine hepatitis virus-4 (JHM strain) were tested for their ability to alter the course of a normally lethal intracerebral virus challenge. Three monoclonal antibodies directed against two distinct epitopes on the E2 glycoprotein of MHV-4 protected mice against lethal virus challenge and converted the infection from fatal encephalomyelitis to demyelination. A single neutralizing antibody directed against a third epitope on E2 as well as seven nonneutralizing antibodies to E2, E1, and N polypeptides did not protect against challenge. In mice which received protective antibody, MHV-4 infection was not blocked, however, virus grew to lower titers in liver and brain, and virus replication in the CNS was more restricted than in unprotected mice. Decrease involvement of neurons in the brains of protected mice was observed, and no evidence of neuronal infection in the spinal cords was found. In contrast, oligodendrocytes were infected in the presence of protective antibody, and evidence of demyelination associated with mononuclear cell infiltration was found. These studies demonstrate that antibody to a single epitope on a viral glycoprotein can substantially alter the course and phenotype of disease.

INTRODUCTION

Murine hepatitis virus-4 (MHV-4) strain JHM is a neurotropic member of the coronaviridae. Infection by MHV-4 in the mouse is associated with encephalitis and demyelination (Bailey et al., 1949; Waksman and Adams, 1962; Weiner, 1973). Fatal encephalitis associated with significant loss of neurons is the normal outcome of intracerebral inoculation, with resistance of mice to fatal disease apparently being controlled by a single autosomal recessive gene (Knobler et al., 1981a) expressed at the level of the neuron and macrophage. Recovery from the acute encephalitis is rare, and the infrequent survivors exhibit demyelination (Lampert et al., 1973; Weiner, 1973). Haapel and co-workers (1978) demonstrated that a temperature sensitive mutant of MHV-4, designated ts 8, produced demyelination in over 90% of infected mice while producing encephalitis in less than 5%. The ts 8 mutant infects oligodendrocytes leading to cell death, degeneration, and removal of their myelin sheaths by macrophages (Knobler et al., 1982).

Mouse hepatitis viruses contain at least three major classes of structural proteins including the 50,000-Da nucleocapsid protein (N), and two glycoproteins E1 and E2 (Wege et al., 1979; Sturman et al., 1980; Holmes et al., 1981). E1 is integrally associated with the membrane in both a non-glycosylated 23,000-Da form and an O-glycosylated 25,000-Da form. E2 consists of two approximately 95,000-Da subunits which form a 180,000-Da dimer constituting the large "petal" (peplomer) on the virion envelope (Sturman et al., 1980). In our laboratory we have raised a library of hybridoma antibodies to these viral proteins to study the biology and biochemistry of...
MHV and to probe the in vivo events in disease. With these antibodies we were able, in our initial studies, to assign the viral components responsible for attachment and cell-cell fusion to the E2 glycoprotein (Collins et al., 1982), and to map the epitopic regions of these molecules (Talbot et al., 1984). In this report we have studied the ability of antibodies to specific viral proteins to alter the course of disease in vivo, and have mapped the property of passive protection against lethal encephalitis to specific epitopes on the E2 glycoprotein.

**MATERIALS AND METHODS**

**Virus and cell culture.** MHV-4 (JHM strain) was originally obtained from Dr. Leslie Weiner and is routinely propagated on L-24 cells as previously described (Haspel et al., 1978; Collins et al., 1982). The ts 8 mutant MHV-4, which produces a high frequency of demyelinating disease without encephalitis, was isolated by Haspel et al., (1978). Virus was enumerated by plaque assay on L-24 cells.

**Hybridoma cells.** Hybridoma cells producing monoclonal antibodies to MHV-4 were generated and characterized in this laboratory as previously described (Collins et al., 1982). All lines were cloned by limiting dilution in 96-well plates at a ratio of 0.1 cell per well. Specificity for viral polypeptides and epitopes was established as described elsewhere (Collins et al., 1982; Talbot et al., 1984).

