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Retrovirus Antigens in Brains of Mice with Scrapie- and Murine Leukemia Virus-Induced Spongiform Encephalopathy

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Wild mouse ecotropic virus-induced spongiform encephalomyelopathy pathologically similar to scrapie was associated with the expression of retrovirus antigens in mouse brains. However, scrapie-infected mice with spongiform encephalopathy showed no increased expression of retrovirus antigens in brain. Thus, the pathogenesis of the scrapie spongiform lesion does not appear to involve activation of endogenous retrovirus.

The unconventional agents causing scrapie in animals and kuru and Creutzfeldt-Jakob disease in humans produce a spongiform encephalopathy, the pathogenesis of which is still unknown. The spongiform lesions are seen in most but not all experimental and natural hosts and do not appear to be an obligatory correlate of clinical disease (5, 10, 11, 13). Similar spongiform lesions have been observed in mice naturally or experimentally infected with some strains of murine leukemia virus (MuLV) (1, 3, 4, 14, 17). In these MuLV-infected mice, spongiform encephalomyelopathy was associated with the expression of high levels of MuLV p30 and gp70 antigens in the brain (6, 9, 17). Since endogenous retrovirus can be activated after exogenous virus infection (7), we examined the possibility that the spongiform encephalopathy in scrapie-infected mice might also be associated with retrovirus antigen expression in the brain.

NFS/N mice were obtained from the Small Animal Section, Veterinary Resources Branch, National Institutes of Health, Bethesda, Md.

Neonatal NFS/N mice were inoculated 1 day after birth with 0.03 ml of a neurotropic wild mouse ecotropic MuLV (Cas-Br-M) as previously described (9). Symptoms of neurological disease, including tremor and hind limb weakness, and evidence of spongiform encephalomyelopathy developed by 9 weeks of age (9).

Weanling female outbred Swiss Webster mice (Takonic Farms Inc.) were inoculated intracerebrally in the left hemisphere with 0.03 ml of 10% scrapie or normal brain homogenate in phosphate-buffered physiological saline (pH 7.4). Infected mice received 5×10^5 infectious doses of passage 4 of mouse scrapie strain C506.

Mice showing acute clinical evidence of neurological disease were sacrificed, and brains from scrapie-infected mice or brains and spleens from Cas-Br-M-infected mice were stored at −70°C. Pools consisting of brains or spleens from five mice were homogenized in 0.01 M potassium phosphate buffer containing 1% Triton X-100. Ten percent suspensions were diluted serially (1:4 to 1:32) and assayed in duplicate for MuLV p30 and gp70 by competition radioimmunoassay (RIA) (20). Briefly, p30 was determined in assays with iodinated Rauscher MuLV p30 and a goat anti-interspecies p30 obtained from the Division of Cancer Cause and Prevention, National Cancer Institute, Bethesda, Md., or a goat anti-wild mouse MuLV antiserum kindly provided by Suraiya Rasheed and Murray Gardner, University of Southern California, Los Angeles. MuLV gp70 was detected in competition RIA with ^{125}I-labeled Friend MuLV gp70 and a goat anti-feline leukemia virus antiserum prepared as previously described (21). All three assays were performed in replicate on different days, and repeat assays were similar to those presented here.

In both a broad competition RIA with an anti-interspecies p30 antiserum (Fig. 1A) and a narrow assay with an anti-wild mouse ecotropic antiserum (Fig. 1B), Cas-Br-M-infected mice had significant levels of cross-reactive p30 in the brain and spleen, whereas scrapie-infected mice had no more p30 in the brain than did uninfected control mice. In a broad competition RIA with goat anti-feline leukemia virus gp70 antiserum,

