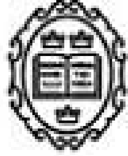

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Monoclonal Antibody to the Receptor for Murine Coronavirus MHV-A59 Inhibits Viral Replication In Vivo

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Because many strains of mouse hepatitis virus (MHV) infect laboratory mice, no effective vaccine has yet been developed. An alternative approach to control MHV disease is the use of a host cell receptor-targeted ligand. To address the potential usefulness of this approach, a monoclonal antibody directed against the host cell receptor for the coronavirus MHV-A59 was administered to infant mice that were then challenged oronasally with 10^4 intracerebral infant mouse median lethal doses of MHV-A59. Antibody treatment of virus-challenged mice resulted in lower proportions of mice with MHV-A59 in target organs and markedly reduced viral titers in these organs compared with mock-treated infected mice. Some antibody-treated infected mice survived for 7 days after viral challenge, whereas no mock-treated, infected mice survived beyond day 3 after viral inoculation. These results support a receptor-targeted approach to intervention in coronavirus disease.

Viral receptors play important roles in determining the species specificity, tissue tropism, and pathogenesis of animal viral infections in vivo [1]. Receptors for viruses in many different groups have been recently identified, leading to efforts to inhibit viral infection in vitro and in vivo by blocking virus-receptor interactions [2–5]. A receptor-targeted approach appears most likely to be effective in viral diseases in which the virus enters the body and first replicates in epithelial cells, such as those of the respiratory or intestinal tracts, which may be most accessible to treatment with receptor-blocking substances.

An excellent model for analysis of such a receptor-targeted approach to prevention of viral disease is the murine coronavirus, mouse hepatitis virus (MHV). MHV is the most prevalent virus infecting laboratory mouse colonies worldwide [6], and many MHV strains with tropisms for either respiratory or intestinal mucosal surfaces have been identified [7]. The receptor for the A59 strain of MHV (MHV-A59) has been identified as a 110- to 120-kDa glycoprotein [8, 9]. Virus-overlay protein blot assays (VOPBA) reveal that this glycoprotein is expressed on intestinal brush border membranes and hepatocyte membranes of MHV-susceptible BALB/c mice [8, 9]. Comparable membrane preparations from genetically resistant SJL mice do not bind MHV-A59 [8, 9]. These studies suggest that genetically based susceptibility to MHV-A59 may be determined, at least in part, by expression of the virus-binding moiety on the plasma membranes of normal target tissues for MHV.

A panel of monoclonal antibodies (MAbs) reactive with the putative receptor for MHV-A59 was developed. One, designated MAb-CC1, prevented binding of MHV-A59 to murine fibroblasts and blocked infection of mouse cell lines with MHV-A59 [9] and with four other prototype MHV strains (unpublished data) that do not cross-neutralize [10]. Specificity of MAb-CC1 inhibition of MHV infection was supported by the fact that titers of vesicular stomatitis (Indiana serotype), Sendai, and Theiler's mouse encephalomyelitis viruses were identical in mock-treated and MAb-CC1-treated MHV-susceptible NCTC 1469 cells (data not shown). Further evidence that MAb-CC1 was an MHV receptor-specific antibody was derived from the fact that an MAb-CC1 affinity-purified 110- to 120-kDa protein eluted after preparative SDS-PAGE specifically bound MHV-A59 in VOPBA [9]. Thus, we sought to determine whether MAb-CC1 modulated the course of MHV-A59 infection in the natural host exposed by a presumed natural route.

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Materials and Methods

Virus. MHV-A59 was originally obtained from the ATCC (Rockville, MD) and was used in the form of an infected 17Cl 1 cell lysate.

Mice. Pregnant BALB/cJ and BALB/cByJ mice were obtained from Jackson Laboratory (Bar Harbor, ME). Pooled results from the two stocks of BALB/c mice are shown, since no differences in pathogenesis of MHV infection were noted (unpublished data). Infant Cr:ORL Sencar random-bred mice (Animal Genetics and

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Production Branch, National Cancer Institute, Bethesda, MD) were used for quantification of infectious virus in BALB/c tissues.