Ascites fluids were produced in BALB/c St mice. Briefly, mice were pretreated by ip injection with 1 ml of Pristane (2, 6, 10, 14-tetramethylpentadecane, Aldrich Chemical Co., Milwaukee, Wisc.). Seven days later, 5 \( \times 10^6 \) to 1 \( \times 10^7 \) hybridoma cells were injected intraperitoneally. Ascites developed 10–14 days later, and were harvested daily from unanesthetized mice by puncture with a 20-gauge needle. Ascites fluids were clarified by centrifugation at 2500 g for 20 min, aliquoted, and stored at \(-20^\circ\). Immunoglobulin concentrations in ascites fluids were estimated by radial immunodiffusion.

**Virus neutralization.** Virus neutralizing capacity of ascites fluids containing monoclonal antibody was quantitated by a plaque reduction neutralization assay. Ascites fluids were diluted in MEM as indicated and mixed with an equal volume (0.5 ml) of virus dilution containing 120 to 200 PFU of MHV-4. The virus-antibody mixtures were incubated at 37° for 30 min, divided in half, and plated in duplicate on monolayers of L-24 cells in 60-mm culture dishes. After adsorption for 1 hr, overlay medium was added and the plates were further incubated 72 hr in a 37° CO2 incubator. Cells were fixed by the addition of 2 ml of 25% formalin in PBS for 4–18 hr. Agar overlays were removed and monolayers stained with 0.1% crystal violet. Neutralization was expressed as the reciprocal of the antibody dilution giving 50% reduction in plaque number compared with virus incubated in parallel with culture medium not containing antibody. Ascites fluids directed against nonneutralizing antigens of MHV-4 (nucleocapsid protein) or against other unrelated viruses often exhibited low levels of an MHV-4 neutralizing activity (<300 PRD/50/ml); thus a control sample consisting of nucleocapsid antibody was included in all assays.

**Passive antibody protection and assessment of demyelination.** BALB/c St mice aged 4–6 weeks are exquisitely sensitive to intracerebral (ic) infection with MHV-4 (Knobler et al., 1981a), and develop a rapidly fatal encephalitis due to infection of neurons. We have used such ic infected mice as a test system to assess the protective effect of passively administered monoclonal antibodies on MHV-4 infection. Two protocols of antibody administration were employed. In initial experiments, we gave mice daily doses of 25 \( \mu l \) of undiluted ascites on Days \(-2, -1, 1, 2, 3, \) and 4 relative to virus challenge on Day 0. In order to minimize handling of mice after infection, we subsequently adopted a protocol of a single dose of 200 \( \mu l \) of ascites on Day \(-1 \) and found that this regimen was as efficient as the first at conferring protection. In all cases, virus challenge consisted of 10–50 PFU (approximately 50–150 LD\(_{50}\)) of wild-
type MHV-4 intracerebrally. This dose reproducibly yielded 100% mortality by 6 days after infection.

To assess demyelination in passively protected mice, we allowed survivors to live for 14–21 days after virus challenge, then sacrificed and perfused them via the left ventricle with glutaraldehyde–paraformaldehyde fixative in PBS as previously described (Knobler et al. (1981b). Following perfusion, spinal cords and brains were dissected out and 1-μm Epon sections were prepared and stained for myelin with p-phenylenediamine. Surveys for demyelinating foci routinely included examination of two sections each from cervical and lumbar spinal cord and one section of thoracic cord. An animal was judged demyelinated if foci of axonal demyelination were observed in the white matter of any of these five sections. Immunoperoxidase staining for viral antigen was performed on 30-μm vibratome sections as previously described (Knobler et al., 1981b) using protein A peroxidase and monospecific rabbit antibody to MHV-4 (kindly supplied by K. V. Holmes, USPHS, Bethesda, Md.). For antigen studies, glutaraldehyde was omitted from the perfusion fixative.

For virus titration, 10% homogenates of specified tissues were prepared from mice infected and treated as indicated and assayed on monolayers of L-24 cells. Virus titers were expressed as PFU per gram of tissue.

