Six New Isolates of Human Immunodeficiency Virus Type 2 (HIV-2) and the Molecular Characterization of One (HIV-2CAM2)

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SUMMARY

We report the isolation of human immunodeficiency virus type 2 (HIV-2) from each of six West Africans with AIDS-related complex or AIDS. One isolate (HIV-2CAM2) was molecularly cloned and shown by restriction mapping to differ in seven out of 22 sites from the prototype HIV-2ROD. Nevertheless, by a number of serological criteria these isolates are all clearly HIV-2.

AIDS was recognized as a distinct human disease in 1981. The causative agent, the first lentivirus known to infect man, was discovered in 1983 (Barré-Sinoussi et al., 1983; Karpas, 1983) and was later designated human immunodeficiency virus (HIV) (Brown, 1986). Although the various isolates of HIV share similar biological and serological properties, and their various genes are of the same size, the molecular characterization of distinct isolates revealed that each isolate has a different restriction enzyme map (Benn et al., 1985). Nucleic acid sequence studies revealed consistent differences between independent isolates which ranged between 7 and 25% (Alizon et al., 1986).

A distinct lentivirus was isolated on 1986 from West African patients with AIDS, whose sera failed to react with the known HIV (Clavel et al., 1986). This new virus was named HIV type 2 (HIV-2) and the original virus isolates HIV-1. Comparison of the complete sequence of HIV-1 with HIV-2 showed that there was only 40% homology between the two viruses and that the genome of the latter was longer by nearly 500 nucleotides (Guyader et al., 1987).

Numerous groups have characterized HIV-1 isolates but, to date, the sequence of only one isolate of HIV-2 has been published (Guyader et al., 1987). We now report the isolation of HIV-2 from six West African AIDS-related complex (ARC) and AIDS patients and the molecular characterization of a Guinea-Bissau isolate which produces persistent high-level expression in a permanent cell line.

Ten ml of peripheral blood was obtained in August 1987 from each of eight patients in the AIDS ward of the Hopital de Egas Moniz in Lisbon, six of whom had come from West Africa (Guinea-Bissau) for treatment. The white blood cells were separated in Cambridge and were cocultivated with phytohaemagglutinin-stimulated white blood cells and the T cell line Karpas 45 (Karpas et al., 1987). The plasma was tested for the presence of antibodies to HIV-1 and HIV-2 using the Karpas cell test (see Table 1), the principles of which were reported earlier (Karpas et al., 1985, 1987). Briefly, 1:10 diluted serum was placed in each well containing acetone-fixed HIV-1- or HIV-2-infected Karpas T cells. Following a 30 min incubation in a humidified chamber, the slides were washed for 5 min in saline solution. Thereafter, the slides were immersed in a solution of Protein A–horseradish peroxidase conjugate diluted 1:100 in phosphate-buffered saline for 30 min. After a further 5 min wash, the slides were immersed in a reconstituted 3-amino-9-ethylcarbazole substrate solution for 3 min. The reaction was stopped


Table 1. Summary of serological and virological studies

<table>
<thead>
<tr>
<th>Patient</th>
<th>HIV-1</th>
<th>HIV-2&lt;sub&gt;ROD&lt;/sub&gt;</th>
<th>HIV-2&lt;sub&gt;CAM2&lt;/sub&gt;</th>
<th>HIV-2</th>
<th>HIV-1</th>
<th>HIV-2 isolate</th>
<th>Western blot</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>±*</td>
<td>++ + +</td>
<td>--</td>
<td>+</td>
<td>--</td>
<td>CAM1 HIV-2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>±</td>
<td>++ + +</td>
<td>++</td>
<td>+</td>
<td>--</td>
<td>CAM2 HIV-2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>±</td>
<td>++ + +</td>
<td>++</td>
<td>+</td>
<td>--</td>
<td>CAM3 HIV-2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>++ +</td>
<td>+ + ++</td>
<td>++</td>
<td>--</td>
<td>+</td>
<td>CAM4 HIV-1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>±</td>
<td>++ + +</td>
<td>++</td>
<td>+</td>
<td>--</td>
<td>CAM4 HIV-2</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>-- --</td>
<td>++</td>
<td>+</td>
<td>--</td>
<td>--</td>
<td>ND†</td>
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<tr>
<td>7</td>
<td>±</td>
<td>++ + +</td>
<td>++</td>
<td>+</td>
<td>--</td>
<td>CAM5 HIV-2</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>±</td>
<td>++ + +</td>
<td>++</td>
<td>+</td>
<td>--</td>
<td>CAM6 HIV-2</td>
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</tr>
<tr>
<td>1379‡</td>
<td>++ +</td>
<td>+ + ++</td>
<td>++</td>
<td>+</td>
<td>--</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>1457‡</td>
<td>++ +</td>
<td>+ + ++</td>
<td>++</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>5553§</td>
<td>+</td>
<td>+ + + +</td>
<td>++</td>
<td>+</td>
<td>--</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>5554§</td>
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<td>++</td>
<td>+</td>
<td>--</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* + and ++ + indicate a positive and strong positive reaction respectively; - indicates a negative reaction.
† ND, Not done.
‡ Sera 1379 and 1457 are HIV-1-positive controls.
§ Sera 5553 and 5554 are HIV-2-positive controls (Karpas et al., 1987).

by dipping the slides in water. ELISA was performed according to the manufacturer's instructions.

As can be seen in Table 1, six of the eight patients had antibodies to HIV-2, while only one had antibodies to HIV-1. The plasma of one patient did not contain antibodies to either. It was subsequently confirmed in Lisbon that patient 6 was not infected by HIV. Following 2 weeks of co-cultivation, giant cells and ballooning of cells became noticeable in six flasks. The number of affected cells increased, together with a wider c.p.e. including dying granular and pyknotic cells. Electron microscopic examination of one of the infected cultures revealed numerous particles which were indistinguishable from HIV-1 (Fig. 1).