MAb-CCl. MAbs directed against the 110- to 120-kD receptor for MHV were developed as described [9]. Briefly, spleen cells from receptor-negative SJL mice that had been immunized with intestinal brush border membranes from MHV-susceptible BALB/c mice were fused with SP_2-0 myeloma cells [9]. MAbs specific for the MHV receptor were selected by reactivity in an ELISA with receptor eluted after preparative SDS-PAGE of BALB/c brush border membranes and by reactivity in immunoblots with BALB/c, but not SJL, brush border membranes [9]. Pretreatment of mouse cell lines with any of these MAbs blocked infection with MHV-A59, suggesting that the 110- to 120-kDa glycoprotein was the only receptor for MHV-A59 on these cells. Anti-receptor MAb-CCl had the highest titer for blocking infection of L2 and 17Cl 1 lines of mouse fibroblasts, and protection resulted from blocking virion binding to cell membranes (unpublished data). This MAb bound specifically to apical brush border membranes of frozen sections of BALB/c mouse small intestine but not to membranes of SJL mouse intestine [11]. MAb-CCl was selected to determine whether infection or disease caused by MHV-A59 in susceptible mice could be prevented in vivo by anti-receptor antibody.

Experimental design. To assess in vivo protection, infant BALB/c mice were treated three times daily with anti-receptor MAb-CCl (100 ng of protein/mouse/day; 5 μl oronasally and 10 μl intraperitoneally for each treatment) beginning on day 1 of life. Control mice were similarly treated with PBS or with a MAb of the same isotype as MAb-CCl directed against an irrelevant antigen (mouse IgGl anti-dengue virus MAb). MAb-CCl–treated and control mice were challenged oronasally with 10^4 infant mouse intracerebral (ic) LD_{50} of MHV-A59 in 5 μl of saline at 3 days of age. Thrice daily oronasal treatment of surviving mice with antibody or buffer was continued for 4 days after challenge. Randomly selected mice were killed with CO_2 gas and necropsied on days 2–7 after viral inoculation. Virus in homogenates of noses, brains, and livers collected from infected mice at days 2 and 3 after viral infection was quantified as $\log_{10}$ virus per gram of tissue in a sensitive LD_{50} assay [12] based on ic inoculation of infant Sencar mice. Tissue sections collected from buffer- or MAb-CCl–treated infected mice at day 2 after viral inoculation and from MAb-CCl–treated infected mice on days 5 and 7 after inoculation were examined microscopically. Buffer-treated infected mice did not live beyond day 3 after viral exposure.

Statistical analysis. Differences in proportions were analyzed by χ^2, and differences in viral titers between treatment groups were analyzed by Student's unpaired t test.

Results

Exposure of susceptible mice to MHV strains having primary tropism for the respiratory tract results in neural transport of virus to the brain and bloodborne dissemination to visceral organs [12, 13]. Therefore, nose, brain, and liver were chosen as indicators of the effect of MAb-CCl treatment on MHV-A59 infection. The proportion of MHV-A59–inoculated mice from which virus could be recovered was lower for MAb-CCl–treated mice in all cases (table 1), with statistically significant differences for brain and liver on day 2 after viral inoculation. Although nasal tissue from most PBS- and MAb-CCl–treated infected mice contained virus on days 2 and 3 after inoculation, viral titers were >1000-fold lower in noses of MAb-CCl–treated mice (figure 1). Viral titers on days 2 and 3 were also lower in brains and livers of infected mice treated with MAb-CCl, with statistically significant differences in brain titers on both days. The relatively high viral titer shown for liver in the MAb-CCl treatment group on day 2 represents only a single mouse from which virus could be recovered. The mean viral titer in the livers of MAb-CCl–treated mice at 3 days after viral exposure was 2.6 $\log_{10}$/g lower than the mean titer in livers of buffer-treated mice; however, this was not statistically significant, primarily due to the small number of surviving buffer-treated infected mice.

MAb-CCl alone did not cause any signs of disease in uninfected infant mice treated as described for 1 week. Results for a small number of mice treated with anti-dengue virus MAb were essentially identical to those shown for PBS-treated infected mice. Of 28 buffer-treated infected mice not scheduled for necropsy, 20 died (average day of death [ADD] = 2.5 ± 0.5), whereas 8 of 30 MAb-CCl–treated infected mice not scheduled for necropsy died (ADD = 2.4 ± 0.5). However, two MAb-CCl–treated infected mice survived until necropsy at 7 days after viral inoculation, and no buffer-treated infected mice survived beyond day 3 after viral challenge.

