Targeted infection of a retrovirus bearing a CD4–Env chimera into human cells expressing human immunodeficiency virus type 1

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We constructed a hybrid Moloney murine leukaemia virus (MoMLV) bearing both a chimeric CD4 and the wild-type MoMLV envelope protein (Env) on its surface. The chimeric molecule, consisting of a surface domain of CD4 and the C-terminal two-thirds of MLV Env, was expressed on the cell surface. When expressed in MoMLV-infected cells, the CD4–Env chimera was incorporated into the virion as efficiently as the wild-type MoMLV Env. The hybrid MoMLV could infect human HeLa cells (although not with high efficiency) only if the cells were expressing human immunodeficiency virus type 1 genome. This method of ligand incorporation into a virion may lead to a development of a cell-specific retroviral vector for targeting gene therapy.

Retroviruses have been widely used as vectors for gene therapy (Friedman, 1989). Selective delivery of transgenes into specific cells such as human immunodeficiency virus type 1 (HIV-1) -infected cells is desirable for this purpose. Because retroviruses infect cells by binding of the envelope protein (Env) to the receptor on the host cell surface (Weiss, 1993), a retrovirus whose surface has a ligand which recognizes a molecule expressed specifically on a particular cell type may be useful for vector targeting (Roux et al., 1989; Etienne-Julan et al., 1992; Valsesia-Wittmann et al., 1994; Kasahara et al., 1994).

To obtain such a vector, the ligand must be efficiently incorporated into the virion surface like the wild-type Env. Retroviral Env is multimerized in the endoplasmic reticulum and transported to the Golgi complex for modification and proteolytic cleavage into the surface protein (SU) and the transmembrane protein (TM) (Einfeld & Hunter, 1988). Correctly processed Env is expressed on the cell surface and assembled into the virion. The mechanism of Env incorporation into the virion remains unclear. Some have claimed that the interaction between Env and core protein (Gag) is necessary for Env incorporation (Yu et al., 1992; Dorfman et al., 1994), whereas others have reported that some heterologous proteins can be efficiently incorporated into the virion (Young et al., 1990; Arthur et al., 1992), suggesting that Env–Gag interaction can be dispensed with. Recently, Suomalainen & Garoff (1994) reported that homologous Env is preferentially incorporated into the virion via a different pathway from that of heterologous proteins.

For targeting of retroviruses through a ligand–receptor interaction, not only is ligand incorporation into the virion necessary but also the internalization of RNA genome with viral core protein into the cell after the interaction between the ligand and the targeted cell receptor. The efficiency of the internalization step appears to depend on the nature of both the ligand and the receptor (Roux et al., 1989; Etienne-Julan et al., 1992). We believe that for such targeting it would be advantageous that the ligand is multimerized, cleaved proteolytically into surface and transmembrane proteins which interact with each other, expressed on the cell surface and incorporated efficiently into the virion, as is wild-type retroviral Env. Here, we made a chimera of CD4, a cell surface receptor for HIV-1, and the C-terminal two-thirds of Friend murine leukaemia virus (FMLV) Env which contained the SU–TM junction and the region considered to be important for SU–TM interaction after the proteolytic cleavage (Kasahara et al., 1994).

A human CD4 gene fragment (nucleotides 76 to 1263 according to the numbering of Maddon et al., 1985; GenBank accession no. M12807) encoding a surface domain of CD4 (amino acids 1 to 373) with the signal peptide was ligated in frame with a Friend murine
leukaemia virus (FMLV) env gene fragment (nt 6548 to 7805 according to the numbering of Koch et al., 1983; GenBank accession no. X02794) encoding the C-terminal two-thirds of Env (aa 224 to 641) to obtain a CD4-env chimera referred to as CD4sFEc (Fig. 1). The chimera construct was introduced into an expression vector, pCXN2, whose insert is driven by a chicken fl-actin promoter with cytomegalovirus immediate early enhancer (provided by J. Miyazaki, University of Tokyo) (Niwa et al., 1991), and transfected into NIH 3T3 cells to obtain a stable cell clone, referred to as NCD4sFEc44, expressing the CD4-env chimera.

The surface expression of the CD4-Env chimera was confirmed by flow cytometric analysis using a monoclonal mouse anti-human CD4 antibody (Cosmo Bio [MOC], IOT4a) (data not shown). The processing of the CD4-Env chimera was examined by immunoprecipitation analysis with the polyclonal goat anti-gp70 (MLV SU) antibody (National Cancer Institute lot 79S000713). A CD4-Env chimera molecule was detected in NCD4sFEc44 cells labelled for 30 min (Fig. 2a, lane 1). After a 30 min ‘chase’, another molecule appeared, which seemed to be the N-terminal product after the proteolytic cleavage at the SU–TM junction in FMLV Env (Fig. 2a, lane 2), which we term the processed chimera or CD4–SU. Thus, it is suggested that the CD4-Env chimera was processed by proteolytic cleavage into CD4–SU and TM and transported to the cell surface in a fashion similar to the wild-type MLV Env.

