Detection of the X gene product of simian T-cell leukemia virus

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The gene product of the X region was examined in simian lymphoid cell lines producing simian T-cell leukemia virus (STLV), which is closely related to human T-cell leukemia virus (HTLV). By use of specific antibodies against pX peptides of HTLV-I, a protein of 41 kDa was identified as a pX product of STLV.

Simian T-cell leukemia virus *HTLV-I* *X region* *Gene product*

1. INTRODUCTION

Simian T-cell leukemia virus (STLV) is the exogenous retrovirus that naturally infects non-human primates of Old World origin [1–3]. This virus immortalizes normal lymphoid cells of human and non-human primates in culture [4–6], and it is possibly associated with lymphoid malignancy in non-human primates [7,8]. The genome of STLV has an additional unique region termed 'X' other than the usual components of the retroviral genome, *gag, pol, env* and 2 LTRs [9,10]. Although there is some species specificity in the STLV genome, the nucleotide sequence of all STLV is highly homologous to that of human T-cell leukemia virus type I (HTLV-I) [6,9–11]. The gene product of the X region of HTLV has been identified as a protein of 40–42 kDa in HTLV-I [12–15] and 37–38 kDa in HTLV-II [14,15], and its function of *trans* activation of transcription has been discussed with regard to its possible relation to oncogenesis [16–18]. However, the gene expression of the X region of STLV has not yet been reported. In this work, we detected the X gene product of STLV in virus-producing cell lines.

2. MATERIALS AND METHODS

2.1. *Cells*

Three cell lines harboring STLV were used, PtM3, BM5 and MtM/RfM26, which all contained STLV from macaque species, that is, a pig-tailed macaque, a bonnet monkey and a red-faced macaque, respectively [5].

2.2. *Antisera*

Antisera were prepared against 2 peptides, OP-1 and bGH-p40^{XI}. OP-1, the tetradecapeptide of Cys-Pro-Glu-His-Gln-Ile-Thr-Trp-Asp-Pro-Ile-Asp-Gly-Arg [14] was synthesized chemically. This sequence corresponds to that from amino acid 49 to 62 of the X gene product of HTLV-I [19]; 12 of the 14 amino acids are identical to that of STLV derived from pig-tailed macaque [10]. bGH-p40^{xI}, which was produced as a fused protein with bovine growth hormone in *Escherichia coli*, covers 54 amino acids located at the COOH terminus of the X gene product of HTLV-I [20], 42 amino acids of which were identical to those of STLV derived from pig-tailed macaque [10]. Antisera against OP-1 and bGH-p40^{xI} were prepared in rabbits and

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guinea pigs. Anti-OP-1 serum reacts with the gene products of the X regions of both HTLV-I and HTLV-II [14] while anti-bGH-p40^{xI} serum reacts with the product of HTLV-I, but not with that of HTLV-II [20].

2.3. *Immunoprecipitation*

3×10^6 cells of a simian cell line were labeled with 100 μCi/ml of [^{35}S]cysteine for 15 h and then lysed according to the method of Yamamoto et al. [21]. The acid-insoluble fraction of each cell lysate ($\sim 1 \times 10^6$ cpm) was incubated with 5 μl of each antiserum or the corresponding preimmune serum for 16 h at 4°C. Then, the immune complex was treated with 5 mg protein A Sepharose for 16 h at 4°C. After extraction with buffer containing 1% SDS for 2 min at 90°C, the immunoprecipitated proteins were subjected to electrophoresis on 12% polyacrylamide gel containing 0.1% SDS [22]. Subsequently the gel was dried and subjected to autoradiography. The specificity of the reaction was examined by a competition experiment with the pX peptides. For this, 10 μg of each polypeptide used as immunogen was incubated with 5 μl of antiserum for 16 h at 4°C before the procedure described above.

