HCoV-OC43–INDUCED ENCEPHALITIS IS IN PART IMMUNE-MEDIATED

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1. INTRODUCTION

HCoV-OC43 and HCoV-229E are the etiological agents for the majority of coronavirus-induced upper respiratory tract infections in humans. HCoV-OC43 was originally isolated from human embryonic tracheal organ cultures; this virus was neurovirulent and caused disease after one passage in suckling mice and encephalitis within 2–4 passages (herein referred to as HCoV-$OC43_{NV}$).[1] This virus was then further adapted for growth in tissue culture cells ("tissue-culture adapted variant," referred to as HCoV-$OC43_{TC}$).

HCoV-OC43 showed increasing neurovirulence with passage through the murine brain; however, most recent studies have used viruses that have been propagated, at least for a few passages, in tissue culture cells. For example, Talbot and co-workers showed, using the mouse-adapted virus after passage in tissue culture cells 5–6 times, that mice infected intranasally with 10^4 to 10^5 $TCID_{50}$ developed encephalitis if inoculated at 8 days but not 21 days postnatally.[2]

For these reasons, we postulated that virus directly harvested from suckling mouse brain would be more virulent than virus passaged, even minimally, in tissue culture. In preliminary experiments, we observed that virus directly harvested from suckling mouse brains is highly virulent and readily caused a lethal infection after intranasal inoculation of adult, 8-week-old mice. When we evaluated the contribution of the host adaptive immune response to HCoV-OC43–induced encephalitis, we found that encephalitis is, in part, immune-mediated.

2. MATERIALS AND METHODS

Viruses and infection of mice: Mouse CNS-adapted (HCoV-$OC43_{NV}$) and tissue culture-adapted (HCoV-$OC43_{TC}$) strains of HCoV-OC43 (VR-759 and VR-1558, respectively) were obtained from the ATCC (Manassa, VA). Eight-week-old, pathogen-free male C57BL/6 mice were purchased from the National Cancer Institute (Bethesda,

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MD). Male and female, 6- to 8-week-old RAG1$^{-/-}$ mice were obtained from breeding colonies maintained by our laboratory. For intranasal infection, mice were lightly anesthetized with halothane and droplets containing 10^7 $SMLD_{50}$ of HCoV-$OC43_{NV}$ or 10^6 $TCID_{50}$ of HCoV-$OC43_{TC}$ were administered to the nares. Mice were monitored for weight loss and survival after infection. All procedures used in this study were approved by the University of Iowa Institutional Animal Care and Use Committee.

Flow cytometry: Single cell suspensions of mononuclear cells from whole brain homogenates were prepared as previously described.[3] Fc receptors were blocked with normal rat serum and anti-CD16/CD32 (clone 2.4G2, BD Biosciences, San Jose, CA). Antibodies used to phenotype cells were fluorescein isothiocyanate-labeled anti-mouse CD4 and phycoerythrin-labeled anti-mouse CD8 (clones GK1.5 and 53-6.7, BD Biosciences, Mountain View, CA). Samples were analyzed on a FACScan flow cytometer (BD Biosciences, Mountain View, CA).

Adoptive transfer: Red blood cell depleted splenocytes were isolated from C57BL/6 mice 7 days after interperitoneal immunization with HCoV-$OC43_{NV}$. 1 x 10^6 HCoV-$OC43_{NV}$-immune splenocytes were adoptively transferred via retroorbital injection to RAG1$^{-/-}$ mice that had been inoculated intranasally with HCoV-$OC43_{NV}$ 4 days previously. Recipient RAG1$^{-/-}$ mice were continuously monitored for weight loss and survival following infection. As a control, some RAG1$^{-/-}$ mice received no cells after intranasal inoculation of virus.

3. RESULTS

3.1. Intranasal Inoculation of 8-Week Mice with HCoV-$OC43_{NV}$ Is Uniformly Fatal

Intranasal inoculation of HCoV-$OC43_{NV}$ resulted in 100% mortality in mice ranging from 5 weeks old (data not shown) to 8 weeks old (Figure 1A). Mice developed signs of acute encephalitis, including hunched posture, lethargy, and wasting by day 7–9 (data not shown). Mortality was associated with a ~35% loss of body mass (Figure 1B). Severe clinical encephalitis was also associated with widespread mononuclear cell infiltration including perivascular cuffing and with loss of CNS architecture (data not shown). In contrast, intranasal inoculation of 8-week-old C57BL/6 mice with the HCoV-$OC43_{TC}$ was not fatal and did not cause any clinical disease, including any weight loss (Figure 1).

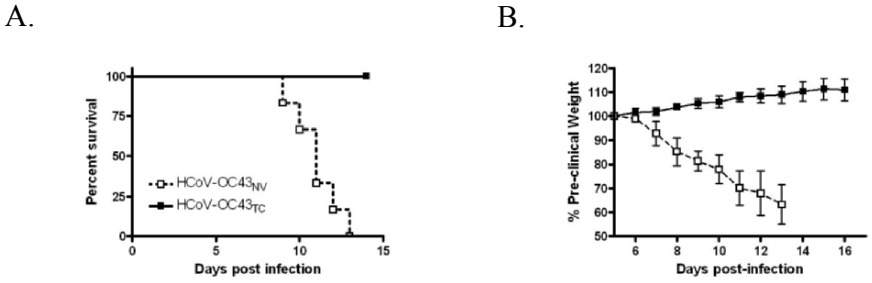

Figure 1. HCoV-OC43–induced lethal encephalitis in 8-week-old mice. Mice were inoculated intranasally with HCoV-$OC43_{NV}$ or HCoV-$OC43_{TC}$ and monitored for survival (A) and weight loss (B). Data represent 6 mice per group. For B, data are expressed as mean +/- SD.

