Unusually High Seroprevalence of Borna Disease Virus in Clade E Human Immunodeficiency Virus Type 1-Infected Patients with Sexually Transmitted Diseases in Thailand

WATTANA AUWANIT, 1, 2 PANASDA I. N. AYUTHAYA, 2 TAKAAKI NAKAYA, 1 SUGURU FUJIWARA, 1, 3 TAKESHI KURATA, 4 KOICHI YAMANISHI, 5 AND KAZUYOSHI IKUTA 1

Section of Serology, Institute of Immunological Science, Hokkaido University, Kita-ku, Sapporo 060, 1 Sapporo I.D.L., Kita-ku, Sapporo 001, 1 Department of Pathology, National Institute of Health, Shinjuku-ku, Tokyo 162, 4 and Department of Microbiology, Osaka University Medical School, Suita, Osaka 565, 3 Japan, and Section of Immunology, Department of Medical Sciences, National Institute of Health, Nonthaburi 11000, Thailand 2

Received 20 February 1996/Returned for modification 26 March 1996/Accepted 9 May 1996

The seroprevalence of Borna disease virus (BDV) in human immunodeficiency virus type 1-infected individuals in Thailand was examined by using recombinant BDV p24. A high (38 to 48%) rate of seroprevalence of BDV was observed in clade E-infected patients with sexually transmitted diseases, compared with those in clade E-infected prostitutes (8.3%), pregnant women (0%), clade B-infected intravenous-drug users (0%), and human immunodeficiency virus type 1-negative blood donors (1.9%).

Borna disease virus (BDV) is a neurotropic, yet unclassified, nonsegmented, negative-sense, single-stranded RNA virus (4, 17). BDV naturally infects horses and sheep and induces a disease characterized by a progressive meningoencephalopathy (7, 8). In addition, BDV, or a related agent, has been suggested to be associated with specific psychiatric disorders, because of the finding of higher-than-expected seroprevalence rates in such patients (more than 10%) compared with that in healthy people (1 to 2%) (1–3, 15, 16). Detection of anti-BDV in healthy people may imply that BDV causes latent infection in humans leading to no expression or an extremely low level of expression of viral antigens. This hypothesis is also supported by previous seroprevalence studies of human immunodeficiency virus type 1 (HIV-1)-infected individuals, in whom a lower seroprevalence rate (4 to 8%) at the early stage of HIV-1 infection is followed by a higher one (13.9%) at later stages of disease (2, 3). Thus, BDV may be more widespread than previously thought, and in immunosuppressive patients BDV could be reactivated.

Two major subtypes (clades) of HIV-1 were identified in Thailand (9, 13): clade B of HIV-1 is similar to the predominant HIV-1 subtype circulating in North America, as well as Europe, and was identified in intravenous-drug users (IDU) (18–20), while a distinct HIV-1 subtype, clade E, was found among female prostitutes (PRO) and male patients with sexually transmitted diseases (STD) (9, 13, 14, 20). Here, we examined BDV seroprevalence among four HIV-1-infected high-risk populations in Thailand by using an enzyme-linked immunosorbent assay (ELISA) with recombinant BDV p24 (phosphorylated protein coded for by the second open reading frame). This was done in order to understand the possible link of BDV reactivation with one or more specific high-risk populations of HIV-1-positive subjects.

A total of 60 asymptomatic carriers (AC) and 67 patients with AIDS were randomly selected for studies of seroprevalence of BDV. The AC were classified into four populations at a high risk for HIV-1 infection (STD patients, PRO, pregnant women [PRE], and IDU). All individuals examined in the STD and IDU populations were male, while all individuals in the PRO population were female. These subjects lived in nine different regions of Thailand, i.e., 17 (9 STD patients, 3 PRO, and 5 PRE) in Chiang Mai, 13 (9 PRO and 4 PRE) in Chon Buri, 10 (6 STD patients and 4 IDU) in Ubon Ratchathani, 7 (PRE) in Khon Kaen, 5 (1 STD patient, 1 PRE, and 3 IDU) in Phitsanulok, 3 (STD patients) in Chiang Rai, 2 (IDU) in Nakorn Ratchasima, 2 (1 STD patient and 1 IDU) in Song Khla, and 1 (STD patient) in Trang. The 67 patients with AIDS also were similarly located in the nine different regions of Thailand. However, information on factors for these patients was not available, since they were diagnosed with AIDS only after admission to a hospital.

