Histopathology of Serum-Transmitted Immune Polioencephalomyelitis in C58 Mice[1] (37724)

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As C58 mice age, there is a progessive loss in their capacity to be immunized to formalin (1:500)-inactivated syngeneic malignant lymphocytes (line I_b leukemia) (1). If mice ten or more months old are immunized to line I_b cells, they develop an acute autoimmune inflammatory disease of the spinal cord and brain stem that leads to paralysis and death (1). We recently described (2) the histopathology of the actively induced disease and referred to it as immune polioencephalomyelitis (IPE). These basic observations provided the basis for planning a systematic series of studies to determine whether IPE in C58 mice can serve as a suitable model for other age-dependent autoimmune diseases, particularly those of the CNS (2). As a working hypothesis, we assumed that IPE resulted from an autoimmune response of old C58 mice to theta or theta-like antigens (3) common to both line I_b cells and CNS tissues. One apparent paradoxical feature of IPE was its serum-transmissibility. This communication briefly describes the histopathologic features of serum-transmitted IPE and discusses possible mechanisms of its induction.

Materials and Methods The origin of the C58/wm strain of mice was described (1). "Young" and "old" mice were 4 and 12 months old, respectively. Young and old test mice received a 1 ml ip injection of a 1:100 dilution of IPE serum. Serum was diluted in phosphate-buffered saline (1). Control mice received a 1 ml ip injection of the diluent alone. Immune polioencephalomyelitis serum was obtained by immunizing old mice with formalinized (1:500) line I_b cells as previously described (1). Blood was collected at the peak of paralytic disease (10 days after immunization), allowed to clot for 18 hr at 4°, filtered through a 0.22 μm Millipore filter to remove any residual cells, diluted 1:100 with phosphate-buffered saline, and stored in individual ampoules (1 ml) at −70°.

For histologic studies, tissue specimens were obtained from mice from 4 to 13 days after they received a 1 ml ip injection of IPE serum. Specimens of brain, cord, liver, spleen, mesenteric lymph nodes, kidney, heart, and lung were obtained from mice immediately after they were killed with diethyl ether. The vertebral column and spinal cord were removed *en bloc* and transverse sections processed at each 2nd–3rd vertebra. Cross sections of the brain were made at the level of the cerebral hemispheres, midbrain, cerebellum, pons, and medulla. Specimens were fixed in 10% neutral formalin, dehydrated, embedded in paraffin, and sections cut. Sections were stained with hematoxylin and eosin. To detect demyelination, selected specimens of brain and cord were stained by the Kluver-Barrera, luxol-fast-blue, and cresyl violet method (4).

Results. Development of lesions. Beginning four days after the ip injection of IPE serum, two old mice each were killed on days 4, 6, and 8; six were killed on days 10 and 12; four were killed on day 13. Mice of the latter group had severe clinical disease for three or more days before death. The earliest histologic evidence of disease occurred 7–8 days after

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injection of serum. All mice sacrificed later showed typical histologic evidence of disease except 1 mouse which remained disease-free. Among the 12 animals sacrificed on days 10 and 12, pathologic evidence of disease ranged from patchy to confluent CNS lesions, usually confined to the cord. All four animals killed 13 days after the ip injection of serum, and which had had CNS disease for 3 or more days, had extensive lesions both in the cord and brain stem. Serum taken from old or young C58 mice before immunization did not induce disease (1, 2).

Gross and microscopic pathology. The hyperplastic changes in lymphoid tissues that occurred in mice with actively induced CNS disease (1) were minimal in the passively induced disease. There was no histologic evidence of glomerulonephritis or systemic vasculitis nor macroscopic evidence of disease in the cords or brains of mice with CNS disease. The topography of the microscopic CNS lesions was distinctive. Lesions were confined mainly to spinal cord gray matter (Fig. 1). Only mice with severe cord disease had involvement of the brain or spinal cord white matter. Brain involvement was confined to the medulla, pons, and midbrain

