

BORNA DISEASE VIRUS AND MENTAL ILLNESS

by W. Ian Lipkin, M.D.

The pathogenesis of schizophrenia and affective disorders remains unknown. Although a genetic component is likely, discordance for disease in monozygotic twin studies and epidemiology suggest the presence of co-factors. One candidate risk factor is *brain infection*. Indeed, an infection hypothesis has been proposed throughout the history of psychiatry by prominent investigators including Esquirol, Bleuler, Kraepelin, Menninger and others. This hypothesis fell into disfavor when classical methods for isolation of infectious agents failed to consistently identify pathogens in brains of affected subjects; however, recent advances in molecular microbiology have rekindled interest in an infection-based model for mental illness.

Establishing a causal relationship between infection with a microbial agent and a specific brain disease can be complex. In some instances, for example herpes simplex encephalitis, the agent is readily implicated: the virus is present

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in brain and destroys infected tissue through replication. Alternatively, tissue damage and disease may be the indirect result of a host immune response to microbial gene products present in neural cells. Immune responses to microbial agents can also lead to breakdown of tolerance to host antigens and result in brain damage. The agent responsible for induction of autoimmunity need not be present in brain at the time of clinical presentation. Furthermore, the original infection may have been peripheral, as is the case in Sydenham's chorea, a brain disorder associated with rheumatic fever. Yet another mechanism for brain disease is persistent noncytopathic viral infection. Such infections can profoundly impact neurotransmitter function or brain development yet remain cryptic unless

specific reagents are used for detecting viral gene products.

Several viruses have been implicated in the pathogenesis of human neuropsychiatric diseases including herpes simplex virus, cytomegalovirus, measles, mumps, bovine viral diarrhea virus, influenza virus and Borna disease virus (BDV). Although some viruses like herpes simplex and measles are clearly associated with sporadic cases of psychosis or dementia, respectively, and others like influenza are historically associated with epidemic psychosis, there is no linkage established between infection with any of these agents and either affective disorders or schizophrenia.

Borna disease virus is an RNA virus, worldwide in distribution, that causes movement and behavior disturbances in warmblooded animal hosts ranging from birds to primates. It replicates at lower levels than most known viruses and is dissimilar in nucleic acid and protein sequence to other infectious agents. Thus, BDV eluded characterization until its nucleic acids were cloned by subtractive hybridization. It is recognized as a nonsegmented negative strand RNA virus (order Mononegavirales) but is not yet formally classified and is likely to represent the prototype of a new virus family, *Bornaviridae*.

BDV pathogenesis is most commonly studied in rats because the disease induced in this model system is similar to that observed in naturally infected hosts. Adult rats have an immune-mediated disease that presents clinically

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as hyperactivity and exaggerated startle responses coincident with the appearance of viral proteins in limbic system neurons and infiltration of monocytes into the brain. The inflammation recedes over a period of several weeks but the virus persists. Rats later have stereotyped motor behaviors (the continuous repetition of behavioral elements), dyskinesias, dystonia and flexed seated postures. The extrapyramidal movement and behavior disorder are linked to distinct changes in CNS dopamine systems: (1) infected rats are more sensitive to dopamine (DA) agonists than normal rats; (2) the movement and behavior disorder is improved following treatment with selective DA antagonists; (3) there is partial DA depletion and compensatory metabolic hyperactivity in nigrostriatal and mesolimbic DA systems; (3) D2 and D3 (but not D1) receptor binding is markedly reduced in striatum, prefrontal cortex and nucleus accumbens.

Rats infected as neonates do not mount a cellular immune response to the virus and have a different disease, characterized by hyperactivity, subtle learning disturbances and altered taste preferences. Two studies are reported of BD in experimentally infected primates: tree shrews and rhesus monkeys. Infected tree shrews have disturbed social and sexual behaviors, manifest as abnormal dominance relationships and failure to reproduce. Infected rhesus monkeys are initially hyperactive and subsequently become apathetic and hypokinetic.

