Behavioral Disease in Rats Caused by Immunopathological Responses to Persistent Borna Virus in the Brain

Abstract. *Borna virus replicated persistently in the brains of rats, causing frenzied and apathetic behavioral states in sequence but no mortality. The transient frenzied behavior was caused by an immune-mediated, cytolytic, encephalitic response that was unexpectedly self-limiting. Cessation of active pathological processes coincided with the onset of the passive phase of the disease. This study thus demonstrates suppression of virus-specific inflammation despite continuous viral replication and describes a new mechanism by which chronic encephalitis may become established.*

Borna virus is the cause of a rare paralytic and fatal encephalitis in horses and sheep in certain regions of Germany and Switzerland (*1*). Experimentally, many species of animals can be infected with this virus, with results that vary from acute fatal encephalitis in the rabbit (*2*) to chronic encephalitis, with social and behavioral abnormalities, in *Tupaia*, a subhuman primate (*3*).

Borna virus does not share biological properties with agents from any known virus group (*4*). It replicates slowly in cell culture, causing a persistent noncytopathic infection and spreading mainly by cell-to-cell contact. Intranuclear immunofluorescent viral antigens can be seen in these cultures when reacted with serum from a diseased animal. This fluorescent antibody test is used for measuring both infectivity and antibodies in tissue culture (*5*). The virus is almost exclusively neurotropic in infected animals. It replicates in neurons and astrocytes and disseminates throughout the nervous system in a manner similar to its behavior in cell culture (*6*).

To further explore the potential of the virus to cause chronic behavioral disease, we inoculated mice and rats with the virus. Mice were resistant to infection but rats were uniformly susceptible and developed a persistent, productive viral infection in the central nervous system and behavioral disease characterized by an initial frenzied, aggressive phase that gave way to an inactive, passive state. The disease was caused by the inflammatory cell response to the virus, which selectively infected neurons in the retina and cerebrum. The changes in behavior were associated with a unique modulation of the inflammatory response in the infected brain.

About 200 Lewis rats 4 to 5 weeks old were inoculated intracerebrally with Borna virus. Some were kept for long-term observation while others were killed sequentially for examination of tissues. The first abnormalities were noted about 20 days after inoculation, when the rats became very alert. During the next few days alertness progressed to a state of frenzy, in which the animals showed exaggerated motor responses to minor stimuli. They seemed disoriented and their running and jumping lacked coordination, but none became paralyzed. Some became aggressive and constantly attacked their cage mates. Most developed ravenous appetites, approaching their food and water in the same frenzied manner. Many males had constant erection (priapism). This behavior lasted until day 30 or 40 and then gradually receded. By day 60 behavioral patterns had undergone a reversal from their state in the previous month. The animals became passive and showed progressively less activity during the ensuing weeks. Most were blind by day 100. Some became obese, while others became emaciated despite constant eating. Priapism and the propensity to bite persisted in many animals despite their lethargy. The rats maintained this apathetic behavior for the remainder of the 7-month study, showing no interest in the environment or their cage mates. Except for victims of biting, deaths were very rare.

Sequential examination of tissues showed that virus replication was largely confined to the brain and eyes, with lower levels of infectivity in the spinal cord. The highest titers of virus in the brain were found in the cerebral hemispheres. Nonneural tissues, including lymphocytes, did not contain virus (*7*). As shown in Fig. 1, a high level of virus production in the brain was maintained throughout the study period. The rats developed antibodies in their serum and cerebrospinal fluid, but these immunoglobulins did not neutralize the agent (*7*).