Next, NCD4sFEc44 cells were infected with Moloney murine leukaemia virus (MoMLV). The lysates of the cells labelled for 2 h were immunoprecipitated by the anti-CD4 antibody or the anti-gp70 antibody (Fig. 2a). Both the unprocessed and the processed wild-type MoMLV Env proteins, SU–TM and SU, were confirmed in the MoMLV-infected cells. Both the unprocessed and the processed chimeric molecules were detected by the anti-CD4 antibody and the anti-gp70 antibody.

To examine the incorporation of the CD4–Env chimera into virions, supernatants of cells labelled for 2 h with a 6 h chase were centrifuged at 18 500 g for 15 h and the lysates of the pellets were subjected to immunoprecipitation analysis (Fig. 2b). The anti-gp70 antibody detected the processed CD4–Env chimera (CD4–SU) as well as MoMLV SU in the pellet fraction from the supernatant of the MoMLV-infected NCD4sFEc44 cells but nothing in that of uninfected NCD4sFEc44 cells. These results confirm that the lysates of the pellets from the supernatants represent the proteins incorporated into virions. The incorporation efficiency of the CD4–Env chimera into the virion was estimated by determining the ratio of the band radioactivity (measured with an image analyser; FUJIX, Bas-2000) in virions (obtained from Fig. 2b) to that in cells (obtained from Fig. 2a). As a result, it was revealed that the CD4–SU in the MoMLV-infected NCD4sFEc44 cells was incorporated into virions as efficiently as wild-type MoMLV SU. Thus, the supernatant from the MoMLV-infected NCD4sFEc44 cells, referred to as MoMLV-CD4sFEc, contained the hybrid MoMLV bearing the CD4–Env chimera.

To examine the interaction between the MoMLV Env and the CD4–Env chimera, immunoprecipitates with anti-CD4 antibody were analysed by Western blotting using the anti-gp70 antibody (Fig. 2c). MoMLV Env co-immunoprecipitated with the CD4–Env chimera was detected in the MoMLV-infected NCD4sFEc44 cells, indicating that the CD4–Env chimera was bound to MoMLV Env to make a heteromultimer. This result suggests that the C-terminal two-thirds of FMLV Env contains the region required for multimerization. Efficient incorporation of the CD4–Env chimera into the virion may be due to the multimerization of the molecule or the interaction between the C-terminal two-thirds of Env and the MoMLV matrix protein.