**RESULTS**

Neutralization by monoclonal antibodies. Previous work from our laboratory established that MAb to the MHV-4 glycoprotein E2 neutralized virus in vitro (Collins et al., 1982). We have extended these findings in surveying a larger panel of antibodies to the E1 and E2 glycoproteins using a more sensitive plaque reduction assay in which we determined PRD₅₀ titers for the monoclonal ascites preparations used in the present studies. Titers observed ranged from approximately 8000 PRD₅₀/ml to greater than 158,000 PRD₅₀/ml for various ascites preparations as summarized in Table 1. Note that a low background titer generally less than 300 PRD₅₀/ml was observed in preparations of nucleocapsid specific antibody as well as in unrelated monoclonal ascites and in some normal mouse sera (data not shown). Whether this

**TABLE 1**

| Antibody | Ig subclass | Polypeptide specificity | Neutralization (PRD₅₀/ml) | Passive protection* (survivors/total) |
|----------|-------------|-------------------------|---------------------------|---------------------------------------|
| 5B19.2   | IgG1        | E2                      | 31600                     | 34/45 (76%)                           |
| 5B170.3  | IgG1        |                         | 7950                      | 5/6 (83%)                             |
| 5A13.5   | IgG2A       |                         | 158,500                   | 6/6 (100%)                            |
| 4B11.6   | IgG2A       |                         | 31,600                    | 0/20                                  |
| 5B93.9   | IgA         |                         | 500                       | 0/6                                   |
| 5B21.5   | IgG1        |                         | 250                       | 0/4                                   |
| 5B207.7  | IgG2B       |                         | <100                      | 0/5                                   |
| 5R216.8  | IgG2A       |                         | <100                      | 0/5                                   |
| 5A5.2    | IgG3        | E1                      | 316                       | 3/28 (11%)                            |
| 5B119.4  | IgG2A       |                         | 400                       | 0/4                                   |
| 5B11.5   | IgG2A       |                         | <100                      | ND*                                  |
| 4B6.2    | IgG1        | N                       | 300                       | 1/27 (4%)                             |
| 5B175.6  | IgG2A       |                         | <100                      | ND                                   |
| None     | —           | —                       | —                         | 0/33                                  |

* Passive protection against lethal intracerebral challenge with MHV-4.

*ND, not data available.
naturally occurring activity represents specific antibody or a cross-reacting natural antibody is under investigation.

Passively transferred MAb blocks lethal encephalitis induced by MHV-4. Intracerebrally inoculated BALB/c mice are exquisitely sensitive to MHV-4. Infection of neurons of the CNS results in rapidly lethal encephalitis usually within 6 days. We tested two passive transfer regimens as described under Materials and Methods to attempt to alter the course of this normally lethal encephalitis. Both regimens were effective, and the simpler single antibody dose protocol was adopted. Figure 1 shows the results of one such protection experiment in which we tested five different MAb to the MHV-4 E2 glycoprotein (5B19.2, 5A13.5, 5B170.3, 4B11.6, and 5B93.9) and as a control, a single antibody to the N protein (4B6.2). Groups of six mice were given 200 µl of the indicated ascites on Day -1 then challenged intracerebrally with 20 PFU of MHV-4 on Day 0. Survivors were observed daily for 21 days then sacrificed in order to score demyelinating lesions in the spinal cord. Note that mice receiving nonprotective antibody (clones 4B11.6, 5B93.9, and 4B6.2) as well as mice not receiving MAb (data not shown) all died by Day 6 after infection. We have screened a panel of eight anti-E2, three anti-E1, and one anti-N MAb and the results of these passive protection analyses are summarized with the neutralization data in Table 1. Note that MAb 4B11.6 neutralized virus efficiently in vitro but showed no passive protection of mice against lethal challenge in vivo. None of four nonneutralizing MAb to E2 gave significant protection in vivo.

Timing and dose dependence of passive protection. The effect of antibody given passively prior to infection might be argued to be simply an in vivo neutralization whereby incoming virus in the challenge inoculum is prevented from infecting targets in the CNS. To test this possibility, we altered the time of antibody transfer relative to challenge. Groups of six mice each were given a single 200-µl dose of MAb 5B19.2 on Days -1, 0, +1, and +2 relative to virus challenge on Day 0. Mortality was again scored over a 21-day interval. As evident in Fig. 2, groups of six mice each receiving no antibody or MAb 4B6.2 on Day -1 all died by 6 days after infection as previously observed. In contrast, mice receiving antibody on Days -1, 0, and +1 showed 100, 100, and 83% survival, respectively, while 33% of those receiving antibody 2 days after infection survived for 21 days. Thus, the effect of passively transferred antibody is therapeutic and confers protection even if given after the establishment of CNS infection.