Histological examination of tissues showed that approximately 20 days after inoculation the rats began to develop meningoencephalitis and retinitis consisting of mononuclear cell infiltrations around blood vessels and in the neuropil. The bulk of the inflammatory response was centered around blood vessels in the retina and in the cerebral cortex, thalamus, and hypothalamus, corresponding to the areas of greatest virus production. The inflammation reached its most intense level between days 30 and 40 (Fig. 2), corresponding in time with the development of the hyperactive phase. The inflammation was accompanied by neuronophagia, and there was a gradual loss of neurons from the inner and outer nuclear layers of the retina as well as from the cerebral cortex. The most extensive cell loss was from the frontal to the temporal cortex. Loss of retinal neurons led to blindness, and loss of brain substance led to hydrocephalus ex vacuo (Fig. 3). Despite continuous productive virus replication in the brain, the inflammatory response began to decline after day 50 and continued until only minimal inflammatory lesions could be detected in the brain (by day 200). There was no further loss of brain substance after the inflammatory cells disappeared. The quiescent phase of the disease coincided with a decline in encephalitis without a corresponding decline in infectivity (Fig. 1).

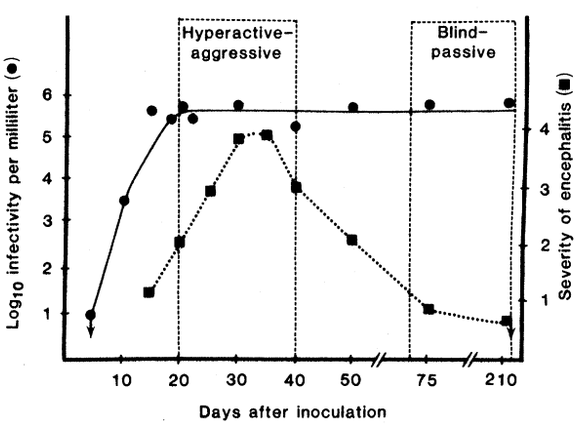

Fig. 1. Sequential effects of Borna virus infection in rats. Two to six rats were killed at the indicated intervals and portions of each brain were examined for infectivity and lesions. Infectivity was measured by inoculating replicate cultures of rabbit glial cells with serial tenfold dilutions of brain homogenates (10 percent, weight to volume). The indicator cultures were examined for virus-specific immunofluorescence 10 days later by using immune Borna virus antiserum (*3*). For histological studies, brain tissue was fixed in 10 percent Formalin and embedded in paraffin. Sections (8 μm) were stained with hematoxylin and eosin and examined by light microscopy. Eyes were fixed in Zenker fluid and examined similarly. Inflammation in the brain was graded on a scale of 1 to 4, with 4 being the most severe (Fig. 2).

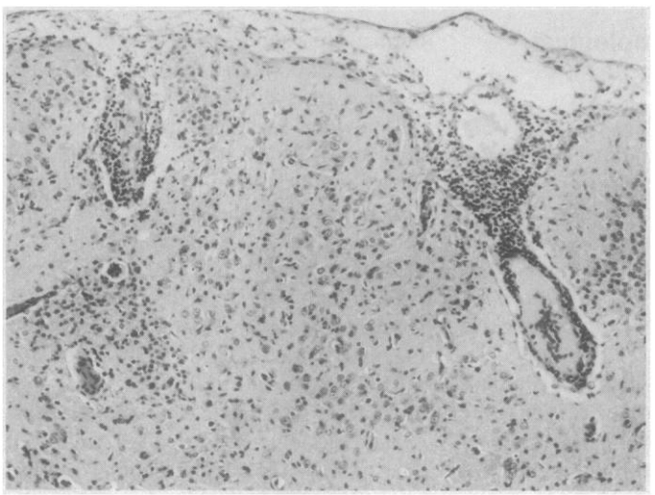

Fig. 2. Section of brain from a rat killed 30 days after inoculation, showing severe mononuclear cell infiltration in the leptomeninges and neuropil (×200).