In order to determine the antigenic relations of the new isolates, the Karpas cell test (Karpas et al., 1985, 1987) was used. Cells from each of the six flasks were acetone-fixed separately on a multispot glass slide, as described previously (Karpas et al., 1985). These fixed cells were incubated with serum containing antibodies to HIV-1 or HIV-2. Likewise, the sera were tested in parallel on HIV-2<sub>ROD</sub>-infected CEM cells (kindly provided by Dr L. Montagnier) or on HIV-2<sub>CAM2</sub>-infected Karpas T cells. Table 1 shows that the sera from these six patients gave strong staining both with HIV-2<sub>ROD</sub>-infected cells, as well as with cells infected with the new isolate (HIV-2<sub>CAM2</sub>), and only a weak reaction with the Cambridge isolates (C-HIV-1)-infected Karpas T cells. This observation confirms that our six isolates are by this serological test indistinguishable from the prototype HIV-2 isolate.

High Mr genomic DNA was extracted from HIV-2<sub>CAM2</sub>-infected Karpas 45 cells (Blin & Stafford, 1976) and shown to contain integrated proviral sequences by Southern blot analysis. This DNA was then used to construct a library in λ2001 (Karn et al., 1984). About 1 x 10<sup>6</sup> recombinant phage were screened using an HIV-2 gag/pol probe [pROD 4.8, which is a HindIII subclone from λROD 4 (Clavel et al., 1986; Guyader et al., 1987)].

Thirteen positive clones were detected and plaque-purified. All 13 clones were examined by Southern blot hybridization using pROD 4.8 and a probe representing the entire HIV-2 genome (pROD derived from λROD 35 and λROD 27; Clavel et al., 1986; Guyader et al., 1987); one clone, λCAM 11/1, was used for restriction map analysis.

A restriction map of the HIV-2<sub>CAM2</sub> provirus was constructed, using single, double and partial digestions with the enzymes BamHI, BglII, EcoRI, HindIII, NheI, PstI and PvuII. (An example is shown in Fig. 2.) After electrophoresis and Southern transfer, the filters were hybridized to one of the three probes, pROD 4.8, pROD or a long terminal repeat (LTR)/gag probe (pCH-1
Fig. 1. Electron micrograph of HIV-2cAM2 produced by infected Karpas T cells. Most particles have a single core, but a few have a double core (indicated by arrows). Cells were fixed in 3% glutaraldehyde and stained in uranyl acetate and lead citrate. Bar marker represents 200 nm.

which is a 2.1 kb BamHI subclone from 2CAM 11/1 representing fragment C in lane 1 of Fig. 2). Using this method, restriction sites could first be mapped to an approximate area of the genome before being determined accurately using the data from several double digests. It was not necessary to use all possible combinations of double digests to construct an unambiguous map of CAM2.

The presence of comigrating fragments (B, C) in lanes 1, 3 and 4 of Fig. 2 were determined using pCH-1, which showed the bands D, E, F and G with the enzymes PstI/BamHI, and bands D, E, F, G and J with PstI/BamHI. BglII cuts within the flanking sequence at the 5' end of the provirus, and this generates a doublet rather than comigrating fragments. In order to resolve the larger fragments more accurately, some small fragments were allowed to run through and out of the gel, and are not labelled.

Calculation of the mean total genome size gave a value of 9.55 kb (BamHI 9.52 kb; BamHI/BglII 9.53 kb; BamHI/EcoRI 9.56 kb; BamHI/HindIII 9.49 kb; BamHI/NheI 9.52 kb; BamHI/PstI 9.60 kb; BamHI/PvuII 9.62 kb) (see Fig. 3a). This compares with 9.67 kb for ROD. However, ROD contains three more closely spaced BamHI sites in the pol region (Guyader et al., 1987) and the two resulting fragments (coding for about 100 bp) have not been included in the mean total genome size for CAM2.

Comparative restriction maps of CAM2 and ROD are shown in Fig. 3(b). The viral genomes differ in seven out of the 22 restriction sites shown (several restriction sites are repeated at each LTR and these are included once).
Fig. 2. Southern blot hybridization of \( \lambda \)CAM 11/1 DNA with the pROD probe. Digests were as follows: lane 1, BamHI; lane 2, BamHI/BglII; lane 3, BamHI/HindIII; lane 4, BamHI/HaeI; lane 5, BamHI/EcoRI; lane 6, BamHI/PstI; lane 7, BamHI/PvuII. The arrows to the left-hand side of lane 1 represent HindIII-cut \( \lambda \) fragments for reference. Digests were run on a 0.8% agarose gel at 30 V for 16 h; DNA was transferred to a nylon filter, hybridized and washed by a standard procedure (Maniatis et al., 1982). Exposure to X-ray film was for 2 h at \(-70^\circ\)C with an intensifying screen. Extra bands most obvious in the PstI/BamHI digestion are due to partial digestions and are not labelled.

Although no data on the biological activity of \( \lambda \)CAM 11/1 are yet available, we believe it to represent the entire HIV-2 genome on the basis of genomic length and comparative restriction map analysis.

In conclusion, we describe the isolation of HIV-2 from each of six West African patients with ARC or AIDS. The molecular cloning and restriction mapping of one isolate shows that seven of 22 sites are different from the prototype strain HIV-2\(_{\text{ROD}}\). Various isolates of HIV-2 may be as distinct from each other as HIV-1 isolates are. Nucleotide sequencing of HIV-2\(_{\text{CAM2}}\) is in
progress and will permit precise determination of the sequence divergence between viral isolates.

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REFERENCES

Short communication


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