To examine the quantity of antibody required to protect against lethal challenge, we gave groups of six mice increasing doses of 5B19.2 ascites 1 day prior to ic challenge with MHV-4 then scored mortality as indicated above. Table 2 summarizes the results of a typical experiment. A single dose of 25 µl of 5B19.2 ascites with a neutralizing titer of 1:10,000 protected half of the challenged mice. This dose of ascites in a 25 g mouse is approximately equivalent to a 1/1000 dilution (1 µl/g).

Effect of passively transferred antibody on virus growth in tissues. The observation that MAb was protective even if given after challenge suggested that the sparing effect
MODULATION OF MHV-4-INDUCED DISEASE BY ANTIBODY

Fig. 2. Effect of time of passive antibody administration on survival following MHV-4 challenge. Groups of six mice each were given 200 µl of ascites fluid containing monoclonal antibody 5B19.2 on the days indicated and challenged with MHV-4 on Day 0 and survival was scored. Note that antibody given on Days -1 (d-1), 0 (d0), and +1 (d+1) was highly efficient in protecting against lethal challenge and antibody given on day +2, (d+2) protected one-third of the challenged mice. Mice given nucleocapsid antibody 4B6.2 or no antibody on Day -1 died by Day 6 after challenge.

TABLE 2

| Group | Volume of passively transferred ascites (n = 6) | Survival at 21 days |
|-------|---------------------------------------------|-------------------|
| I     | 200 d-1 µl                                   | 6/6               |
| II    | 100 d-1 µl                                   | 6/6               |
| III   | 50 d+1 µl                                    | 3/5               |
| IV    | 25 d+2 µl                                    | 3/6               |
| V     | 0 d+2 µl                                     | 0/6               |

*PRD<sub>50</sub>. titer of 5B19.2 ascites determined by neutralization assay was 1/10,000.

Above and tissues were removed for titration 4 days after infection, at a time when sufficient unprotected mice remained alive for study. Table 3 summarizes these results. We noted that titers in the brains of mice receiving MAb 5B19.2 were reduced by a factor of 20 relative to untreated controls. This reduction was not observed in mice receiving nonprotective antibody to E1 (5A5.2) or E2 (4B11.6). In the protected mice (group IV, Table 3), we were unable to detect virus in the livers at a threshold value of 200 PFU per gram whereas control mice (groups I and III) had greater than 2 × 10⁶ PFU per gram in their livers on Day 4. To confirm that this difference reflected actual reduction in virus replication and not merely a damping effect due to high concentration of MAb in the liver homogenates, we examined H and E sections as well as fluorescein-stained cryostat sections of livers from protected and unprotected mice. Mice which received no protective MAb showed numerous large necrotic foci of hepatocytes (Fig. 3A), stained for MHV-4 antigens (Fig. 3B), but only limited isolated foci of infected cells were observed in sections of livers from mice receiving protective MAb 5B19.2 (Figs. 3C, D). Taken together, these findings indicate that virus replication is substantially decreased by passively administered MAb both in the CNS and peripheral compartments.

TABLE 3

| Group | Passive antibody | Liver | Brain |
|-------|------------------|------|-------|
| (n = 4) | d-1              |      |       |
| I     | None             | 2.4 × 10⁶ | 2.5 × 10⁶ |
| II    | 5A5.2            | ND   | 5.4 × 10⁶ |
| III   | 4B11.6           | 2.0 × 10⁴ | 7.3 × 10⁵ |
| IV    | 5B19.2           | <2 × 10² | 1.3 × 10⁴ |

*Mean of four mice per group.

ND, no data available.