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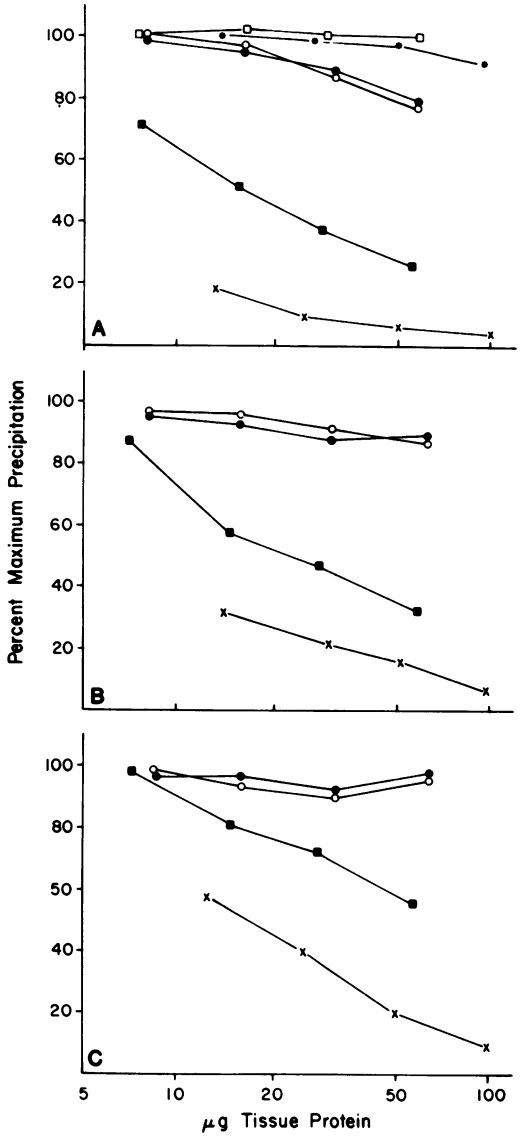

FIG. 1. Detection of MuLV p30 and gp70 related antigens in brain tissue pools from five scrapie-infected (●) and five control (○) Swiss Webster mice and five Cas-Br-M-infected (■) and five control (□) NFS/N mice. Cas-Br-M-infected (X) and control (*) spleens from five NFS/N mice are shown for comparison. Four serial dilutions of tissue extracts were tested in duplicate in competition RIAs for MuLV p30 with ^{125}I-labeled Rauscher MuLV p30 and goat anti-interspecies p30 serum (A) or goat anti-wild mouse ecotropic serum (B). Competition RIAs for gp70 were performed with ^{125}I-labeled Friend MuLV gp70, goat anti-feline leukemia virus serum, and serial dilutions of tissue extracts (C).

Cas-Br-M-infected mice again showed significant levels of cross-reactive gp70 in the brain and spleen whereas scrapie-infected mice showed no cross-reactivity (Fig. 1C).

At the lowest dilution of Swiss Webster mouse brain (scrapie infected or uninfected), small but definite competition in the broad but not the narrow competition RIA for p30 was observed (Fig. 1A and B). This competition was unchanged by sequential absorption of the goat anti-interspecies p30 antiserum with normal NFS/N mouse brain acetone powder, suggesting that the competition could not be attributed to nonspecific cross-reacting brain protein. It seems likely that this competition represents background p30 antigen contained in the brain or in residual serum from brain blood vessels. Normal Swiss Webster mice express low levels of endogenous MuLV antigens in several tissues (15), and we have observed even higher levels of MuLV p30 in the brains of normal AKR and C58 mice (unpublished data), both of which are known to express high levels of endogenous MuLV antigens in other tissues (15).

Spongiform encephalopathy is a principal pathological feature of scrapie, kuru, and Creutzfeldt-Jakob disease, but the pathogenetic mechanism of spongiform degeneration is unclear. The possibility that retrovirus or retroviral proteins were involved in the spongiform lesions of scrapie was suggested by the similarity of the spongiform lesions in MuLV-induced spongiform encephalomyelopathy (1, 3, 4, 14, 17) and the observations that herpesvirus infection enhances endogenous retrovirus expression (7) and lactate dehydrogenase virus infection of mice with high levels of endogenous retrovirus results in an inflammatory polioencephalomyelopathy (18, 19). Although retrovirus virions have been observed in some (1, 3, 4, 14, 17) but not all MuLV-infected brains demonstrating spongiform pathology (14), the relationship of exogenous, endogenous, or recombinant MuLV to spongiform encephalopathy is also unclear (8, 9). In several electron microscopic studies of scrapie mouse brain, no reports of retrovirus virions have appeared (2).

The results of this study support previous observations that MuLV p30 and gp70 antigens are expressed in high titer in the brains of paralyzed MuLV-infected mice (6, 9, 16) and demonstrate that increased expression of MuLV p30 and gp70 does not occur in scrapie-infected mouse brains. The vehicle of transmission in scrapie could be a nonviral, molecular inducer of an endogenous virus rather than a virus particle per se (12). This would not preclude the possibility that the unconventional agents and MuLV may be acting to produce spongiform encephalopathy through similar molecular mechanisms.

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