A 1:100 dilution of plasma samples obtained from the 60 AC and 67 AIDS patients was used in ELISAs with horse BDV-derived recombinant full-length p24 fusion protein with glutathione S-transferase (GST-p24) as the antigen; the negative control antigen was GST alone (5, 6, 10–12). Both GST-p24 and GST proteins were used after purification by glutathione Sepharose 4B (Pharmacia Biotech AB, Uppsala, Sweden) column chromatography. The results are shown as A492 with GST-p24 subtracted from that with GST alone. The samples showing an A492 of more than 0.5 were considered positive, since we previously showed that such samples were also positive by Western blot (immunoblot) analysis (5). As shown in Fig. 1, 9 of 60 AC (15%) and 12 of 67 AIDS patients (17.9%) were positive for antibodies to BDV p24. When BDV seroprevalence was examined separately for each of the four high-risk populations among 60 AC, the highest rate of BDV seroprevalence was seen in the STD group, i.e., 8 among 21 (38%) STD patients. Only 1 of 12 (8.3%) PRO was positive; none of the 17 PRE and none of the 10 IDU were positive (Fig. 1). The mean antibody reactivities calculated by the Mann-Whitney U test were statistically significant in the STD population, compared with those in the PRO (P < 0.05) and IDU populations (P < 0.01). However, there were no statistically significant differ-
ences between the STD and PRE groups ($P < 0.1$), probably because of the low number of samples examined.

Next, we confirmed whether the STD patient samples showing seropositivity to BDV p24 also contained anti-HIV-1 clade E antibodies. HIV-1 serotyping of the plasma samples was performed by an ELISA with four synthetic peptides designated PND-E, PND-B, PND-BR, and PND-MN. These peptides were derived from the principal neutralizing determinant of HIV-1 gp120, according to the method of Pau et al. (14). Peptides PND-E (DTSITIGPGQVFYRT) and PND-B (DKSIHLGPGQAWYTT) were obtained from the consensus sequences of the genes for Thai clades E and B, respectively. In addition, PND-BR (DKSIHLGPGRAWYTT) was derived from the consensus sequence of the gene for clade B in North America, while PND-MN (DKRIHIGPGRAFYTT) was derived from the clade B strain MN. PND-BR and PND-MN were also used for the detection of clade B HIV-1. The N-terminal amino acid, Asp, in all peptides was added in order to accelerate peptide binding to the surfaces of microtiter plate wells. As shown in Table 1, the ELISA with four kinds of synthetic peptides showed that most individuals in the nine regions, i.e., 95.2% (20 of 21) with STD, 83.3% of PRO, and 88.2% of PRE, were infected with clade E. Similarly, most AIDS patients (95.6%) also were infected with clade E. By contrast, 70% of the individuals in the IDU group (7 of 10) were infected with clade B HIV-1; i.e., samples from three reacted with only peptide PND-B; samples from two reacted with PND-B and -BR; a sample from one reacted with PND-B, -BR, and -E; and a sample from one reacted with PND-B, -BR, -MN, and -E. Consequently, all the patients positive for anti-BDV antibody in the nine regions (9 positive AC and 12 positive AIDS patients) were individuals positive for anti-HIV-1 clade E antibody. Thus, an unusually high BDV seroprevalence rate was observed for individuals in the STD group who are also infected with HIV-1 clade E in Thailand.

To confirm this finding, we prepared an additional 50 plasma samples derived from STD patients from Chiang Mai. As a control, 103 plasma samples derived from HIV-1-seronegative blood donors from Chiang Mai were also prepared. Examination by ELISA with the same GST-p24 and GST antigens clearly confirmed the correlation between BDV seroprevalence and HIV-1 clade E infection. Anti-BDV antibodies were detected in 24 of the 50 (48%) individuals with STD but in only 2 of the 103 (1.9%) blood donors (Fig. 3). This difference was statistically significant ($P < 0.001$). Further, infection with clade E HIV-1 was detected in all of the 50 samples from STD patients (Table 1) by using the four synthetic peptides described above.