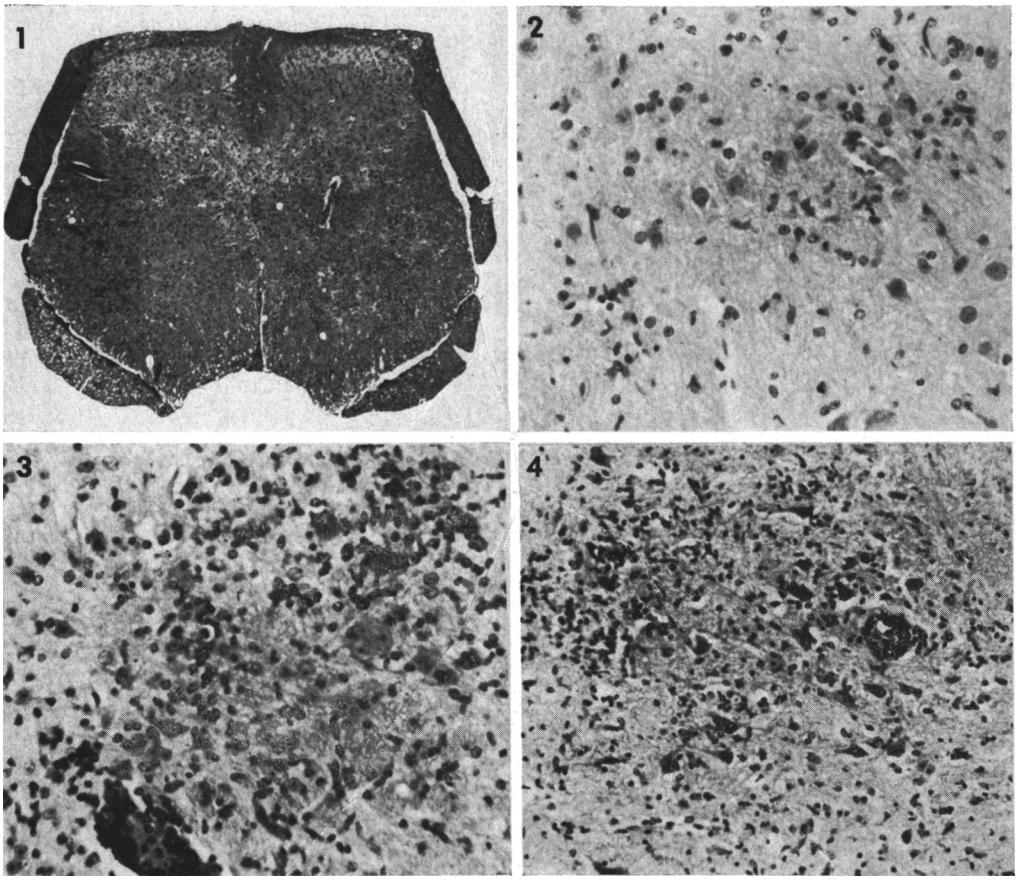

FIG. 1. Spinal cord from an old mouse with IPE. Note the marked involvement of gray matter. White matter is spared except for perivascular lymphocytic infiltrates. ×45.

FIG. 2. Spinal cord from a mouse with mild IPE. Note that cellular infiltration is moderate and neuronal degeneration is absent. ×340.

FIG. 3. Spinal cord from a mouse with severe IPE. Note the neuronal degeneration and that large numbers of lymphocytes (round dark nuclei) and microglia (elongated lighter-staining nuclei) have infiltrated the gray matter. ×340.

FIG. 4. Brain stem from a mouse with severe IPE. ×125. Note the similarity to Fig. 3.

tegmentum; *i.e.*, there was no evidence of disease in the cerebral hemispheres, cerebellum, or meninges. Spinal nerve roots and ganglia also were not involved.

The typical cord lesion was characterized by neuronal degeneration and the infiltration of inflammatory cells. The number of hyperchromatic pyknotic neurons varied directly with the severity of inflammation (Figs. 2, 3) and the duration of disease. Early lesions featured perivascular aggregates of lymphocytes with some neuronal degeneration. More advanced lesions had lymphocytes dispersed throughout the gray matter with extensive neuronal damage. Microglial proliferation also was characteristic. Microglial cells were most numerous in areas of severe inflammation and neuronal destruction. When inflammation occurred in the white matter, inflammatory cells were confined to the perivascular area. Brain stem lesions (Fig. 4) closely resembled cord lesions. No inclusions were seen in the CNS tissue sections. There was no proliferation of astrocytes nor were bizarre astrocytes found. The cerebrum and cerebellum were free of inflammatory changes or histologic evidence of neuronal damage. There was no evidence of demyelination in cresyl violet and luxol-fast-blue stains of appropriate tissue sections.