Passively transferred MAb blocks infection of neurons but not oligodendrocytes. The
Fig. 3. Hepatic lesions in unprotected (A, B) and antibody-protected (C, D) mice, following intracerebral challenge with 20 PFU of MHV-4. Antigens were stained using a rabbit antibody to MHV which recognizes all of the viral polypeptides. (A) One of multiple necrotic foci with infiltrating polymorphonuclear leukocytes, in an unprotected mouse. 250×, H & E. (B) Fluorescein-labeled MHV antigens in an MHV-4-induced hepatic lesion in an unprotected mouse. 250×. (C) An isolated focus of necrotic hepatocytes in a 5B19.2-protected mouse. 250×, H & E. (D) Fluorescein-labeled MHV antigens in an isolated MHV-4-induced hepatic lesion in a 5B19.2-protected mouse. 250×, H & E.

Observation of lower virus titers in the brains of protected mice suggested that virus replication was restricted to fewer cells in these animals. To test this, we compared the distribution of MHV antigens in sections of spinal cord among infected and untreated, infected and unprotected, and infected and protected mice. Figure 4 demonstrates peroxidase-labeled MHV antigens in a section from an MHV-4-infected untreated mouse. There are MHV antigens in neuronal cells in the gray matter (Fig. 4A) as well as in oligodendrocytes with their processes surrounding multiple myelin sheaths in the white matter (Fig. 4B).

The MHV-4 antigen distribution in infected animals given nonprotective antibody is virtually identical. In contrast, mice infected with MHV-4 and protected by antibody have a different distribution of MHV antigens. Figure 5 demonstrates peroxidase-labeled MHV antigen in oligodendrocytes, identified by their cytoplasmic processes surrounding multiple myelin sheaths (Knobler et al., 1981b, 1982), in the white matter. Neurons containing MHV antigen were not usually found in the spinal cord of antibody-protected mice.

These findings were corroborated by histopathology. Both MHV-4-infected, un-
treated mice and MHV-4-infected mice treated with nonprotective MAb had histopathological evidence of necrotizing encephalomyelitis with neuronophagia. They showed evidence of white matter involvement as well, but did not survive long enough to allow the demonstration of demyelination. Neurons of mice infected with MHV-4 and protected by antibody were spared, but these animals did have definite areas of demyelination (Fig. 6A). These demyelinated foci were frequently associated with perivascular infiltration by mononuclear cells (Fig. 6B).

Demonstration of demyelination in the presence of MHV-4 protective antibody suggested that the effects of antibody were different on neurons and oligodendrocytes. Since the ts 8 mutant of MHV-4 causes infection of oligodendrocytes, we evaluated the effect of protective antibody on the demyelinating disease induced by ts 8 virus. Two groups of seven mice each received either protective MAb 5B19.2 or no antibody, and both groups were challenged 1 day later with 10,000 PFU of ts 8 virus ic. Demyelination was found in 100% of the mice examined from each group suggesting that MAb 5B19.2 can protect neuronal cells from histopathologic disease, but does not similarly protect oligodendrocytes.

DISCUSSION

Factors which influence pathogenesis of virus infections are complex and may be determined at the level of virus or host. In the case of MHV-4 infection, previous studies have established that an attenuated
ts mutant of MHV-JHM (Haspel et al., 1978) virus showed decreased encephalitogenicity while retaining the ability to induce CNS demyelination. In the present study, we have investigated the influence on MHV-4 infection of passively transferred antibodies to single epitopes on the viral glycoproteins. Our results demonstrate that specific monoclonal antibodies to the E2 glycoprotein block encephalitis and convert a normally lethal infection to nonfatal demyelinating disease. This sparing effect upon passive transfer is restricted to a subset of MAb which react with the A (E2) and B (E2) epitopes but is not a property of antibody to epitope C (E2) or of MAb to the MHV E1 or N polypeptides (Talbot et al., 1984). Further, although all of the protective antibodies examined neutralized virus infectivity in vitro, one MAb (4R11.6) directed against epitope C (E2) neutralized virus efficiently in vitro but showed no sparing effect in vivo, thus the properties of in vitro neutralization and in vivo protection can be distinguished and appear to map to distinct subsets of epitopes on E2.