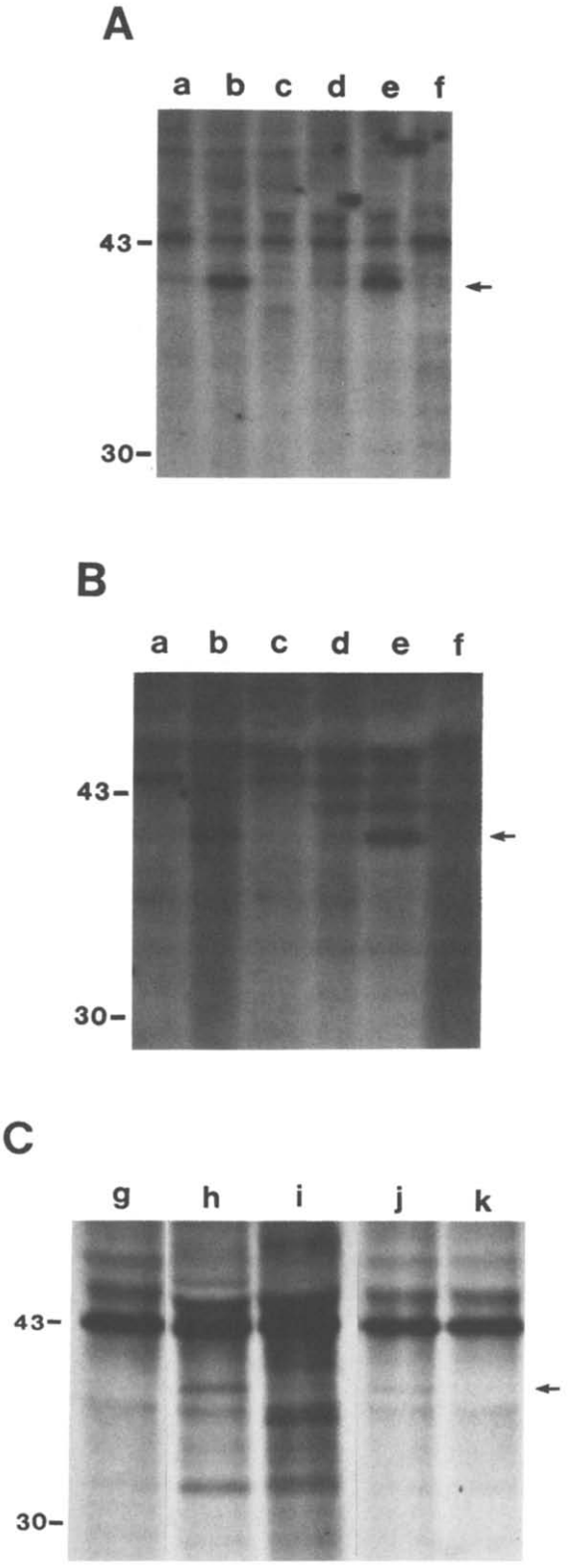

Fig. 1. Immunoprecipitation of cell lysates with antisera against peptides corresponding to parts of the amino acid sequence of the X gene product of HTLV-I. (A) [^{35}S]Cysteine-labeled cell lysate of BM5. The lysate was precipitated with rabbit preimmune serum (lane a), rabbit anti-OP-1 serum (lane b), rabbit anti-OP-1 serum previously treated with OP-1 (lane c), guinea-pig preimmune serum (lane d), guinea-pig anti-bGH-p40^{xI} serum (lane e) and guinea-pig anti-bGH-p40^{xI} serum previously treated with bGH-p40^{xI} (lane f). (B) [^{35}S]Cysteine-labeled cell lysate of RfM/mfm26. The sera used for immunoprecipitation were arranged in the same order as in A. (C) [^{35}S]Cysteine-labeled cell lysate of PtM3. In this case, anti-OP-1 as well as anti-bGH-p40^{xI} was prepared in guinea pigs. Then, the lysate was precipitated with guinea-pig preimmune serum (lane g), guinea-pig anti-OP-1 serum (lane h), guinea-pig anti-Op-1 serum previously treated with OP-1 (lane i), guinea-pig anti-bGH-p40^{xI} serum (lane j) and guinea-pig anti-bGH-p40^{xI} previously treated with bGH-p40^{xI} (lane k). Arrows indicate the position of 41 kD protein. Numbers represent sizes of ovalbumin (43 kDa) and carbonic anhydrase (30 kDa) used as size markers.

3. RESULTS AND DISCUSSION

As shown in fig. 1A, in BM5 cells carrying STLV from a bonnet monkey a protein of 41 kDa was detected with antisera against OP-1 and bGH-p40^{xI}, but not with preimmune sera. Its detection was due to a specific antigen-antibody reaction, since its detection was completely prevented by the relevant peptides. This 41 kDa protein was not detected with either of these antisera against pX peptides in cultured lymphocytes of a STLV-negative bonnet monkey. These results showed that this 41 kDa protein was a gene product of the X region of STLV. Furthermore, this X gene product of STLV was almost the same size as that of HTLV-I [12–15]. As shown in fig.1B and C, the putative X gene product of each STLV was also found in MtM/RfM26 and PtM3 cell lines, which produce STLV from a red-faced macaque and a pig-tailed macaque, respectively. In both cell lines, a protein of 41 kDa was detected with both anti-OP-1 and anti-bGH-40^{xI}, and immunoprecipitation of this protein was completely inhibited by the relevant peptides, like that of the 41 kDa protein in BM5 cells.

From these results, this 41 kDa protein was concluded to be a product of the X region of STLV derived from these 3 macaques. Furthermore, this product of the X region of STLV seems to be more closely related to the product of HTLV-I than to that of HTLV-II since it was detected with antiserum that reacted with the X gene product of HTLV-I, but not with that of HTLV-II. In addition to this protein, a faint band of 44 kDa was observed when a lysate of PtM3 cells was precipitated with anti-OP-1, and this immunoprecipitation reaction was inhibited competitively by the OP-1 peptide. However, the 44 kDa protein in this cell line was not detected with anti-bGH-p40^{xI}. The nature of the protein in this band is unknown, but it might be another X gene product of STLV in PtM3 cells.

The X region of STLV provirus does not show significant homology with simian cellular DNA free of STLV (not shown), and sero-epidemiological data indicate that there are many healthy carriers of STLV [1–3]. Thus the X region of STLV is unlikely to be a typical type of oncogene. In other retroviruses of the HTLV family, the X gene product has been shown to have a function of *trans* activation, and the possible relation between this function and the oncogenesis by the viruses has been discussed [16–18]. Further studies on the function of the X gene product of STLV should provide information on the mechanism of leukemogenesis by HTLV family viruses.

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