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Infection of mesangial cells with HIV and SIV: Identification of GPR1 as a coreceptor

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Infection of mesangial cells with HIV and SIV: Identification of GPR1 as a coreceptor.

Background. Mesangial cells are an important component of the glomerulus. Dysfunction of mesangial cells is thought to be involved in the development of human immunodeficiency virus type 1 (HIV-1)-associated nephropathy (HIVAN). HIVAN is a structural renal failure frequently observed in patients with acquired immune deficiency syndrome. However, the susceptibility of mesangial cells to HIV-1 is disputable. More than ten G protein-coupled receptors, including chemokine receptors, have been shown to act as HIV-1 coreceptors that determine the susceptibilities of cells to HIV-1 strains with specific cell tropisms.

Methods. We examined the susceptibility of mesangial cells to various HIV-1, HIV type 2 (HIV-2) and simian immunodeficiency virus (SIV) strains. Expression of CD4 and HIV/SIV coreceptors was examined by Western blotting and polymerase chain reaction.

Results. Mesangial cells were found to be susceptible to HIV-1 variant and mutants that infect brain-derived cells, but highly resistant to T-tropic (X4), M-tropic (R5) or dual-tropic (X4R5) HIV-1 strains. In addition, mesangial cells were also susceptible to HIV-2 and SIV strains that infect the brain-derived cells. Among HIV/SIV coreceptors we tested, the expression of GPR1 mRNA was detected in mesangial cells. Expression of CD4 mRNA and protein was also detected in them. Mesangial cells and GPR1-transduced CD4-positive cells showed similar susceptibilities to the HIV-1 variant and mutants and HIV-2 and SIV strains.

Conclusions. CD4 and GPR1 mRNAs were detected in mesangial cells. Mesangial cells were susceptible to HIV/SIV strains that use GPR1 as a coreceptor. Our findings suggest that an orphan G protein-coupled receptor, GPR1, is a coreceptor expressed in mesangial cells. It remains to be investigated whether the interaction of mesangial cells with specific HIV-1 strains through GPR1 plays a role in the development of HIVAN.

Key words: HIVAN, AIDS, mesangium, CD4, immune deficiency syndrome, FSGS, apoptosis.

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Patients infected with human immunodeficiency virus type 1 (HIV-1) often develop a chronic renal disease known as HIV-1-associated nephropathy (HIVAN) [1–7]. The pathological change commonly observed in the lesions of HIVAN is focal and segmental glomerulosclerosis (FSGS). However, it is still not clear how HIV-1 is involved in the development of HIVAN. The principal point requiring elucidation is whether HIVAN is caused directly by infection or an interaction of renal cells with HIV-1, or indirectly by the response of renal cells to something such as cytokines released from other nonrenal cells infected with HIV-1. Successful infection of mesangial cells with HIV-1, and, on the contrary, resistance of renal cells to HIV-1 has been found in studies in which T cell line (T)-tropic and macrophage (M)-tropic (X4 and R5) HIV-1 strains were used as inocula. As for the susceptibility of other renal cells to HIV-1, discrepant results have also been reported [8–10]. It has been shown that HIV-1-transgenic mice develop progressive renal lesions that are similar to those observed in human HIVAN, and that kidneys transplanted into normal mice from HIV-1-transgenic mice develop FSGS [11, 12]. These findings strongly suggest that HIVAN is caused by direct infection of renal cells with HIV-1 or by interaction of renal cells with gene products of HIV-1.

The early predominant features observed in HIVAN are mesangial cell hyperplasia and mesangial expansion [4, 6, 13]. In contrast, the primate model of HIV-1-associated glomerulopathy showed that damage to mesangial cells occurs in the lesions of monkeys infected with simian immunodeficiency virus (SIV) [14]. The remnant kidney model of rats as well as the primate model demonstrated that apoptosis of mesangial cells possibly plays an important role in the development of FSGS [14, 15].

More than ten G protein-coupled receptors (GPCRs), including chemokine receptors, have been shown to act as coreceptors for HIV-1, HIV type 2 (HIV-2), and SIV [16–28]. Coreceptors mediate the infection processes together with the first receptor, a CD4 molecule. Thirteen

GPCRs have been identified as coreceptors that potentiate infection of cell-free HIV/SIV [16–28] when this study was started.