Recognition of BDV's broad host and geographic range led to the proposal that it might cause human neuropsychiatric disease. Because the behavioral disturbances in the rat model were considered to be reminiscent of affective disorders, particularly bipolar depression, initial studies were targeted toward these disorders. Over the past 10 years investigators in the United States, Germany and Japan used several methods for serology including indirect immunofluorescence assays of infected cells, western immunoblot of proteins expressed in infected cells or as

recombinants in prokaryotic or baculovirus systems, ELISA and radioimmunoprecipitation. All published reports noted an increased prevalence of antibodies to BDV proteins in patients, however, prevalence rates varied from 1.6–38% in patients and 1–16% in controls. With the objective of establishing a correlation between immunoreactivity to BDV and the duration and severity of psychiatric disease, Bode et al. performed serial IFT on sera from 70 German patients with a variety of diagnoses including minor or major depression, paranoid psychosis, schizophrenia, anxiety disorder and personality disorder. Overall, the prevalence of immunoreactivity to BDV was greater than 20%, a marked increase from the 2–4% found in the earlier study by assay of each subject at one timepoint. Thirty-seven percent of major depressives, 25% of paranoid psychotics, but only 6% or less of patients with reactive depression and other neurotic conditions were seropositive by day 17 of illness.

Because BDV is found in peripheral blood mononuclear cells (PBMC) of persistently infected rats, PBMC of these patients were examined for the presence of viral antigens by fluorescence activated cell sorting (FACS) analysis. More than 40% of subjects with neuropsychiatric disease were found to be antigen carriers, twice the number predicted by the previous serologic survey.

Other investigators focused on potential associations between BDV and schizophrenia. Waltrip et al. examined the prevalence of antibodies to three viral proteins in 90 American schizophrenic subjects and 20 controls by western blot analysis. Over 14% of patients and no controls had antibodies to two or more viral proteins. This definition of immunoreactivity was significantly associated with abnormal brain morphology to magnetic resonance image analysis (MRI) and the clinical diagnosis of deficit syndrome, a schizophrenia subgroup characterized by social withdrawal, neurological dysfunction and neuroanatomic abnormalities. Similar findings were reported by Bechter et al. indicating a

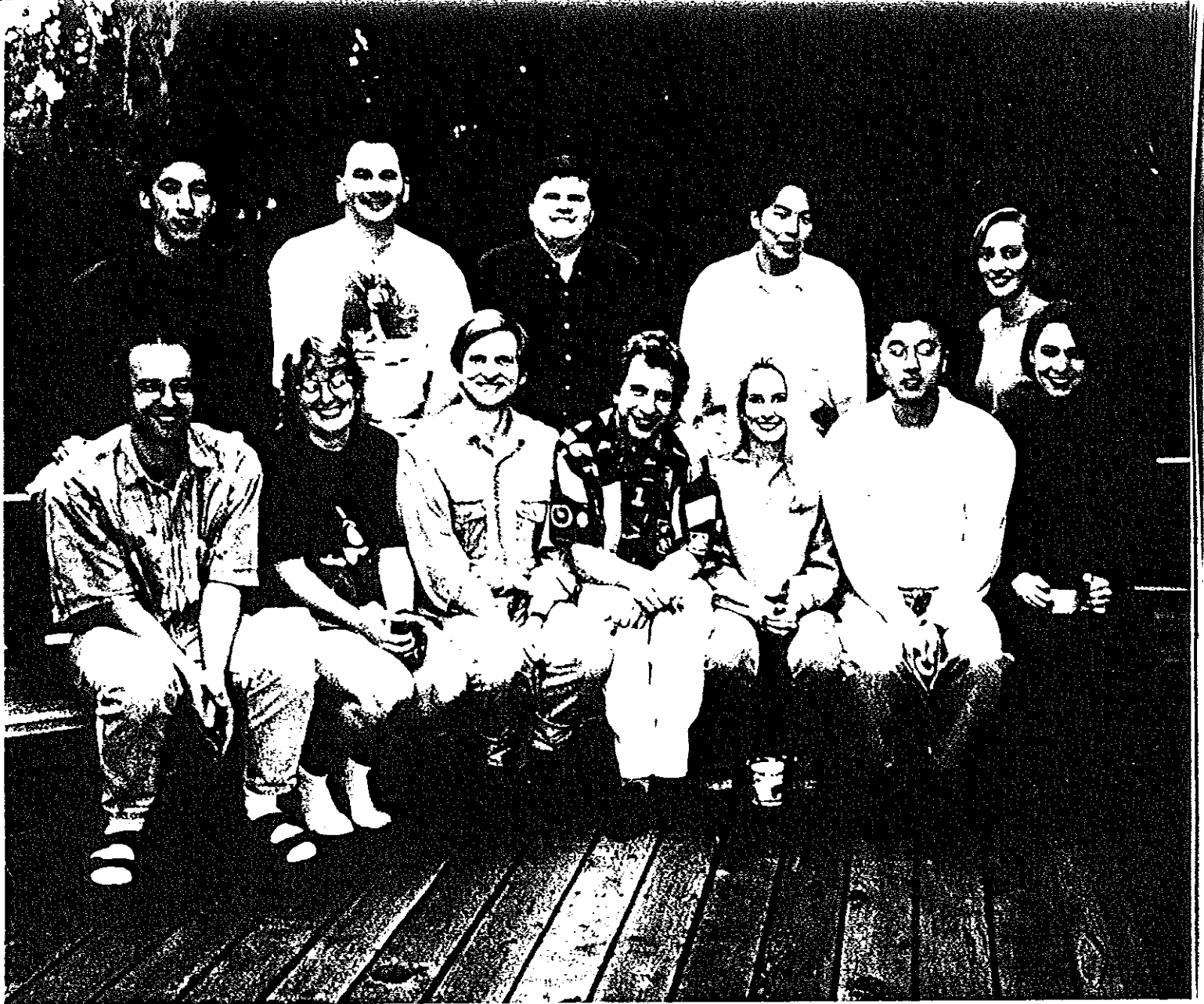
correlation in German schizophrenic patients between the presence of antibodies to BDV and MRI evidence of cerebral atrophy.