We examined whether the hybrid MoMLV bearing the CD4–Env chimera as well as the wild-type MoMLV Env could infect human cells expressing HIV-1, although the wild-type MoMLV is known to infect murine cells only. The 24 h culture supernatant of the MoMLV-infected NCD4sFEc44 cells, MoMLV-CD4sFEc, had an infectious virus titre of 1 x 10⁸ p.f.u./ml estimated by the UV-XC test on NIH 3T3 cells (Rowe et al., 1970; Matano et al., 1993). The supernatant containing the hybrid MoMLV was frozen at –80 °C after eliminating contaminating cells by centrifugation at 2000 g for 10 min before use. HeLa cells were plated at a density of 10⁴ cells per well. The supernatant was used to infect HeLa cells (5 x 10⁴ cells per well).
Fig. 2. (a) Immunoprecipitation analysis. Cells were plated at a density of 5 x 10^5 cells/6 cm dish and grown overnight. The cells were labelled with 50 µCi/ml of [35S]methionine and 20 µCi/ml of [35S]cysteine (> 1000 Ci/mmol; Amersham) for 30 min (lane 1), 30 min with a 30 min chase (lane 2), 30 min with a 30 min chase (lane 2), or 2 h (lanes 3 to 8) and lysed with the triple-detergent lysis buffer (50 mM-Tris-HCl, pH 8.0, 150 mM-NaCl, 0.02% sodium azide, 0.1% SDS, 0.5% sodium deoxycholate, 0.1 mg/ml PMSF, 1 µg/ml aprotinin, 1% Triton X-100) (Sambrook et al., 1989; Matano et al., 1994). The lysates were immunoprecipitated with the anti-gp70 antibody (lanes 1 to 5) or the anti-CD4 antibody (lanes 6 to 8) and subjected to SDS-PAGE (8% polyacrylamide). Lanes 1 and 2, NCD4sFEc44 cells; lanes 3 and 7, NIH 3T3 cells; lane 4, MoMLV-infected NIH 3T3 cells; lanes 5 and 6, MoMLV-infected NCD4sFEc44 cells; lane 8, the wild-type CD4-expressing NIH 3T3 clone cells (referred to as NCD4) infected with MoMLV. The solid triangle indicates the unprocessed CD4-Env chimera and open triangle indicates the processed CD4-SU. Molecular mass markers on the right. (b) Immunoprecipitation analysis of the proteins incorporated into the virion. Cells were labelled for 2 h, and after a 6 h chase, the supernatants were harvested. After eliminating contaminated cells by centrifugation at 2000 g for 10 min, 0.8 ml of the supernatants were centrifuged at 18,500 g for 15 h at 4 °C. The lysates of the pellets were immunoprecipitated by the anti-gp70 antibody (lanes 1 to 3) or the anti-CD4 antibody (lanes 4 to 6) and subjected to SDS-PAGE (8% polyacrylamide). Lanes 1 and 6, MoMLV-infected NIH 3T3 cells; lanes 2 and 5, NCD4sFEc44 cells; lanes 3 and 4, MoMLV-infected NCD4sFEc44. (c) Western blot analysis using the anti-gp70 antibody of the immunoprecipitates by the anti-CD4 antibody. The cells were lysed with the single-detergent lysis buffer (50 mM-Tris-HCl, pH 8.0, 150 mM-NaCl, 0.02% sodium azide, 0.1 mg/ml PMSF, 1 µg/ml aprotinin, 1% Triton X-100) (Sambrook et al., 1989; Matano et al., 1994). Western blot analysis was performed with an ECL kit (Amersham). Lane 1, MoMLV-infected NCD4 cells; lane 2, MoMLV-infected NCD4sFEc44 cells; lane 3, NCD4sFEc44 cells. Solid triangles indicate the unprocessed and processed MoMLV Env and open triangles indicate the unprocessed and processed CD4-Env chimera.

HIV-1 Env expression was confirmed in the HeLa cells 48 h after HIV-1 DNA transfection by immunostaining and Western blotting using an anti-HIV-1 antiserum (provided by N. Yamamoto, Tokyo Medical and Dental University) (data not shown). The efficiency of the HIV-1 DNA transfection, which was estimated by counting cells immunostained with the anti-HIV-1 antiserum, was about 8%.
MoMLV gene expression was examined by reverse transcription and PCR (RT-PCR) amplification of an MoMLV env-specific sequence (nt 6272 to 7062) (Fig. 3a). MoMLV RNA expression was detected in the HIV-1-transfected HeLa cells cultured with MoMLV-CD4sFEC, but was undetectable in the control untransfected HeLa cells cultured with MoMLV-CD4sFEC. Northern blot analysis using an MoMLV-specific DNA probe (nt 7674 to 8229) detected MoMLV RNA in the HIV-1-transfected HeLa cells cultured with MoMLV-CD4sFEC (Fig. 4b, lane 3). RT-PCR amplification from titrated RNA (Fig. 3b and c) suggested that MoMLV RNA expression in the HIV-1-transfected HeLa cells cultured with MoMLV-CD4sFEC was about 1% of that in the HeLa clone cells expressing MoMLV genome.

Thus, the hybrid MoMLV bearing both the CD4-Env chimera and the wild-type MoMLV Env, MoMLV-CD4sFEC, could infect the human cells expressing HIV-1. This result may lead to anti-HIV-1 gene therapy with a retroviral vector targeting HIV-1-infected cells. However, there are still unresolved problems. Firstly, the MoMLV-CD4sFEC must be unable to infect non-dividing cells like the wild-type MLV. Secondly, it might be possible that the MoMLV-CD4sFEC could infect the cells expressing class II major histocompatibility complex (MHC) molecule through the interaction between the CD4-Env chimera and class II MHC. Thirdly, the MoMLV-CD4sFEC may bind to free HIV-1 virions and thus lose infectivity for the cells expressing HIV-1.

Our work provides the first step toward a development of a vector targeting HIV-1-infected cells. Recently, Kasahara et al. (1994) reported that a chimera of erythropoietin with MLV Env is efficiently incorporated into an ecotropic MLV, which is able to infect human cells expressing the erythropoietin receptor. Thus, it may be possible to obtain many kinds of targeted retroviral vectors by generating chimeras of a variety of ligands with MLV Env.

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References


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