Buffer-treated infected mice had necrotizing rhinitis at 2 days after viral exposure. These mice also had severe hepatitis that was evident grossly and microscopically, whereas livers of MAb-CCl–treated infected mice were histologically normal on days 2, 5, and 7 after inoculation. Lesions noted for MAb-CCl–treated infected mice consisted of necrosis of the anterior nose at 5 days after viral inoculation and nasal lesions and encephalitis on day 7 after viral challenge.

Table 1. Proportions of infant BALB/c mice with virus in target organs at 2 and 3 days after challenge with mouse hepatitis virus (MHV)-A59.

Day after MHV challenge, treatment	Nose	Brain	Liver
2, PBS	10/10	10/10	10/10
MAb-CC1	7/9	1/9*	1/9*
3, PBS	2/2	2/2	2/2
MAb-CC1	4/6	3/6	2/6

NOTE. Results are number of virus-positive tissues/number of tissues tested from mice inoculated intranasally with 10^4 infant mouse intracerebral median lethal doses of MHV-A59. MAb = monoclonal antibody.

* $P < .005$ (χ^2 analysis).

Discussion

Earlier studies showed that MHV given intranasally gains access to the brain by neuronal pathways, whereas transport to other organs is via viremia [12, 14]. The reported experi-

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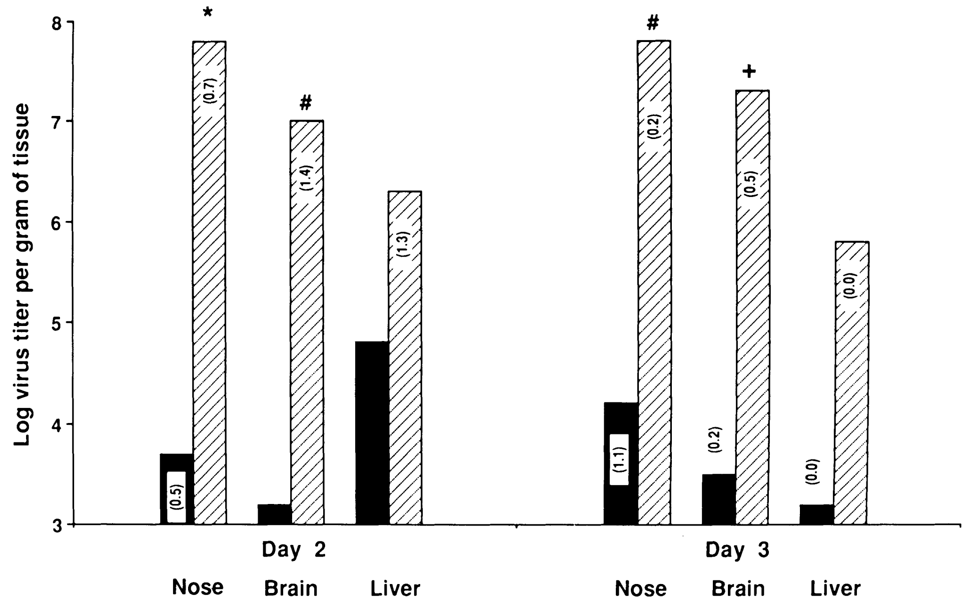

Figure 1. Mean virus titers ($\log_{10}$ infant mouse intracerebral [ic] LD_{50}/g of tissue) in virus-positive BALB/c tissues at 2 and 3 days after challenge with 10^4 infant mouse ic LD_{50} of mouse hepatitis virus A59. Titers for monoclonal antibody (MAb)-CC1–treated infected mice are solid bars; buffer-treated infected mice are cross-hatched bars. By Student's unpaired t test, differences between titers in tissues of PBS- versus MAb-CC1–treated mice: $* P < .001$; $+ P < .005$; $\# P < .025$; ( ) = SD.

ments show that MAb-CC1 treatment reduced primary MHV-A59 replication in the nose and delayed or prevented viral spread to or replication in secondary target organs such as brain and liver. The absence of nasal lesions among MAb-CC1–treated mice at day 2 and their later appearance (at days 5 and 7) suggests that MHV replication in the nose was delayed by this treatment.