In this study we examined the expression of coreceptors for HIV-1, HIV-2 or SIV in human mesangial cells cultured in vitro, and then we examined the susceptibilities to various HIV-1 strains with different cell tropisms [T-tropic (X4), M-tropic (R5), and dual-tropic (X4R5) strains] and to HIV-1 mutants. That is, we previously isolated several HIV-1 variants that infect primary brain-derived cells (B) such as BT-3 or BT-20/N cells, and a CD4-transduced glioma cell line, U87/CD4, as well as T cell lines (T). These brain-derived cells are highly resistant to X4, R5 and X4R5 viruses [21, 28–30]. In this article, these variants are referred to as "BT-tropic." We found that mesangial cells are susceptible to BT-tropic HIV-1 mutants but not to the T-, M- or dual-tropic HIV-1 strains. Among the coreceptors for HIV/SIV tested, the expression of GPR1 mRNA was detected in mesangial cells. We recently reported that GPR1 is a coreceptor for these variants or mutants [30]. The possible mechanism for the damage or dysfunction of mesangial cells by HIV-1 is discussed.

METHODS

Cells

Primary human mesangial cells, HMes and NHMC 2845 (normal human mesangial cell), and the human primary skin fibroblasts, TT-5 and SF-TY [31], were maintained in Dulbecco's modified Eagle's minimum essential medium (DMEM) supplemented with 20% (vol/vol) fetal calf serum (FCS). The HMes strain was isolated from a Japanese as previously reported [32]. The NHMC 2845 (NHMC) strain was purchased from BioWhittaker (Walkersville, MD, USA) through TAKARA Shuzo Co. Ltd. (Kyoto, Japan). The TT-5 strain was derived from the skin of a five-year-old boy, and SF-TY cells were obtained from the Japanese Cancer Research Resources Bank. BT-3 and BT-20/N cells were fibroblast-like cells isolated from a meningioma and normal human brain tissue next to a glioblastoma, respectively, as described previously [21, 28–30, 33]. BT-3 and BT-20/N cells were negative for specifically differentiated cell markers in the brain, such as Factor VIII-related antigens or glial fibrillary acidic protein, but were considered to have originated from blood vessel cells in the brain, as monoclonal antibodies against BT-3 cells reacted with blood vessels (unpublished data). These brain-derived BT-3 and BT-20/N cells were maintained in RPMI 1640 medium containing 10 μg/mL of endothelial cell growth supplements (ECGS; Collaborative Biomedical Products, Bedford, MA, USA), 10 ng/mL of epidermal growth factor, and 90 μg/mL of heparin (HEE medium) in gelatin-coated cell culture dishes. HEE medium supported the growth of mesangium cells well. These brain-derived cells and mesangial cells stopped growing after 15 to 20 cell passages and showed senescent properties, suggesting that they originated from normal cells. A human T-cell line, C8166 [34], was maintained in RPMI 1640 medium supplemented with 10% (vol/vol) FCS. NP-2 is a human glioma cell line. CD4-transduced NP-2 cells (NP-2/CD4), GPR1-expressing NP-2/CD4 cells (NP-2/CD4/GPR1) and CXCR4-expressing NP-2/CD4 cells (NP-2/CD4/CXCR4) were made as previously described [21, 30, 35].

HIV/SIV strains

A T-tropic (X4) HIV-1 strain, IIIB [36], a dual-tropic (T- and M-tropic) (X4R5) HIV-1 strain GUN1WT [37], an M-tropic (R5) HIV-1 strain, BaL [38], and an HIV-1 variant, GUN1v [21, 28–30], were propagated in MOLT-4#8 [39] cells, phytohemagglutinin (PHA)-stimulated human peripheral blood lymphocytes (PBL) or U87/CD4 cells as described previously [21, 28–30, 35]. HIV-1 mutants, GUN1/A, GUN1/L, GUN1/R, GUN1/S and GUN1/T strains, were constructed by changing the GPGR (glycine-proline-glycine-arginine) sequence at the V3 tip of the *Env* protein of the GUN1WT strain to GA (alanine) GR, GL (leucine) GR, GR (arginine) GR, GS (serine) GR and GT (threonine) GR, respectively, by site-directed mutageneses [30, 40]. GUN1/L and GUN1/R were dual-tropic (X4R5) strains, while GUN1v, GUN1/A, GUN1/S and GUN1/T were infectious to brain-derived cells such as BT-3, BT-20/N or U87/CD4 cells, which are resistant to the R5, X4 or R5X4 HIV-1 strains, as well as to C8166 or Molt-4 T cell lines [40]. GUN1v is a variant isolated from GUN1WT in tissue culture [28]. As for HIV-2 strains, ROD/B [41], CBL20, CBL21 and CBL23 [42], and as for SIV strains, mac251 [43], mndGB-1 [44] and agmTYO-1 [45] were used.