To test the role of the immune response to the virus in the causation of inflammatory lesions and disease, we inoculated two new groups of rats with the virus and examined them as before. One group was inoculated with virus at 1 day of age, when they were immunologically immature. The other group was inoculated at 4 weeks of age and given cyclophosphamide (150 mg/kg, intraperitoneally) 1 day later—a classic method of inducing immunological tolerance (*8*). Again, the virus replicated persistently and productively in the central nervous system in both groups (*7*). However, none of the animals in either group became ill; they did not develop retinitis, encephalitis, or hydrocephalus. Whereas the newborn animals developed high levels of antibodies, the cyclophosphamide-treated group developed neither antibodies nor encephalitis up to 40 days after treatment. These results suggest that the inflammatory cells were responsible for the disease and that antibodies did not play a role.

We confirmed the immune basis for the disease in experiments in which we transferred spleen cells (*9*) from 4-week-old rats at various intervals after infection to infected 4-week-old animals treated with cyclophosphamide. These cells had no effect when injected into immunologically normal rats. However, cells taken from rats 6, 14, and 30 days after inoculation were effective in inducing antibody production, encephalitis, and behavioral disease in the tolerant recipients. In contrast, cells taken from rats 75 days after inoculation reconstituted only antibody production. These recipients did not develop encephalitis or otherwise become ill. Thus, the cells that caused disease were derived from animals in a preencephalitic stage or with active encephalitis, whereas the cells that failed to cause encephalitis were derived from persistently infected animals that had recovered from acute encephalitis. Cytotoxic cells apparently were generated early in the infection, but at the peak of inflammatory disease the rats presumably produced factors that suppressed further generation of these cells. Since antibody production was normal in recipients of cells from rats with chronic disease, this suppressor activity was probably directed only to the effector T lymphocytes, explaining the lack of disease potential of these cells after transfer.

In summary, the pathogenesis of Borna disease in rats seems to depend on three factors. First, not only was the virus neurotropic but it apparently had a selective tropism for neurons in the limbic system, in which virus replication occurred without causing significant viral cytopathology. Second, the virus-specific cellular immune response characterized by encephalitis had a role in the induction of behavioral disease. This pathogenesis closely resembles that of lymphocytic choriomeningitis virus infection in mice with respect to the role of immunopathological responses (*10*). The third factor and the most intriguing is the unexpected decline in virus-specific inflammation despite the constant production of viral antigen in the persistently infected brain. This delayed tolerance, which was activated in such a reproducible and predictable manner, has not, to our knowledge, been observed in other viral infections and constitutes a new mechanism by which chronic encephalitis may become established. The fact that these events were reflected clinically only by changes in behavior provides a new perspective for evaluating pathological behavior and suggests that in some cases such behavior could originate from persistent viral infections of the brain.

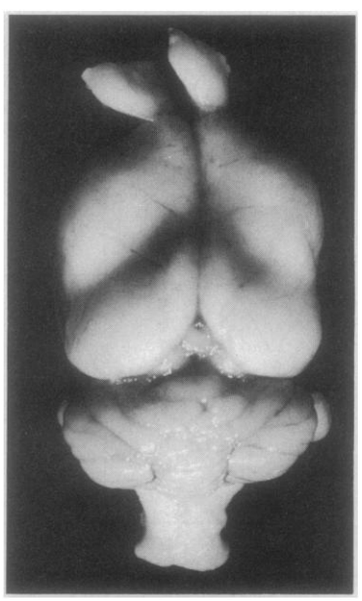

Fig. 3. Brain of a rat killed 200 days after inoculation, showing collapse of the cerebral hemispheres due to loss of brain substance earlier in the infection.

Although a role for this agent in human disease is speculative, a similar disease, characterized by sequential acute and chronic encephalitis with hydrocephalus and dementia, has been observed among isolated peoples in the Soviet Union (*11, 12*). Given the wide host range of this agent, a search for an association between a Borna-related agent and human psychosomatic disease should be undertaken.

