Molecular Confirmation of Human Immunodeficiency Virus (HIV) Type 2 in HIV-Seropositive Subjects in South India

R. KANNANGAI,1 S. RAMALINGAM,1 K. J. PRAKASH,1 O. C. ABRAHAM,2 R. GEORGE,3 R. C. CASTILLO,4 D. H. SCHWARTZ,4 M. V. JESUDASON,5 AND G. SRIDHARAN1*

Departments of Clinical Virology,1 Internal Medicine Unit 1,2 Dermatology,3 and Clinical Microbiology,5 Christian Medical College Hospital, Vellore, India, 632004, and Department of Molecular Microbiology and Immunology, The Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland 212054

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NestEd PCRs for human immunodeficiency virus type 1 (HIV-1) and HIV-2 were compared with immunoblot test results. Twelve of 13 immunoblot-positive HIV-2 samples were positive by PCR. There were five INNO-LIA (Innogenetics, Zwijnaarde, Belgium) and/or HIVBLot 2.2 (Genelabs, Singapore) samples that tested positive for dual infection. HIV-1 PCR was positive in all samples, while HIV-2 PCR was positive in two and RIBA (Chiron Corporation, San Diego, Calif.) was positive for HIV-2 in 3 samples. Thus the prevalence of HIV-2 is accurately estimated by the use of immunoblotting, but that of HIV-1 and -2 dual infection may be overestimated.

Human immunodeficiency virus type 2 (HIV-2) was first detected in West Africa, where it is seen as a major problem (2, 9, 10, 18). In Asia, 95% of the reported HIV-2 cases come from India (17, 19). The prevalence of HIV-2 in India varies regionwise, ranging between 2% and 33% of all HIV infections; these reports identify HIV-2 infection serologically (1, 11, 13, 20). Some reports indicate that immunoblotting may overestimate the prevalence of HIV-2 and HIV-1 and -2 dual infections (14, 16); thus, it is important to use molecular techniques for confirmation. We used a PCR to ascertain true HIV-2 infection in seropositive individuals and evaluated the Chiron RIBA HIV-1/HIV-2 strip immunoblot assay.

All study participants were diagnosed HIV positive at the Christian Medical College Hospital in Vellore, a tertiary care center in southern India. Initial diagnoses were performed during the period of 1993 to 1999, as patients checked into the hospital with some other ailments or with a suspicion of HIV infection. Contact information was available on only 67 HIV-seropositive individuals and evaluated the Chiron RIBA HIV-1/HIV-2 strip immunoblot assay.

Thirty HIV-1 immunoblot- and PCR-positive samples were compared with immunoblot test results. Twelve of 13 immunoblot-positive HIV-2 samples were positive by PCR. There were five INNO-LIA (Innogenetics, Zwijnaarde, Belgium) and/or HIVBLot 2.2 (Genelabs, Singapore) samples that tested positive for dual infection. HIV-1 PCR was positive in all samples, while HIV-2 PCR was positive in two and RIBA (Chiron Corporation, San Diego, Calif.) was positive for HIV-2 in 3 samples. Thus the prevalence of HIV-2 is accurately estimated by the use of immunoblotting, but that of HIV-1 and -2 dual infection may be overestimated.

HIV-1 PCR product was 700 bp, while that of HIV-2 was 542 bp. All products were detected by gel electrophoresis. The expected size of HIV-1 PCR product was 700 bp, while that of HIV-2 was 542 bp. All products were detected by gel electrophoresis.

In 18 study subjects, 12 of 13 dual-reactive samples by LIA and/or HIVBLot 2.2, only three were positive for both HIV-1 and HIV-2 by RIBA. The remaining two samples were positive only for HIV-1. Information, including clinical status and CD4 counts of the dual infection, is shown in Table 1.

The LIA and/or HIVBLot (n = 13) HIV-2-positive samples tested negative by HIV-1-specific PCR, while 12 (92.3%) of these samples tested positive by HIV-2 PCR. Two of these samples were amplified only after increasing the DNA input to

by increasing the input DNA to 20 μl. The expected size of HIV-1 PCR product was 700 bp, while that of HIV-2 was 542 bp. All products were detected by gel electrophoresis.