Virus infection assay

BT-3, BT-20/N or mesangial cells (HMes and NHMC) were seeded into 24-well cell culture plates at 3 to 5×10^4 cells per well. On the following day, the cells were washed with heparin-free culture medium and inoculated with one of HIV/SIV strains at 37°C for one to several hours. Amounts of HIV/SIV inoculated were adjusted to be 1×10^4 or 1×10^5 cpm by reverse transcriptase (RT) activities of inocula as previously described [30, 35, 40]. Then the cells were cultured in a suitable heparin-free medium. After incubation for 7 to 10 days, the expression of HIV/SIV antigens in the infected cells was detected by indirect immunofluorescence assay (IFA) as described previously [21, 28–30, 35]. HIV-1-seropositive human serum or SIVmac-infected monkey serum [30] was used as the first antibody and fluorescent isothiocyanate (FITC)-conjugated rabbit anti-human immunoglobulins or anti-simian immunoglobulins (DAKO Co. Ltd., Clostrup, Denmark) was used as the second antibody in IFA. Ex-

pression of HIV-1 antigens was also confirmed by IFA using a monoclonal antibody (mAb) against p24 [33]. SF-TY, TT-5, U87/CD4, NP-2/CD4, NP-2/CD4/GPR1 and NP-2/CD4/CXCR4 cells were also seeded and infected with HIV/SIV, and they were examined for their susceptibilities to HIV/SIV strains by IFA five to seven days after infection as described above. Production of HIV-1 protein p24 into the culture supernatants of mesangial cells infected with GUN1v or GUN1wt strain was detected by enzyme-linked immunosorbent assay (ELISA) as described elsewhere [33].

Monoclonal antibodies

A monoclonal antibody against CD4 Nu-T H/I (Nichirei Co. Ltd., Tokyo, Japan) was used to detect expression of CD4 in mesangial cells by flow cytometry (FCM) as described elsewhere [35]. This mAb was also used to examine whether it inhibits infection of mesangial cells with HIV-1 strains. Cells were treated with mAb for 30 minutes and then were infected with HIV-1. The culture supernatants of infected cells were harvested six days after infection and examined by ELISA for production of HIV-1 protein p24 as previously described [33].

Production of CD4 in mesangial cells was detected by Western blotting using Nu-T H/I mAb. mAb bound to filters was detected by the enhanced chemiluminescence (ECL) detection kit (GIBCO BRL Co. Ltd., Rockville, MD, USA) according to the recommended protocol of the supplier.

