ISOLATION OF HUMAN IMMUNODEFICIENCY VIRUS FROM A JAPANESE HEMOPHILIA B PATIENT WITH AIDS

Yasuhiro Takeuchi,*1 Minoru Inagaki,*2 Norio Kobayashi*3 and Hiroo Hoshino*1,*4

*1Department of Hygiene and *2Third Department of Internal Medicine, Gunma University School of Medicine, 39-22, Showamachi 3-chome, Maebashi, Gunma 371 and *3Ogikubo Hospital, Imagawa-cho, Suginami-ku, Tokyo 167

Human immunodeficiency virus (HIV) was isolated from a Japanese hemophilia B patient with AIDS. This isolate, HIV[GUN-1], was infectious to several mature T-cell lines. Proteins with apparent molecular weights of 160, 55 and 25 kilodaltons were detected. Restriction enzyme cleavage patterns of the proviral genome indicated that HIV[GUN-1] is related to but clearly different from HTLV-III or ARV-2.

Key words: AIDS — Human immunodeficiency virus — Infectivity — Genetic variation

Human immunodeficiency virus (HIV) is etiologically associated with acquired immune deficiency syndrome (AIDS). Various names such as lymphadenopathy-associated virus (LAV), human T-lymphotropic virus type III (HTLV-III) or AIDS-associated retrovirus (ARV) have been used for HIV. Hemophiliacs represent an AIDS risk group, and many HIVs have been isolated from peripheral blood lymphocytes (PBL) of hemophiliacs. Gomperts et al. reported the isolation of HIVs from one-third of hemophiliacs who were positive for antibody against HIV antigens. Japanese hemophiliacs also carry antibody against HIV. So far, four HIVs, namely, YU-1, YU-2, YU-3 and YU-4 viruses have been isolated from Japanese hemophiliacs: YU-1 virus was from a patient with hemophilia A. These isolates were reported to have narrow host range. They were able to infect interleukin-2-dependent human T cells but not H9, Molt-4 or MT-4 cells, although HTLV-III, LAV and ARV could infect all these T cells. Genetic variation of Japanese HIV isolates has not yet been characterized. We isolated HIV from a Japanese patient with hemophilia B. This isolate had a wider host range than YU viruses. Its relatedness to HTLV-III or ARV was examined by Southern blot analyses.

PBL of two Japanese patients with hemophilia B (patients 1 and 2) positive for antibody against HIV were collected by Ficoll-Paque gradient centrifugation. Antibody titers of these patients against HIV antigens were determined by indirect immunofluorescence assay (IFA) using acetone-fixed H9/HTLV-IIIb cell smears as described elsewhere. Their titers were: patient 1, 1/320; patient 2, 1/320. Patients 1 and 2 were brothers and had been treated with factor IX concentrates prepared from plasma collected in the United States. The OKT4/OKT8 ratios of their PBL were lowered to 0.02–0.08. Patient 1 was a 19-year-old male and had been suffering from pneumonia caused probably by *Protozoa* or fungi; he died four months after the beginning of this experiment. The presence of *Pneumocytis carinii* was confirmed by postmortem examination. Thus, patient 1 met the criteria of AIDS.

PBL (2×10⁶ cells) were cocultivated with 4×10⁵ ATL-3I, H9, TALL-1 or Molt-4 cells. ATL-3I cells had been derived from a patient with adult T-cell leukemia (ATL). These cells express CD4 antigen and contain human T-cell leukemia virus type 1 genomes. Cocultivated cells were passaged serially and examined for expression of HIV antigens by IFA as described. About 1% of ATL-3I cells cocultivated with PBL of patient 1 but not patient 2 became positive for HIV antigens by IFA after cultivation for 2 weeks. Sera from not only Japanese hemophiliacs but also British patients with AIDS gave similar results on IFA. In another 2 weeks, almost all ATL-3I...
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cells became immunofluorescent. Cytopathic effects were evident and all ATL-3I cells were lysed. Therefore, fresh ATL-3I cells were added to cocultivated ATL-3I cells every 3-4 days in order to maintain HIV antigen-positive ATL-3I cells. No HIV antigen-positive cells were detected in H9, TALL-1 or Molt-4 cells during cocultivation for a month.

ATL-3I, ATL-1K, MT-4, H9, and HUT 78 cell lines were then inoculated with cell-free culture fluid of HIV antigen-positive ATL-3I cells. In a few weeks, all cell lines became positive for HIV antigens. Inoculation of the culture fluid was cytopathic to ATL-3I, ATL-1K and MT-4 cells, whereas HIV antigen-positive H9 and HUT78 cells could be maintained by serial passage. These findings suggested that the virus isolated from patient 1 shared many biological properties with HIV. Thus, this isolate was named HIV[GUN-1]. Reverse transcriptase assay of culture fluid was carried out using polyethylene glycol as described elsewhere. Incorporation of [3H]-TMP into DE81 filter-binding fractions were: ATL-3I cells, 854 cpm; ATL-3I/HIV[GUN-1] cells, namely, ATL-3I cells infected with HIV[GUN-1], 334,350 cpm; medium alone, 166 cpm. Furthermore, incorporation of [3H]-TMP was markedly inhibited in the presence of serum of a Japanese hemophiliac positive for antibody against HIV (antibody titer, 1280), but not in the presence of sera of ATL patients or a normal human (data not shown). Thus, HIV[GUN-1] was produced abundantly by ATL-3I/HIV[GUN-1] cells.