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References and Notes

1. A. Mayr, *Ann. Inst. Pasteur Paris* **123**, 545 (1972); A. Metzler, U. Frei, K. Danner, *Schweiz. Arch. Tierheilkd.* **118**, 483 (1967).
2. W. Zwick and J. Witte, *Z. Infektkrankh. Parasit. Krankh. Hyg. Haustiere* **30**, 42 (1927); M. Gierend and H. Ludwig, *Arch. Virol.* **67**, 217 (1981).
3. H. Sprankel, K. Richarz, H. Ludwig, R. Rott, *Med. Microbiol. Immunol.* **165**, 1 (1979).
4. A. Mayr and K. Danner, *Proc. Soc. Exp. Biol. Med.* **140**, 511 (1972); H. Ludwig, H. Becht, L. Groth, *Med. Microbiol. Immunol.* **158**, 275 (1973).
5. S. Herzog and R. Rott, *Med. Microbiol. Immunol.* **168**, 153 (1980).
6. H. Ludwig and H. Becht, in *Slow Virus Infections of the Central Nervous System*, V. ter Meulen and M. Katz, Eds. (Springer-Verlag, New York, 1977), p. 75; H. Krey, H. Ludwig, R. Rott, *Arch. Virol.* **61**, 283 (1979).
7. S. Herzog, O. Narayan, R. Rott, in preparation; O. Narayan, S. Herzog, K. Frese, H. Scheefers, R. Rott, *J. Infect. Dis.*, in press.

8. N. Nathanson and G. Gole, *Adv. Virus Res.* **16**, 397 (1970).
9. Spleens were removed aseptically from donor rats and placed in petri dishes, and the splenocytes were teased into Dulbecco's minimum essential medium containing heparin (5 I.U. per milliliter). The cells were passed through a stainless steel screen (100-μm mesh), washed once by centrifugation, and injected intraperitoneally into recipients on a basis of one spleen equivalent per recipient.
10. D. H. Gilden, G. A. Cole, A. A. Monjan, N. Nathanson, *J. Exp. Med.* **135**, 860 and 874 (1972).
11. P. Petrov, *Am. J. Trop. Med. Hyg.* **19**, 146 (1970).
12. D. C. Gajudsek, *Adv. Neurol.* **36**, 363 (1982).
13. We thank H. Becht for helpful discussions. Supported by the Deutsche Forschungsgemeinschaft (Sonder forschungsbereich 47). A guest professorship awarded by the DFG to O.N. while on sabbatical at the Giessen Institut für Virologie is also acknowledged.
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Ecology and Catastrophic Mortality in Wild Horses: Implications for Interpreting Fossil Assemblages

Abstract. *The identities, sexes, and reproductive status of groups of wild horses (*Equus caballus*) living in the Great Basin Desert of North America were known prior to their deaths on ridgelines. Another group of very young horses died on a quagmire. Snow accumulation or drought was apparently responsible for the mass deaths. These data have implications for reconstructing some aspects of the social structure of fossil mammals on the basis of skewed sex or age ratios in bone assemblages.*

Socioecological interpretation of fossil mammalian assemblages is a fairly recent field of study (*1*), although grouped remains of fossil bison, camels, pigs, rhinos, prongbucks, horses (*2*), and dinosaurs (*3*) have been used as evidence of complex social structures because of biased sex ratios or adult females situated in proximity to young. Such group remains potentially provide information about social structure of a species. Because groups varying in sex or age composition can become fossilized in the same quarries for many different reasons (for example, death traps), realistic grouping patterns may be obscured. Data on sex, age, and reproductive status of wild (feral) horses (*Equus caballus*) that died in groups or individually on high altitude windswept ridges in the Great Basin Desert of North America indicate that (i) snow and mud are prominent forces influencing mortality and (ii) death in groups may be more common than has been assumed. This information has implications for determining some patterns of mortality in extant wild horse populations and assessing the validity of paleoethological reconstructions of social groupings.

Horses played prominent roles in community dynamics during the middle and late Tertiary in North America (*4*). Today, feral horses are conspicuous elements of the mammalian fauna of the Great Basin, where an estimated 35,000 to 40,000 range. Since 1979 I have studied populations of horses in the Granite Range of northwestern Nevada. Most horses migrate seasonally to the highest peaks, some of which exceed 2780 m. They are organized into distinct year-round bands, each with at least one stallion and a harem (females and young) (*5*). Nonharem males live alone or form bachelor groups.