The pathogenesis of infection with parental MHV-A59 has been compared with that of virus in a nasal homogenate of an MAb-CC1–treated infected mouse collected during these studies. After oronasal exposure of infant mice, both inocula induced fatal disease with qualitatively similar lesions in the same spectrum of organs (data not shown). This finding suggests that virus recovered from organs of MAb-CC1–treated mice did not represent a selected variant population. Further studies are required to determine if complete protection from viral infection can be afforded by challenge with a lower dose of virus or by administration of more concentrated anti-receptor antibody or more frequent administration of the antibody. However, a relatively small amount of anti-receptor antibody yielded a protective effect against an overwhelming inoculum of MHV-A59 administered by a natural route.

There are many strains of MHV, and infection with one strain does not afford protection against challenge with a heterologous strain [7]. The likelihood of developing an effective vaccine against this prevalent murine virus is, therefore, remote. MAb-CC1 blocking activity is generic in the sense that it protects cultured cells from infection with a variety of MHV strains in vitro (unpublished data). The current in vivo experiments suggest that treatment with anti-receptor antibody or receptor-targeted drugs could protect at least limited numbers of valuable laboratory mice that are at risk during MHV epizootics.

Very few studies have demonstrated significant protection against viral infection in vivo by antibody directed against the host cell receptor [2]. A possible complication of this approach is that the normal cellular function of the receptor for MHV is unknown. Blocking of the receptor by antibody could interfere with its function(s). However, these experiments are promising in that no adverse effects of treatment with MAb-CC1 were observed in vitro (unpublished data) or in vivo. Possibly the virus-binding domain of the MHV receptor glycoprotein differs from the domain for host cell function(s). Evidence for this hypothesis stems from the observation of a homologous protein antigenically related to the BALB/c 110- to 120-kDa MHV receptor on SJL intestinal and hepatocyte membranes; however, the SJL protein fails to bind MHV-A59 or MAb-CC1 [9].

Numerous coronaviruses have been identified, and most are very host species–specific. Human coronaviruses, like those of the mouse, are ubiquitous, and identification of the host cell receptor(s) for these agents will have a significant impact on human health. Human coronaviruses commonly cause respiratory infections and account for ~15% of colds [14]. The remaining 85% of colds are predominantly caused by rhinoviruses [15]. Vaccines against rhinoviruses have not proved practicable because there are >100 serotypes in the genus; however, drugs targeted against the receptors for rhinoviruses might prove useful since there are only two receptors for all of the serotypes [3]. Nose drops containing antibody against the receptor for the major group of rhinoviruses did not reduce the overall infection or illness rates among challenged human volunteers but reduced viral titers and delayed viral shedding [4]. Soluble intracellular adhesion molecule-1 has recently been shown to inhibit binding of rhinovirus to cultured cells and to protect against cytopathic effect induced

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by picornaviruses that use the major group rhinovirus receptor [5]. Our studies suggest that a receptor-targeted approach to preventing coronavirus infection is worthy of further study. If the receptor for human coronaviruses is homologous to the glycoprotein receptor for murine coronavirus, oronasal treatment with a receptor-targeted ligand might also be a useful approach to prevention of human coronavirus infections.

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Neutralizing Antibodies to Interferon-α: Relative Frequency in Patients Treated with Different Interferon Preparations

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The frequencies of antibody development so far reported in patients treated with different interferons (IFNs) are not readily comparable because of differences in treatment regimens and assay methods. Thus the frequency of neutralizing antibody development was analyzed in a large sample of sera derived from a relatively homogeneous group of patients treated with different IFN-α preparations. The frequency of developing neutralizing antibody to IFN varied according to the IFN given. Particularly, the seroconversion frequency was significantly higher in patients treated with recombinant IFN-α2a (20.2%) than in patients treated with either recombinant IFN-α2b (6.9%) or IFN-αN1 (1.2%), a lymphoblastoid IFN-α. Furthermore, sera obtained from patients treated with either recombinant IFN neutralized both types of recombinant IFNs but failed to neutralize IFN-αN1.

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There are several reports of patients forming neutralizing antibodies while under treatment with interferon (IFN) preparations [1–7]. These antibodies may be clinically important, as shown by concomitant loss of beneficial effects of treatment [8–12]. Unfortunately, the data in these studies are heterogeneous in terms of the patients and diseases involved, the types and doses of IFN used, and the methods used to mea-

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