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PATHOGENESIS OF CNS WHITE MATTER (WM) INJURY IN SEMLIKI FOREST VIRUS (SFV) ENCEPHALITIS. R.A. Sobel¹, A.W. Elahi², T. Smith-Norowitz², F. Mokhtarian². ¹VA Hlth. Care Sys., Palo Alto and Stanford U. Schl. of Med., Stanford, CA; ²SUNY Hlth. Sci. Ctr. and Maimonides Med. Ctr., Brooklyn, NY.

Intraperitoneal inoculation of susceptible mice with SFV induces acute encephalitis followed by the appearance of WM lesions after SFV is cleared from the CNS. Immunization of B6 mice with SFV proteins or killed SFV induces T cell responses to an SFV peptide (E2 115-129) with homology to a myelin oligodendrocyte glycoprotein peptide (MOG 18-32). Immunization with E2 115-129 induces cross-reactive T cell and antibody (Ab) responses to MOG 18-32 and a late-onset EAE-like disease with WM vacuolation (Mokhtarian *et al.* 1999). To determine if Abs contribute to WM injury in active infection, wild type (WT) and B cell deficient (BCD) B6 and WT BALB/c mice were infected with SFV A774 strain and killed from days 7 to 35. Clinical disease was from days 7-10 in WT and days 14-21 in BCD mice. All mice had CNS inflammatory and PAS+ macrophage foci after day 7, maximal from days 7-14. From day 10 on, WT mice had more CNS WM vacuolation than BCD mice. WT B6 mice made Abs to E2 115-129, other SFV peptides, MOG 18-32 and MBP 64-75 (another E2 115-129-mimicked peptide); the latter were higher than other anti-MOG and -MBP Abs. BCD B6 mice made some anti-SFV, but no cross-reactive Abs. These data suggest that antiviral Abs that recognize CNS myelin epitopes through molecular mimicry contribute to WM injury after inflammation subsides and virus is cleared. Supported by NS 26773 and Maimonides Res. and Devel. Foundation.

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Demyelination determinants map to the spike glycoprotein gene of coronavirus MHV by targeted RNA recombination. J. Das Sarma and E. Lavi^{*}. University of Pennsylvania School of Medicine, Philadelphia, PA.

Coronavirus mouse hepatitis virus (MHV), strain A59, causes chronic inflammatory demyelinating disease in mice which mimics many of the pathologic features of the human multiple sclerosis. A closely related virus MHV-2 is incapable of producing demyelination. Previous studies including sequencing comparison between demyelinating and non-demyelinating MHVs suggested that the spike glycoprotein (S) gene may contain determinants of pathogenesis. To map the genomic control of demyelination, we used targeted RNA recombination to replace the S gene of A59 with the S gene of MHV-2. We recombined the genome of a recipient temperature sensitive(ts) mutant of A59 (Alb4) with an RNA transcribed from a plasmid containing the 3' end of A59 in which the S gene was replaced with a cloned S gene of MHV-2. Two recombinant viruses, which were named Penn98-1 and Penn98-2, were selected and characterized. Both contained the S gene of MHV-2, as confirmed by sequencing. A control recombinant virus, in which the S gene of A59 was recombined into an A59 background (wtR13), produced demyelination in 100% (5/5) of the mice. Penn98-1 and Penn98-2 were highly virulent in comparison to wtR13, wt A59 and wt MHV-2, but were unable to produce demyelination (in 0/7 mice for each virus), given the same dose of virus (5 PFU). Thus targeted RNA recombination revealed for the first time that the S gene of MHV contains important determinants of demyelination.

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PERSISTENCE OF REOVIRUS TYPE 3 IN THE MOUSE BRAIN. T. Valyi-Nagy, S. Olson, D. Wetzel, C. Tucker, K. Valyi-Nagy, M. Grundy, T. Montine^{*}, and T. Dermody, Vanderbilt University, Nashville, TN.

To determine whether type 3 reoviruses (RV-3) can establish long-term persistence in the nervous system, newborn mice were inoculated by the peroral, subcutaneous, or intracranial routes with RV-3 strains T3A or T3D and brain tissues were examined 5, 8, 13, 50, 100, and 240 days after infection for evidence of reovirus protein and mRNA expression. Mice infected with high doses of both strains developed acute encephalitis within 1-2 weeks of inoculation as evidenced by paralytic symptoms and immunohistochemical and *in situ* hybridization detection of RV-3 protein and mRNA expression in the CNS. Sites of viral infection were most prominent in neurons of limbic structures, the brainstem, and cingulate cortical areas. Most mice surviving beyond three weeks of inoculation became asymptomatic, with only a few demonstrating persistent growth retardation. However, brain tissues obtained from mice 50, 100, and 240 days after infection demonstrated focal, chronic encephalitis as well as viral mRNA expression in a low number of neurons and non-neuronal cells. RV-3 protein expression also was detected in the CNS of a few long-term survivors. These results indicate that reovirus mRNA and protein are detectable in long-term survivors of acute reovirus infection, which suggests that reovirus persists in the CNS for prolonged periods.

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CHOROID PLEXUS HARBORS HIV IN AIDS AND IN ASYMPTOMATIC HIV-INFECTED PATIENTS: A MOLECULAR AND IMMUNOHISTOCHEMICAL STUDY. Carol K. Petito^{*}, Hexin Chen, Colleen Hanna and Charles Wood. University of Miami School of Medicine, Miami, FL and University of Nebraska at Lincoln, Lincoln, NB.

The choroid plexus (CPx) often contains HIV-infected cells in AIDS patients; prior studies indicate an infection rate of 44% and localization of HIV infection to CPx monocytes and dendritic cells. To further examine the role of the CPx in the neuropathogenesis of HIV encephalitis, (HIVE), we extracted DNA from paraffin-embedded brain, spleen and CPx of 4 AIDS patients and analyzed HIV sequences by amplifying the V3 region of HIV env gene, characterizing at least 3 clones from each sample. We found that viral sequences from brain and spleen form 2 distinct clusters, while CPx sequences contained admixtures of splenic and brain isolates in 3 of 4 cases and were similar to brain sequences in the 4th case. In addition, we looked for the presence of CPx infection during the asymptomatic period of HIV infection by immunohistochemistry for HIV gp41 and by sequence analysis of the V3 region in DNA extracted from paraffin blocks. We found HIV-infected cells in the CPx in 2 of 7 ASY cases and confirmed the presence of virus in CPx in 1. None of the 7 had HIVE or HIV infected cells in brain although many had mild lymphocytic infiltrates in leptomeninges and perivascular spaces. In contrast, 7 of 14 AIDS cases had HIV-infected CPx and 3 of the 14 had HIVE. These results support the hypothesis that the CPx may be a site of viral entry into the CNS since it contains viruses of brain and spleen genotypes. They also suggest that the CPx may be a viral sanctuary for HIV since virus infects this structure during the ASY period, a time when brain infection is absent. (Supported by NS35331,CKP).