The studied population has increased from 58 to 129, and 15 horses (yearlings or older) have disappeared. All individuals have been identified and the ages of more than 90 percent are known (*6*). Of the 15 animals that disappeared, the bodies or skulls of one bachelor male (9 years), three stallions (15+, 13, and 10 years), two younger males (2 and 3 years), five females (12, 6, 5, 5, and 4 years), and a male yearling have been located. Remains of nine of these animals (75 percent) were discovered along windswept ridgelines (Fig. 1A) at about 2600 m. From hair fragments of tails, manes, and fur, the animals were identified as members of two bands, last observed alive during the summer of 1979. One band of six animals (a male, 4 females, and a juvenile) was found in June 1981, and the other with three horses (two females and a juvenile), which had been part of a band of 12, was found in June 1982.

Three lines of evidence suggest not only that these two groups perished as a result of severe winter snowstorms but also that high altitude snow-induced mortality may be more common than has been assumed among some populations of mountain dwelling wild horses. First, because of their ages (*7*), one would not expect the dead animals to be in poor condition, and when last observed alive they appeared to be healthy and vigorous. Second, 80 to 85 percent of the animals migrated every year from winter ranges at low altitudes (around 1400 m) to higher altitudes for the summer and fall, where some remained into winter. The bands observed at high-altitude sites (2100 m, 2470 m, and 2560 m) during December 1980 and January 1981 were not subordinate bands and, thus, social factors did not appear to explain why they remained in summer ranges into early winter. Although it is likely that

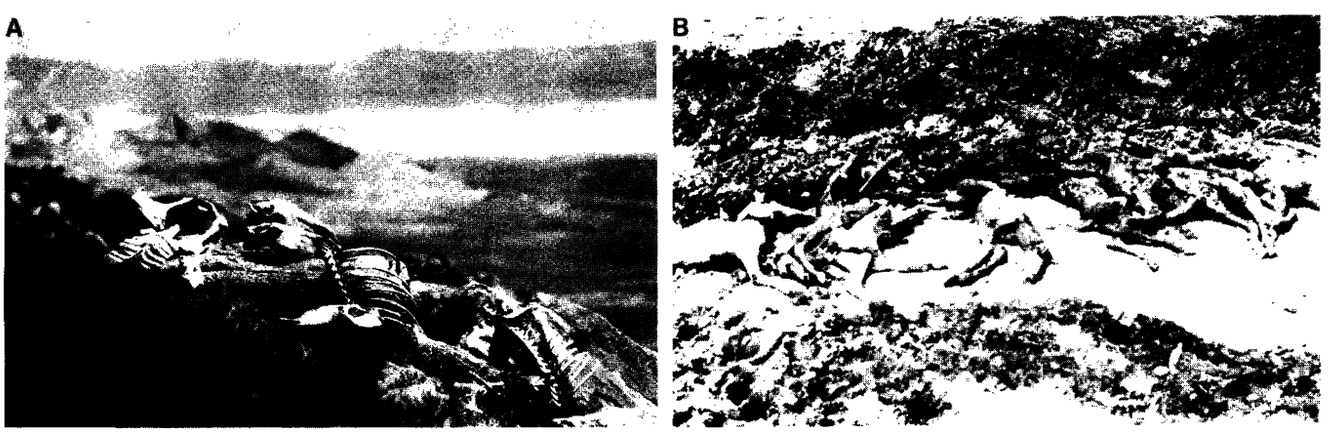

Fig. 1. (A) Skulls and skeletons on a ridge (about 2600 m) in the Granite Range, Nevada. Two of the skulls were removed from underlying vegetation and placed on rocks. (B) Foals in the Owyhee Desert, Nevada. Animals were pulled from the quagmire. [Desert photograph courtesy of the U.S. Department of the Interior]