The susceptibilities of established T-cell lines to HIV[GUN-1], HTLV-III and ARV-2 were compared (Table I). Culture fluids of Molt-4 cells infected with HIV[GUN-1] or HTLV-III and H9 cells infected with ARV-2 were used as inocula. H9, MT-4, and Molt-4 cells (2 x 10^6) were inoculated with 0.2 ml of the culture fluids. After 6 days, HIV antigen-positive cells were examined by IFA. H9 and MT-4 cells were immunofluorescent. After prolonged cultivation, Molt-4 cells infected with HIV[GUN-1] also became positive for HIV antigens. Thus the new isolate was infectious to established T-cell lines. This property is different from those of the previous Japanese HIVs isolated by Yoshiyama et al.

Expression of HIV antigens in cells infected with HTLV-III, LAV, ARV-2 or HIV[GUN-1] was analyzed by immunoprecipitation assay as described elsewhere. That is, H9/HTLV-III, TALL-1/LAV, HUT78/ARV-2 (E line) and ATL-3I/HIV[GUN-1] cells were labeled with [3H]leucine (100 μCi/ml) for 8 hr in leucine-free RPMI1640 medium supplemented with 20% dialyzed fetal calf serum. Each cell lysate was absorbed with normal human serum and then reacted with hemophiliac serum positive for antibody against HIV antigens. Immunoprecipitates thus prepared were subjected to 10% polyacrylamide gel electrophoresis containing sodium dodecyl sulfate (SDS-PAGE) (Fig. 1). Proteins with apparent molecular weights of about 25, 54-55 and 160 kilodaltons (kd) were detected (lanes 1-4). These proteins were reported to be encoded by HIV. Apparent molecular weights of env precursor protein gp160 or gag precursor protein p55 were slightly different among different HIV isolates. A protein of 60 kd was expressed in ATL-3I/HIV[GUN-1] cells (lane 4) while a protein with similar molecular weight was not detected in H9 cells infected with HIV[GUN-1] (data not shown). ATL-3I/HIV[GUN-1] cell lysates were also reacted with serum of patient 1 from whom HIV[GUN-1] had been isolated. This serum reacted with gp160 predominantly but not (or hardly) with p55 or p25 (lane 5). Faint bands that represented p19, p24 and p53 of HTLV-1 were also detected by serum of an ATL patient (lane 6).

Table I. Infection of Established T-cell Lines with HIVs

<table>
<thead>
<tr>
<th>Cell</th>
<th>HIV[GUN-1] (%)</th>
<th>HTLV-III (%)</th>
<th>ARV-2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H9</td>
<td>0.5</td>
<td>16</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>MT-4</td>
<td>82.5</td>
<td>&gt;95</td>
<td>3.8</td>
</tr>
<tr>
<td>Molt-4</td>
<td>&lt;0.5</td>
<td>1.8</td>
<td>&lt;0.5</td>
</tr>
</tbody>
</table>

Cells were infected with HIVs, smeared onto slide glasses 6 days later and examined by IFA.
DNA was subjected to electrophoresis through 0.7% agarose gel, transferred to Zeta probe membranes and hybridized with the nick-translated BH-10 probe\(^{15}\) (Biotech Res. Lab., Md., USA) as previously described.\(^ {14}\) Hybridized membranes were washed at 65° with 0.1×SSC (0.015M NaCl, 0.0015M sodium citrate) and exposed to X-ray films. SacI digestion of Molt-4/HTLV-III DNA gave a very faint band of 8.9 kilobase pairs (kbp), which could not be reproduced by photography (Fig. 2A, lane 1) and two discrete bands (5.3 and 3.6 kbp) as described by Shaw et al.\(^ {17}\) Both HUT78/ARV-2 and ATL-3I/HIV[GUN-1] cell DNAs were digested with SacI (A), SacI and HindIII (B), SacI and BglII (C) and SacI and KpnI (D) and subjected to Southern blot analysis.
committee supported by the Ministry of Health and Welfare of Japan has confirmed 7 AIDS cases with hemophilia up to April 1986: 4 cases were hemophilia A and 3 cases were hemophilia B. The incidence of AIDS or ARC cases with hemophilia B may be much higher in Japan than in the United States, because most hemophilia patients who had contracted AIDS in the United States were hemophilia A. So far four HIVs have been isolated from Japanese patients with hemophilia, probably hemophilia A.\textsuperscript{6,7} These isolates are not infectious to permanent T-cell lines, whereas HIV\textsuperscript{[GUN-1]} could infect these cells readily. It is probable that HIVs infectious to permanent T-cell lines are more pathogenic than those not infectious to these cell lines. Restriction enzyme digestion of HIV\textsuperscript{[GUN-1]} genome indicated that HIV\textsuperscript{[GUN-1]} was appreciably different from HTLV-III or ARV-2. It will be important clinically to determine whether there is an association between the pathogenicity of each HIV isolate to the Japanese people and the host range or restriction enzyme cleavage patterns of the virus. Further isolation and characterization of HIVs from Japanese hemophiliacs or homosexuals may be needed to answer this question.

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