FIG. 1. Gel Doc (Bio-Rad, Hercules, Calif.) picture showing specific bands for HIV-1 (700 bp) and HIV-2 (542 bp) on a 2.5% agarose gel with DNA Molecular Weight Marker IX (72-1353). Lanes 1, 3, 5, 9, and 11 show bands specific for HIV-1, amplified from the dual-reactive samples. Lanes 2 and 4 show HIV-2 PCR product of the dually positive samples, while lanes 6, 10, and 12 show the three HIV-2 PCR-negative samples. Lanes 14 and 15 show product from pure HIV-1 and HIV-2 samples, respectively. Lane 8 is the molecular weight marker. Lanes 7 and 13 are distilled-water PCR controls.
HIV-2 nPCR is highly specific. Since the closely related virus HIV-1 is not amplified, this highly sensitive method for the detection of HIV-2 DNA from PBMC. If the V3 region of HIV-1, the V3 region of HIV-2 is more conserved. This PCR technique successfully amplified HIV-2 DNA from two patients who were on triple-drug antiretroviral therapy for 4 to 6 months and who could reasonably be expected to have extremely low viral burdens. Considering these factors, this technique can be considered a highly sensitive method for the detection of HIV-2 DNA from PBMC. Since the closely related virus HIV-1 is not amplified, HIV-2 nPCR is highly specific.

The primary motivation for using a PCR-based diagnosis of HIV-2 infection is that in serologically detected HIV-1- and -2-positive samples, dual reactivity may be due to one of the following reasons in addition to true dual infection: extensive cross-reactivity of antibodies of either HIV type, infection by one virus and exposure to a second one, and infection with a putative intermediate virus (10). The earlier reports on PCR confirmation of dual-positive samples showed a PCR-positive rate varying from 18 to 62% (6–14–16). Immunoblot data from this institution (the Christian Medical College Hospital), collected between 1993 and 1997, showed a prevalence of 2.1% for dual infections among the HIV infections, which was higher than that of HIV-2 infection alone (1.8%) (11). Reports from western parts of India showed prevalence rates in high-risk groups varying from 5 to 20% for dual infection, by serological methods. Such high proportions of dual infection might be inconsistent with HIV-2 infection serving a protective role against HIV-1 infection (22). However, because of cross-reactivity in immunoblotting, these figures may be falsely high. Although PCR is considered the “gold standard” for the detection of HIV-2, RIBA appears to be more specific in detecting HIV-2 than the other two immunoblot assays. Despite the limitations of the sample size, in conclusion we think that immunoblots may overestimate the prevalence of HIV-2 and that the data require reassessment with PCR testing.

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**TABLE 1.** The clinical status and CD4 counts of 5 HIV-1 and -2 dual-infected individuals and comparison of LIA and/or HIVBLOT 2.2 patterns with RIBA reactivity and PCR findings

| Sample no. | Clinical status | CD4 countsa | Band pattern on LIA and/or HIVBLOT 2.2 | Band pattern on RIBA | HIV-2 PCR result |
|------------|----------------|-------------|----------------------------------------|---------------------|------------------|
| 1          | Asymptomatic   | NA          | gp120, gp41, **gp36**d, p31, p24, and p17b | gp120, gp41, p31, p24/26 | Negative         |
| 2          | Symptomatic    | 173         | gp160, gp120, gp41, p31, p24, **p27**    | gp120, gp41, p31, p24/26 | Negative         |
| 3          | Symptomatic    | 874         | gp41, **gp36**, p31, and p24b            | gp120, gp41, gp36, p31, p24/26 | Negative         |
| 4          | Symptomatic    | 457         | gp160, gp120, gp41, p31, p24, **p27**    | gp120, gp41, **gp36**, p31, p24/26 | Positive         |
| 5          | Asymptomatic   | 572         | gp41, **gp36**, p31, p24, and p17b       | gp120, gp41, gp36, p31, p24/26 | Positive         |

a CD4 counts estimated by Capcella (Sanofi Diagnostic Pasteur, Marne la Coquette France), an immunocapture enzyme linked immunosorbent assay-based technique. The mean CD4 cell counts for asymptomatic (n = 35) and symptomatic (n = 19) HIV-1-positive individuals were 698 ± 266 and 429 ± 203, respectively. In asymptomatic (n = 8) HIV-2-infected individuals, it was 963 ± 369. In healthy (n = 44) South Indian adults, the CD4 count was 1,048 ± 210 (12). NA, not available.

b Results from LIA.

c Results from HIVBLOT.

d Figures in bold indicate HIV-2-specific bands.

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**TABLE 2.** Immunoblot reactivity results compared to HIV-1- and -2-specific PCR findings

| Immunoblot result | No. of samples tested | No. of HIV-1 PCR positive samples (%) | No. of HIV-2 PCR positive samples (%) |
|-------------------|-----------------------|---------------------------------------|---------------------------------------|
| Positive for HIV-1| 30                    | 30 (100)                              | 0                                     |
| Positive for HIV-2| 13                    | 0                                     | 12 (92.3)c                             |
| Positive for HIV-1 and -2| 5           | 5 (100)                              | 2 (40)                                |

The PCR-negative individual had exposure to her HIV-2-positive spouse for 8 months (unprotected sex) prior to PCR testing.
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