The advent of BDV molecular biology provided new, sensitive tools for epidemiology. Unfortunately, results have been inconclusive. Yolken and colleagues examined brain tissue and cerebrospinal fluid from schizophrenic subjects using a sensitive method known as reverse transcription polymerase chain reaction (RT-PCR) and found no viral nucleic acids. Included in this group were 9 sets of monozygotic twins discordant for disease. Similarly, examining PBMC of neuropsychiatric patients by RT-PCR, Richt and colleagues found no evidence of infection in 10 schizophrenic subjects from Philadelphia or 16 schizophrenic subjects and 9 affective disorder subjects from Ulm Germany. In contrast, two groups in Germany and one in Japan have independently reported isolation of viral nucleic acid from PBMC of patients with assorted neuropsychiatric diseases.

In Germany, Bode and colleagues found BDV nucleic acids in PBMC of 4 of 6 patients: 1 subject with obsessive compulsive disorder, 2 with major depression and one organic mood disorder. In a larger survey in Japan, Kishi et al., reported prevalence rates for BDV nucleic acids in PBMC of 37% and 6.5% in 60 neuropsychiatric patients and 77 controls, respectively. Sauder and colleagues examined PBMC of neuropsychiatric patients from Homburg Germany by RT-PCR and found BDV sequences in 13 of 26 subjects. BDV sequences are now also reported in PBMC of Japanese subjects with chronic fatigue syndrome, and in hippocampus of 4 of 5 U.S. patients with diagnosis of hippocampal sclerosis.

If BDV does indeed infect humans, the source and routes for that infection are not clear. Naturally infected horses, sheep, cattle, cats and birds could serve as reservoirs for the virus, however, no detailed epidemiology has been done in animal populations and there are no studies that demonstrate transmission from domestic animals to humans.

At present there are at least 10 groups



Dr. Lipkin seated, third from the left.

internationally trying to determine whether BDV or a related agent can be linked to human neuropsychiatric disease. However, to date there has been no blinded multicenter analysis of samples from patients with neuropsychiatric diseases and controls using standardized methods for serology or molecular biology.

A Multi-Center Project consisting of laboratories actively engaged in BDV research (W. Ian Lipkin, University of California, Irvine; Hilary Koprowski, Thomas Jefferson University; Juan Carlos de la Torre, The Scripps Research Institute; Kathryn Carbone, Food and Drug Administration; Royce W. Waltrip II, University of Maryland) and clinical centers with expertise in affective

disorders and schizophrenia research (Steven Potkin and William Bunney, University of California-Irvine; Mady Hornig-Rohan and Jay Amsterdam, University Pennsylvania; Micheal Egan, St. Elizabeth's Hospital/National Institute of Mental Health), has been established under the auspices of the Stanley Foundation which represents the first concerted effort to rigorously address the epidemiology of BDV in human populations.

The Project will determine the prevalence of serum antibodies to BDV in patients and controls, the extent to which the various assays for antibodies are in accord and whether the presence of antibodies reactive with individual BDV proteins in any assay varies with

clinical course. The Project will also determine the prevalence of BDV nucleic acids in brains and PBMC of patients and controls, and whether the presence of viral nucleic acids can be correlated with serology or clinical course.

In the event that a link is established between BDV and neuropsychiatric disease we will be poised to explore potential strategies to prevent and treat illness due to Bornaviral infection. Future work would include identification of animal reservoirs for BDV and mechanisms for transmission of the virus to humans, vaccine development, cloning of human Bornaviral genomes and therapeutics for BDV infections. ■