CORRESPONDENCE

Demonstration of Borna Disease Virus Nucleic Acid in a Patient with Chronic Fatigue Syndrome

To the Editor—Czygan et al. [1] reported the detection of Borna disease virus (BDV) nucleic acid in 3 cases of a rare form of hippocampal degeneration, whereas the brains of patients with other neuropsychiatric disorders tested negative for BDV. Chronic fatigue syndrome (CFS) is another, more frequently diagnosed neuropsychiatric disease that is associated with BDV infection. However, the published findings are highly controversial. Nakaya et al. [2, 3] and Kitani et al. [4] showed both BDV-specific antibodies and RNA in a high percentage of Japanese patients with CFS. Bode et al. [5] isolated BDV from peripheral blood mononuclear cells (PBMC) of an American patient with CFS; however, in an earlier publication, Bode et al. [6], as well as Evengård et al. [7] and Yamaguchi et al. [8] in recent publications, did not find serologic evidence for BDV in patients with CFS. A possible explanation for the controversial results is that the term "chronic fatigue syndrome" probably includes several similar clinical conditions that may have different etiologies. In the study by Czygan et al. [1], brain tissue samples from patients who had CFS were not included. Unfortunately, none of the BDV sequences of the CFS cases mentioned above are available in the GenBank database.

We report a 30-year-old man who was diagnosed with CFS. After a sudden onset 5 years ago of a disease with influenza-like symptoms, mild fever, myalgia, and muscle weakness, he had debilitating fatigue, impaired memory and concentration, and recurrent headache and was unable to work efficiently. Phases of temporary recovery alternated with relapses. During a more acute course of the disease, he saw a psychiatrist and had an EDTA blood draw. PBMC were separated by Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden), aliquoted, and stored frozen.

To test for the presence of BDV nucleic acid, RNA was extracted by use of the QIAamp Viral RNA kit (Qiagen, Chatsworth, CA), and a 1-tube reverse-transcriptase (RT) polymerase chain reaction (PCR) system (Titan; Boehringer Mannheim/Roche Diagnostics, Mannheim, Germany) was used for reverse transcription and PCR. Subsequently, nested and seminested PCR assays resulted in 3 PCR products of 212, 691, and 724 bp in length (figure 1). To minimize possible PCR contamination, RNA extraction was done in a biosafety level 3 (P3) laboratory on a different floor, and RT-PCR and seminested and nested PCR were done in laboratories that had not previously handled BDV. Finally, the whole procedure was repeated with another aliquot of PBMC that had been stored frozen, with the same results. As a negative control, PBMC from a healthy person who was serologically and virologically negative for BDV was tested in parallel (figure 1). The PCR products were sequenced in both directions by direct automated sequencing. The sequences represent part of the p38/40 gene,

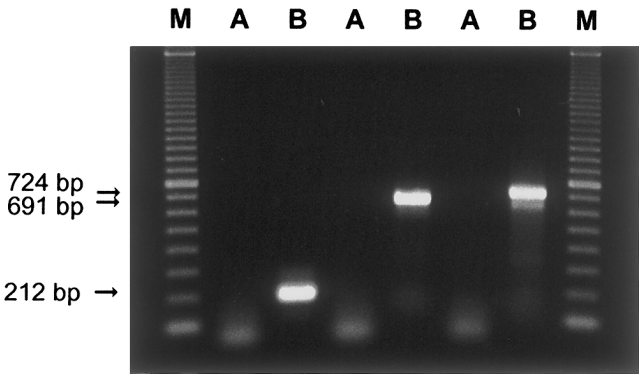

Figure 1. Detection of Borna disease virus (BDV)–specific nucleic acid in peripheral blood mononuclear cells (PBMC) of a man with chronic fatigue syndrome (CFS), by nested and seminested polymerase chain reaction assays. Lanes: A, BDV-negative PBMC; B, BDV-specific amplicates from PBMC of patient with CFS; M, size marker (100-bp DNA ladder).

the complete p23/24 gene, the complete gp18 gene, and part of the p57 gene of BDV for a total of 1398 bp of sequence information. When compared with BDV sequences in the GenBank database, the sequences showed an overall nucleotide identity rate of 96.2%–97.7%. In contrast to the sequences amplified from 3 brains with hippocampal degeneration, which proved to be virtually identical to the sequence of a BDV laboratory strain [1], there was no identity with any human, animal, or laboratory BDV strain in the sequence of the CFS case that we describe.

The BDV sequence of this case exhibits several point mutations, 2 of which lead to amino acid changes, that have not been seen in any other BDV case. Also, 3 different primer pairs were used independently, amplifying different regions of the BDV genome, and all resulted in specific amplifications by PCR.

The patient was retested 4 months later, during a chronic stage of the disease. At this point, no BDV nucleic acid was detected in his PBMC. This corresponds with the finding of Bode and Ludwig [9], who also detected BDV during acute disease only. With regard to the discussion of whether BDV infection should be considered a zoonosis, we note that the patient we describe had no close contact with animals and does not live in an area in which BDV is endemic in animals.

The sequences described in this paper have been reported to the GenBank database (accession numbers AF094477 and AF094478) and are available to the scientific community.

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Reply

To the Editor—Published findings regarding the role of Borna disease virus (BDV) in human psychiatric diseases remain controversial. The findings in the new case reported by Nowotny and Kolodziejek [1] seem to support previous findings in studies of Japanese patients [2, 3], which suggested an association between BDV and chronic fatigue syndrome, a neuropsychiatric disorder not evaluated in our recent studies [4, 5].

Whereas viral nucleic acids persisted in peripheral blood mononuclear cells (PBMC) of 3 of 4 patients in a Japanese study [3], Nowotny and Kolodziejek [1] found BDV-specific RNA in a single blood sample taken in the early phase of disease development but not in blood samples from the same patient during the chronic phase of the disease. Because viral sequences found in the patient's PBMC in the latter study differed from all known BDV isolates by several percent, the authors concluded that sample contamination had not occurred.

In our recent analysis of the brains of 3 American patients with a rare form of hippocampus sclerosis [4], viruses with sequences almost identical to that of the BDV laboratory strain He/80 were identified. Since large amounts of viral nucleic acid were present in the brains, we argued that accidental contamination of the tissue samples in the laboratory probably had not occurred. Although contamination seems unlikely in both studies, independent confirmation of the proposed disease associations is of great importance. We recently demonstrated the essential role of confirmatory work [6] by showing that a novel variant of BDV, which was believed to originate from the blood of a patient with schizophrenia [7], was almost identical to a laboratory strain whose sequence previously had not been determined. Other human bornaviruses also are related strongly to virus strains frequently used for experiments in laboratories that report human bornaviruses, which raises questions about the human origin of BDV isolates known to date [6].

The new report by Nowotny and Kolodziejek [1] highlights the intrinsic problems of diagnostic bornavirus work. If brief, transient viremic phases are the rule rather than the exception in persons infected with BDV, simple retesting of new blood samples may not reveal the true infection status of a patient. Furthermore, if BDV infections do not result in detectable humoral immune responses on a regular basis, as indicated by previous studies [8], and if lifelong virus persistence in the central nervous system occurs infrequently in humans, researchers who try to establish reliable diagnostic methods for bornaviruses are facing enormous technical and intellectual challenges. Obviously, many basic questions of BDV epidemiology, including the critical issue of a possible association between BDV and particular forms of human neuropsychiatric disorders, cannot be answered properly before improved diagnostic tools are available.

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