Protective antibody does not prevent infection of CNS cells by MHV. Further, replication was decreased but not completely blocked by antibody. Virus titers in the brains of mice treated with protective doses of antibody prior to infection were approximately 5% of those reached in control unprotected mice (Table 3). Antibody was found to be effective in conferring protection when given as late as 1–2 days after virus challenge (Fig. 2). Thus, the sparing effect is both therapeutic in that the titer of virus and severity of pathology are lessened, and prophylactic because lethal encephalitis is prevented. It is unlikely that the absence of liver lesions in antibody-protected mice is a significant factor in their survival since much larger doses (1000 PFU) of MHV-4 than used in these studies cause only subacute hepatitis when given intraperitoneally.

The mechanism by which antibody-mediated protection functions appear to be in blocking the spread of infection in neurons. It is apparent from the data in Table 3 that virus replicates in the brains of protected mice but to diminished levels relative to control mice. Only a few neurons bearing viral antigen could be demonstrated in these brains by immunofluorescence (data not shown). Further, no evidence of MHV replication was found in neurons in the spinal cord. The sparing effect of MAb was selective for neuronal cells. Infected oligodendrocytes and resultant demyelination

FIG. 6. Demyelination of the lumbar spinal cord of 5B19.2 antibody-treated animals following intracerebral challenge with 20 PFU of MHV-4. (A) Subpial demyelination is evident in this 1-μm plastic embedded transverse section of spinal cord. 400X, p-phenylenediamine. (B) Subpial demyelination is evident in this 1-μm plastic embedded longitudinal section of spinal cord. Demyelinated axons are present in the right side of this photomicrograph. Perivascular mononuclear cells are observed in this lesion. 400X, p-phenylenediamine.
were observed in spinal cords of infected and passively protected mice (Figs. 5, 6). Further, antibody which protected against lethal encephalitis did not block demyelination induced by the ts 8 mutant of MHV-4. The observed restriction of virus replication in the neuronal cells but not oligodendrocytes suggests that infection spreads via different mechanisms in these two cell types. Whether this is due to differences in the mode of cell-to-cell spread or reflects distinct receptors for MHV-4 on each cell type is a matter for further study. In addition to the altered virus tropism in the presence of antibody, we found that the characteristics of the demyelinating lesions were changed. Lesions in antibody-protected mice showed increased cellularity with frequent perivascular cuffing in contrast to the lesions usually observed (Knobler et al., 1982) following MHV-4 infection.

This model system demonstrates that antibody response to precisely defined regions on a viral glycoprotein may induce profound changes in the pathogenesis of infection and the phenotype of disease. Conceptually, such factors may be of importance following primary infections, where an early response to a critical epitope(s) might influence the course of disease. Stohlman and Weiner (1981) have shown that mice surviving acute MHV-4 encephalomyelitis have neutralizing antibody in the circulation and develop chronic demyelination. Similarly, the repertoire of response to a primary infection may influence the course of subsequent infection by a related virus. Human viral diseases such as subacute sclerosing panencephalitis (Vandvik, 1973) and progressive rubella panencephalitis (Wolinsky et al., 1976) occur in the presence of high titers of antibody to measles and rubella viruses, respectively. In the human demyelinating disease multiple sclerosis, there are elevated titers of antibodies to a number of common viruses such as measles, varicella, herpes, and others (Norrby, 1978). Although causal relationships between the respective viruses, the presence of these antibodies, and demyelinating disease have not been established, the present model system provides new insight into the potential role of selected specific antibodies in leading to host survival from fatal infection, with development of a chronic disease. Finally, this model offers the opportunity to study the factors which govern the infection of and spread within specific populations of CNS cells by MHV-4 at the molecular level.

ACKNOWLEDGMENTS

This is Publication No. 3044-IMM from the Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, California. We are grateful to Ricarda deFries, Linda Tunison, and Gretchen Collins for capable technical assistance, to Lisa A. Flores and Ana M. Garcia for manuscript preparation, and to Michael B. A. Oldstone for helpful discussion. This research was supported by U. S. Public Health Service Grant NS-12428 and AI-16102 from the National Institutes of Health. This work was done during the tenure of an Established Investigatorship granted to M.J.B. by the American Heart Association, and in part while R.L.K. was the Ralph I. Straus Fellow of the National Multiple Sclerosis Society. R.L.K. is a recipient of Teacher Investigator Award NS00803 from the National Institute of Neurological, Communicative Disorders and Stroke. P.J.T. is a recipient of a Medical Research Council of Canada Fellowship.