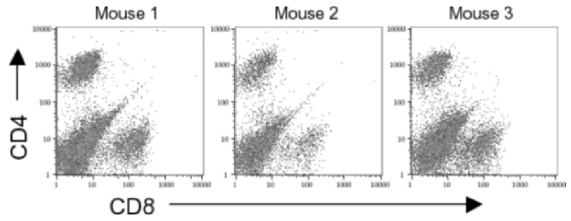

Figure 2. CD4 and CD8 T cells infiltrate the HCoV-OC43$_{NV}$–infected CNS. 7 days postinfection, mononuclear cells were prepared from brain homogenates, stained for CD4 and CD8, and analyzed with flow cytometry. Samples from 3 representative mice are shown.

3.2. Encephalitis Is Partly Immune-Mediated

As described above, HCoV-OC43$_{NV}$–induced encephalitis was associated with a large infiltration of mononuclear cells into the brain parenchyma. When we immunophenotyped CNS infiltrates from HCoV-OC43$_{NV}$–infected mice, we found that a large proportion of the infiltrating mononuclear cells was comprised of CD4 and CD8 T cells (Figure 2). When we examined viral titers and RNA burden in the CNS, we found that virus was in the process of clearance at the time of death (data not shown), suggesting that the host immune response both cleared virus and contributed to a fatal outcome.

To probe the role of this T-cell response in pathogenesis, we infected immunodeficient mice lacking normal T- and B-cell responses (RAG1$^{-/-}$) and monitored these mice for weight loss and survival. HCoV-OC43$_{NV}$–infected RAG1$^{-/-}$ mice lost weight and developed signs of encephalitis (lethargy, hunching, weight loss) similar to those observed in infected wild-type mice, but with delayed kinetics. Moreover, infected RAG1$^{-/-}$ mice also survived longer than did their B6 counterparts (data not shown).

To confirm the pathological role of T cells, we adoptively transferred HCoV-OC43–immune splenocytes to RAG1$^{-/-}$ mice that had been infected with 10^7 SMLD$_{50}$ HCoV-OC43$_{NV}$ intranasally 4 days earlier. The adoptive transfer of HCoV-OC43–immune splenocytes to RAG1$^{-/-}$ mice hastened the onset of clinical disease and death (Figure 3).

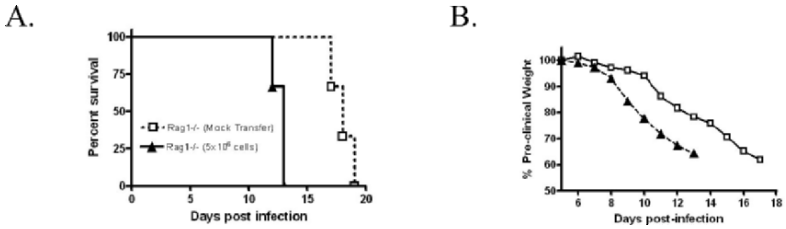

Figure 3. Adoptive transfer of HCoV-OC43$_{NV}$–immune splenocytes hastens the onset of mortality and morbidity in recipient RAG1$^{-/-}$ C57BL/6 mice. RAG1$^{-/-}$ mice were infected intranasally with HCoV-OC43$_{NV}$ 4 days prior to serving as recipients of the adoptive transfer of HCoV-OC43–immune splenocytes isolated from wild type mice. Mice were monitored for survival (A) and weight loss (B).

4. DISCUSSION

These data show that HCoV-OC43 that has been exclusively passaged in suckling mouse brain is highly neurovirulent relative to other strains reported to only cause disease in 1- to 3-week-old mice. These latter strains of HCoV-OC43, which have been through 5–6 passages in HRT-18 cells (Dr. Pierre Talbot, personal communication), are likely less virulent than virus isolated directly from infected suckling mouse brains because coronaviruses often become attenuated after passage *in vitro*.[4]

Our data also show that HCoV-OC43-induced encephalitis is in part mediated by the anti-viral T-cell response. During the course of our investigation we found that HCoV-OC43 infection appears restricted to neurons (data not shown). Neurons do not normally express MHC class I or II antigen and express only low levels of the machinery required for loading peptide onto MHC class I antigen.[5] Therefore, neurons generally do not serve as targets for activated T- cells. However, electrically silent or damaged neurons do express MHC class I antigen[6-8] and it is possible that infection with HCoV-OC43 makes neurons into suitable targets for CD8 T cells.

Together, these data suggest that for some human coronavirus infections, such as with HCoV-OC43, the ensuing pathology may often include an immune-mediated component. Future studies will be directed at determining how the anti-viral T cell response, while important for virus clearance, also contributes to more severe disease. These studies may also be relevant to understanding disease outcome in patients with SARS, since neurons are infected in some patients.[9]

This work was supported in part by a grant from the National Institutes of Health (AI60699).

5. REFERENCES

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