Our findings thus suggest that the infection of the STD
group with clade E virus is highly associated with an active infection by BDV or a BDV-related agent. Specifically, the rate of seroprevalence of BDV infection in AC with STD group in Thailand was 38% for samples from the nine regions (Fig. 2), while it was 48% for the Chiang Mai samples (Fig. 3). Further, the rate of seroprevalence of BDV was low in all other AC groups, i.e., IDU (no positive case among 10), PRE (no positive case among 17), and PRO (only 1 positive case among 12) (Fig. 2). Thus, a great difference in BDV seroprevalence rates exists among HIV-1-infected AC within different groups. This result extends previous reports which showed an overall rate of 14% correlation of BDV and HIV-1 (2, 3). One possible reason for the higher seroprevalence rate observed may be the improved sensitivity of an ELISA with recombinant BDV antigen. An alternative explanation may be that there are differences in BDV expression dependent on the infecting HIV-1 clade. It seems that HIV-1 clade E-infected patients with STD progress to AIDS rapidly after initial infection (21), indicating a higher virulence of clade E than clade B. Consequently, the mechanism for a higher rate of seroprevalence of BDV in HIV-1 clade E-infected AC with STD could be reactivation of BDV from latency under a stronger immunosuppression induced by clade E infection. Therefore, BDV might be more widespread in healthy individuals than previously believed. It is unknown whether BDV can be transmitted by sexual contact. Also, the BDV infection observed here might not be correlated with psychiatric disorders, since the individuals with STD examined did not show any clinical signs of mental disease.

We thank R. B. Luftig, Louisiana State University Medical Center, New Orleans, for a critical review of our manuscript. This work was partly supported by a Grant-in-Aid for BDV Research from the Ministry of Health and Welfare, Tokyo, Japan; Grants-in-Aid for Scientific Research (A and B) and International Scientific Research (Joint Research) from the Ministry of Education, Science, Sports and Culture, Tokyo, Japan; the Japan Society for the Promotion of Science (Dissertation Ph.D. Program); and an annual budget provided by the Ministry of Public Health, Nonthaburi, Thailand.

REFERENCES


TABLE 1. BDV seroprevalence and HIV-1 serotypes in four high-risk groups from nine regions in Thailand

<table>
<thead>
<tr>
<th>Sample source</th>
<th>Risk group</th>
<th>% BDV seroprevalence</th>
<th>HIV-1 clade(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All nine regions</td>
<td>AC-STD*</td>
<td>38.1 (8/21)</td>
<td>E 20 (8) B 0 0 0 E and B 1 (0) Other 0</td>
</tr>
<tr>
<td></td>
<td>AC-PRO</td>
<td>8.3 (1/12)</td>
<td>10 (1) 0 0 2 (0)</td>
</tr>
<tr>
<td></td>
<td>AC-PRE</td>
<td>0 (0/17)</td>
<td>15 (0) 0 0 2 (0)</td>
</tr>
<tr>
<td></td>
<td>AC-IDU</td>
<td>0 (0/10)</td>
<td>1 (0) 5 (0) 2 (0) 2 (0)</td>
</tr>
<tr>
<td></td>
<td>AIDS</td>
<td>17.9 (12/67)</td>
<td>64 (12) 0 0 0</td>
</tr>
<tr>
<td>Chiang Mai†</td>
<td>AC-STD†</td>
<td>48 (24/50)</td>
<td>50 (24) 0 0 0</td>
</tr>
</tbody>
</table>

* Values in parentheses are the number positive for BDV/number of samples tested.
† Each number in parentheses shows the number seropositive for BDV.
‡ No reactions with PND-E, -B, -BR, and -MN peptides.
§ The original results for AC with STD in nine regions and additional results for AC with STD in Chiang Mai were derived from those in Fig. 2A and 3A, respectively.

FIG. 3. Comparative seroprevalence of BDV in AC with STD and blood donors from Chiang Mai. A total of 50 AC with STD (A) and 103 HIV-1-seronegative blood donors (B) were examined for seroprevalence of BDV. An ELISA was carried out as described in the text, and the results are expressed as in Fig. 1. The mean antibody reactivities were calculated as for Fig. 2.