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NEWS AND COMMENTARY

Detective work in neuropsychiatric disease

The game's afoot: seeking viruses that cause chronic and degenerative neurologic and psychiatric disorder

MBA Oldstone

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A major discovery of the last half century is that, in addition to the well-defined acute diseases viruses produce such as measles, poliomyelitis, yellow fever, flu, smallpox, and so on, viruses can also cause persistent infections in humans. Indeed, the many millions of humans who have lifelong viral infections represent both a major health issue for the 21st century, but also a unique opportunity for investigative virologists, the virological detectives of this era.

Persistent viral infections come in two flavors. First is the expected proof that chronic inflammation and associated tissue injury follow infection as with Hepatitis C virus. The second and unexpected evidence came from an experimental model in the early 1980s of lymphocytic choriomeningitis virus infection in its natural murine host that, without causing noticeable tissue damage, viruses can disrupt normal homeostatic functions like growth, cognitive learning and immune regulation by interfering with production of hormones, neurotransmitters, cytokines and the formation of antibody- or cell-mediated T cell responses.[1–3] This latter phenomenon consists of minimal or negligible structural damage to the infected cells and enables those cells to escape immune detection. For this type of a persistent infection to endure, two ingredients are required. For the first, viruses engage in a unique strategy of replication; instead of killing the cells they infect the pathogens cause little-to-no damage, yet set up lifelong residency inside such cells. This often occurs in cells specialized to perform specific physiological functions. The continuous replication of viruses' foreign genes then alters the differentiated function of the specialized cells, where they reside without disturbing the cells' housekeeping or vital genes required for survival.[3] With the second ingredient, the persisting viruses abort or modulate the immune response so it does not recognize and/or react to infected cells, thereby also allowing the viruses to survive undetected. Overall, these findings illuminate the possibility that a multitude of biochemical alterations, chemical aberrancies and cognitive dysfunctions, currently of unknown etiology, enable infecting viruses to escape the hallmarks, for example, fever, inflammation, tissue injury, usually in place to warn the physician, pathologist or epidemiologist of a viral infection.[3]

To evaluate whether such hormonal, immunological or neuropsychiatric disorders were associated with a persistent viral infection(s) required an armamentarium of novel and sophisticated approaches and techniques to uncover viral genes and/or viral gene products, and discriminate those findings from normal host genes and their products. This issue's paper from Ian Lipkin's laboratory, 'Absence of evidence for bornavirus infection in schizophrenia, bipolar disorder and major depressive disorder,' by Hornig *et al.*[4] displays the usage of such an approach and the controls required to critically define or refute that a pathogen is the cause of a chronic psychiatric disorder.

Borna disease was first described as episodes in Germany in the Saxony town of Borna during the late 1800s when horses behaved erratically. Not until the 1920s, however, did transmission experiments show that the infectious agent from infected horses passed to multiple species of animals. The experimental tactic was testing material from the infected horses for passage through filters too narrow to retain bacteria, but sufficient for collecting virus. Identification of the collected viruses thereby indicated that Borna disease was caused by a virus (Borna disease virus (BDV)). However, it was 50–60 years later that heightened interest in BDV infection developed following reports that BDV infection in tree shrews and in rats was accompanied by inappropriate grooming and nursing behavior (tree shrews), and by a biphasic phenomenon of hypermotility and excitability followed by depressed locomotion (rats). The odd behaviors suggested schizophrenic disorders in the former animals and a biphasic disease with similarities to bipolar disorder in the latter. These actions mimicked human neuropsychiatric disorders and led to a plethora of investigations to evaluate the possibility that BDV infection of humans was associated with human psychiatric diseases.[5–8]

But what was one to make of the morass of results supporting or rejecting the BDV/human neuropsychiatric finding and the controversies that followed (reviewed in ref. 9). As the philosopher Sherlock Holmes stated, 'It is a capital mistake to theorize before one has data.',from the unequivocal data collected and reported in this issue by Hornig *et al.*[4] using careful, controlled and extensive studies of blinded serological and molecular analyses coupled with standardized

methods for clinical assessment, one finds little evidence to support a correlation between BDV and human psychiatric illness.

The senior author of this report,[4] Lipkin, pioneered the use of purely genetic methods of subtraction hybridization as a probe for pathogen discovery, which allowed a detailed description of the BDV genome and analysis of its gene order.[10] This was accomplished by obtaining virus-specific complementary DNAs from diseased brain tissue and using subtractive hybridization against the background of uninfected brain tissue. He then looked for a relationship between the viral genes and the disease by *in situ* hybridization. Others also obtained the BDV genome at this time and showed a connection with human behavioral diseases.[8] With the viral gene on hand, Lipkin *et al.*[9,10] definitively located BDV in cells and determined its transcription, replication and genomic sequence. The result was to establish BDV as the first member of a new family, Bornaviridae, in the order of Mononegavirales. Temporally, the uncovering of the BDV sequence occurred with the development of PCR. Both complimentary techniques, along with serology, now made it possible to critically evaluate the association of BDV within human tissue in patients with neuropsychiatric diseases.

In the current report, Hornig *et al.*[4] addresses the controversial issue of a possible association of BDV infection with psychiatric illness using samples from multiple centers involving a well-defined patient population and carefully matched control cases, coupled with strict protocols for sample collection and experimental procedures to detect BDV markers (antibodies and RNA). The experimental design and its execution are excellent and represent a true milestone among the other less controlled studies. The results obtained provide strong evidence that markers (antibodies and RNA) of BDV exposure were absent from the peripheral blood of all subjects (patients and controls) examined, thus strongly questioning any role for BDV in the pathogenesis of schizophrenia, mood disorders and other neuropsychiatric illnesses.[4]

The authors present reasonable arguments to account for the differences they report in this issue,[4] with other previously published reports that suggested an association of BDV infection with psychiatric illness. One could argue that a variety of reasons, including subject recruitment and sampling procedures, may have contributed to the inability to detect BDV markers in the individuals examined in this study. For clarity, there is a need for a better exchange of samples from multiple laboratories. Nevertheless, the design and experimental procedures carried out in the Hornig[4] study provide a gold standard for future investigations of the suspected association between persisting viral infection and human disease. With caution, the authors correctly acknowledge that, although their study did not provide evidence of an association of BDV infection with neuropsychiatric disease, it may still be feasible that certain psychiatric disorders could be attributed to a virus(es). Such associations, if they occur, between viruses and neuropsychiatric diseases await future, carefully controlled studies.

Conflict of interest

The author declares no conflict of interest.

MBA Oldstone is at Department of Immunology and Microbial Science, The Scripps Research Institute, La Jolla, CA, USA.
E-mail: mbaobo@scripps.edu

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