REFERENCES

BAILEY, O. T., PAPPENHEIMER, A. M., and CHEEVER, F. S. (1949). A murine virus (JHM) causing disseminated encephalomyelitis with extensive destruction of myelin II pathology. J. Exp. Med. 90, 195-212.

COLLINS, A. R., KNOBLER, R. L., POWELL, H., and BUCHMEIER, M. J. (1982). Monoclonal antibodies to murine hepatitis virus-4 (strain JHM) define the viral glycoproteins responsible for attachment and cell-cell fusion. Virology 119, 353-371.

HASPEL, M. V., LAMPERT, P. W., and OLDSTONE, M. B. A. (1978). Temperature sensitive mutants of mouse hepatitis virus produce a high incidence of demyelination. Proc. Nat. Acad. Sci. USA 75, 4033-4036.

HOLMES, K., DOLLER, E., and BISHNKE, J. (1981). Analysis of the functions of coronavirus glycoproteins by differential inhibition of synthesis with Tunicamycin. In "Biochemistry and Biology of Coronaviruses" (V. ter Meulen, S. Siddell, and H. Wege, Eds.), pp. 133-142. Plenum, New York.

KNOBLER, R. L., HASPEL, M. V., and OLDSTONE, M. B. A. (1981a). Mouse hepatitis virus type-4 (JHM strain)-induced fatal central nervous system disease. I. Genetic control and the murine neuron as the susceptible site of disease. J. Exp. Med. 153, 832-843.
KNOBLER, R. L., DUBOIS-DALCQ, M., HASPEL, M. V., CLAYSMITH, A. P., LAMPERT, P. W., and OLDSTONE, M. B. A. (1981b). Selective localization of wild type and mutant mouse hepatitis virus (JHM strain) antigens in CNS tissues by fluorescence, light and electron microscopy. J. Neuroimmunol. 1, 81-92.

KNOBLER, R. L., TUNISON, L. A., LAMPERT, P. W., and OLDSTONE, M. B. A. (1982). Selected mutants of mouse hepatitis virus type 4 (JHM strain) induce different CNS diseases. Amer. J. Pathol. 109, 157-168.

LAMPERT, P. W., SIMS, J. K., and KNIAZEFF, A. J. (1973). Mechanism of demyelination in JHM virus encephalomyelitis. Electron microscopic studies. Acta Neuropathol. 24, 76-85.

NORRBY, E. (1978). Viral antibodies in multiple sclerosis. Prog. Med. Virol. 24, 1-39.

STOHLMAN, S. and WEINER, L. P. (1981). Chronic central nervous system demyelination in mice after JHM virus infection. Neurology 31, 38-44.

STURMAN, L., HOLMES, K., and BEHNKE, J. (1980). Isolation of coronavirus envelope glycoproteins and interaction with the viral nucleocapsid. J. Virol. 33, 449-462.

TALBOT, P. J., SALMI, A. A., KNOBLER, R. L., and BUCHMEIER, M. J. (1984). Topographical mapping of epitopes on the glycoproteins of murine hepatitis virus-4 (strain JHM): Correlation with biological activities. Virology, 132, in press.

VANDVIK, B. (1973). Immunopathological aspects in the pathogenesis of subacute sclerosing panencephalitis, with special references to the significance of the immune response in the central nervous system. Ann. Clin. Res. 5, 308-315.

WAKSMAN, B. H., and ADAMS, R. D. (1962). Infectious leukoencephalitis: A critical comparison of certain experimental and naturally-occurring viral leukoencephalitides with experimental allergic encephalomyelitis. J. Neuropathol. Exp. Neurol. 21, 491-518.

WEGE, H., WEGE, H., NAGASHIMA, K., and TER MEULEN, V. (1979). Structural polypeptides of the murine coronavirus JHM. J. Gen. Virol. 42, 37-47.

WEINER, L. P. (1973). Pathogenesis of demyelination induced by mouse hepatitis virus (JHM virus). Arch. Neurol. 28, 298-303.

WOLINSKY, J. S., BERG, B. O., and MAITLAND, C. J. (1976). Progressive rubella panencephalitis. Arch. Neurol. 33, 722-723.