Age-related tissue changes. Four normal young and old mice, representative of a much larger group examined at various times (1, 2, 5), were studied in detail to determine whether some of the lesions observed were attributable to the aging process *per se.* Neuronal degeneration, as a function of uncomplicated aging, was not found. The most consistent changes in tissues of old C58 mice were focal hepatic necrosis and mononuclear cell infiltration in hepatic portal areas. Hemosiderin accumulation in spleen and lymph nodes also was a consistent finding in old C58 mice. We did not find histopathologic changes in old C58 mice different from those already described (6).

Discussion. Since autoimmune CNS diseases like EAE (experimental allergic encephalitis) ordinarily are not serum-transmissible (7), we initially sought to determine whether the age-dependent CNS disease induced in C58 mice by immunization with inactivated line I_b cells was caused by a latent neurotropic virus or a C-type virus present in serum. The disease could not be actively induced (1) with a C-type virus derived from our strain of mice or by virus-rich spleen cells obtained from C58 mice with spontaneous leukemia. Cell-free tissue preparations from normal old C58 mice did not induce the disease (1) in young C58 or BALB/wm mice. Serum from C58 mice with IPE did not induce the disease in young or old BALB/wm, C_3H/HeJ, C57BL/J, or AKR/J mice. Characterization studies (5) of the active serum factor showed that it had a molecular weight between 64,000 and 150,000, was inactivated by pronase but not RNAse, or DNAse, was stable at 37° but not at 56°, and was not a major immunoglobulin. These data from a number of lines of investigation (1, 2, 5) were consistent with the hypothesis that IPE was an autoimmune disease. Its relevance to human diseases of an analogous histologic pattern and possible pathogenetic mechanisms was discussed previously (2).

Two features of IPE that appear to be paradoxical are its age-dependence and serum-transmissibility. In recently completed studies, conclusive evidence was obtained (Martinez, Sager, and Murphy, unpublished data) that IPE can be induced in appropriately X-ray- or drug-immunosuppressed young mice. These results are in accord with the view that drug- or age-induced immunosuppression can break tolerance (8) so that mice can respond immunologically to antigens in common between line I_b cells and CNS tissue. The current studies show that the inflammatory nature of the CNS lesions in serum-transmitted IPE did not differ significantly from the actively induced disease in terms of the topographical distribution or the time sequence of lesion development. Results from parallel studies designed to characterize the active serum component (5) indicate that it may be line I_b antigen (processed or not) or a lymphokine produced in the immune response of sensitized animals. In brief (5), serum taken from either young or old C58 mice was positive within 24 hr after immunization and remained positive for up to 15 days. Thus, it is the reactivity of old mice to im-

munization (occurrence of IPE) that distinguished them from young mice. In the context of the current paper, it is important to recognize that "serum transmissibility" means transmission due to a serum factor that appears to be line I_b cell antigen or a lymphokine, *i.e.*, not an immunoglobulin. Thus in studies of the serum transmissibility of autoimmune disease, it is important to distinguish between serum transfer of immunoglobulins, sensitizing antigens, or lymphokines, or other serum factors.

Summary. A histopathologic study of serum-transmitted immune polioencephalomyelitis in C58 mice showed that the inflammatory nature of the CNS lesions in the serum-transmitted disease did not differ significantly from the disease actively induced by inactivated syngeneic malignant lymphocytes. The histopathology was characterized by perivascular lymphocytic infiltrates in the gray matter of the spinal cord and brain stem with accompanying microglial proliferation and neuronal destruction. Demyelination, inclusion body formation, astrocyte proliferation, and cerebral and cerebellar involvement were absent. Evidence was summarized indicating that the active serum component was not an immunoglobulin but probably a processed antigen or a lymphokine. The disease appears to result from an age-dependent autoimmune response to theta or theta-like antigens common to CNS tissues and lymphoid cells.

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