Polymerase chain reaction primers for GPCRs

Oligonucleotide primers (Nihon Idenshi Kenkyujo, Co. Ltd., Miyagi, Japan) were used to detect the expression of GPCR mRNA in BT-3, BT-20/N, C8166, HMes, NHMC, and U87/CD4 cells by polymerase chain reaction (PCR) after reverse transcription (RT) of cellular RNA (RT-PCR) as previously described [21, 30]. The GPCRs examined in this study, and the nucleotide sequences, orientations (sense or antisense) and positions of the primers in the open reading frame are as follows: CCR2b, 5′-ATGCTGTCCACATCTCGTTCTCGGTT TATCAG-3′ (sense, the 1st to 32nd nucleotide sites) and 5′-TTATAAACCAGCCGAGACTTCCTGCTCCC CAG-3′ (antisense, the 1,052nd to 1,083rd) (GenBank accession number, U03905), CCR3, 5′-GCCCGGACTG TCACTTTTGGTGTCATCACCAG-3′ (sense, the 433rd to 464th) and 5′-CTTCTCACTAGGAAGGAATGGGA TGTATCT-3′ (antisense, the 982nd to 1,011th) [U49727]; CCR5, 5′-GCCAGGACGGTCACCTTTGGGGTGGT GACAA-3′ (sense, the 415th to 445th) and 5′-AGCCTC TTGCTGGAAAATAAAACAGCATTT-3′ (antisense, the 964th to 993rd) (U54994); CCR8, 5′-GTGAGGAC GATCAGGATGGGCACAACGCTGTG-3′ (sense, the 430th to 461st) and 5′-GCTCTCCCTAGGCATTTGT CTTCCTAGGTA-3′ (antisense, the 973rd to 1,002nd) (U62556); CXCR4, 5′-CCAAGGAAGCTGTTGGCT GAAAAGGTGGTCTA-3′ (sense, the 439th to 470th) and 5′-TCCACCTCGCTTTCCTTTGGAGAGGAT CTT-3′ (antisense, the 979th to 1,008th) (X71635); GPR1, 5′-ATGGAAGATTTGGAGGAAACATTATTT GAA-3′ (sense, the 1st to 30th) and 5′-TTATTGAGCT GTTTCCAGGAGACACAGATTC-3′ (antisense, the 1,038th to 1,050th) (U13666); GPR15, 5′-ATGGACCC AGAAGAAACTTCAGTTTATTTG-3′ (sense, the 1st to 30th) and 5′-TTAGAGTGACACAGACCTCTTC CTCCTCCTGG-3′ (antisense, the 1,052nd to 1,083rd) (U34806); STRL33/Bonzo, 5′-AATCTCGACAAGCTC ATATGTGGTTACCATG-3′ (sense, the 520th to 550th) and 5′-AGATTTCCATTGATGTGAGACCCCAAG GTAAG-3′ (antisense, the 929th to 960th) (AF007545); US28, 5′-CCGCATTTCCAGAATCGTTGCGGTGT CTCAG-3′ (sense, the 624th to 654th) and 5′-TGTGA GACGCGACACGCCTCGTCGGACAGCG-3′ (antisense, the 1,019th to 1,048th) (L20501); and v28, 5′-ATG GATCAGTTCCCTGAATCAGTGACAGAAAAC-3′ (sense, the 1st to 33rd) and 5′-TCAGAGAAGGAGC AATGCATCTCCATCACTCG-3′ (antisense, the 1,037th to 1,068th) (U20350).

Expression of CD4 and GPCRs mRNA

Expression of CD4 and GPCRs mRNA was detected as previously described [21, 30]. That is, total cellular RNA was isolated from BT-3, BT-20/N, C8166, HMes, NHMC, U87/CD4 and C8166 cells (1×10^5 cells) using an RNA extraction kit (SepaGene; Sanko-Junyaku Co. Ltd., Tokyo, Japan) following the manufacturer's protocol. Contaminated DNA in RNA preparations was removed by digestion with 1 U/μL of RNase-free DNase I (Boerhinger Mannheim Japan Co. Ltd., Tokyo, Japan) at 37°C for two hours. cDNA was made using 2 U/μL Superscript II reverse transcriptase (GIBCO BRL) in 20 μL of the reaction cocktail containing 2 μg of total RNA, 0.6 μg oligo-dT (GIBCO BRL), 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$ 2.5 mmol/L of each dNTP, 10 mmol/L dithiothreitol, and 10 U RNasin (Takara Shuzo Co. Ltd.) at 42°C for one hour. Reverse-transcribed GPCRs or CD4 DNA in the cDNA preparations was detected by PCR using the specific primers for each GPCR as described above. PCR was performed in a reaction mixture (20 μL) containing 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 2.5 mmol/L of each dNTP, 5 U *Taq* DNA polymerase (Takara Shuzo Co. Ltd.) and 60 ng of one of the PCR primer pairs sense and antisense as follows: 38 cycles of 93°C for 30 seconds, 60°C for one minute, and 72°C for two minutes. The amount of each cell RNA subjected to RT-PCR was normalized to be equal by densitometry of DNA bands for glyceraldehyde-3-phosphate dehydrogenase (G3PDH) RNA amplified by RT-PCR. PCR-amplified DNA fragments were detected

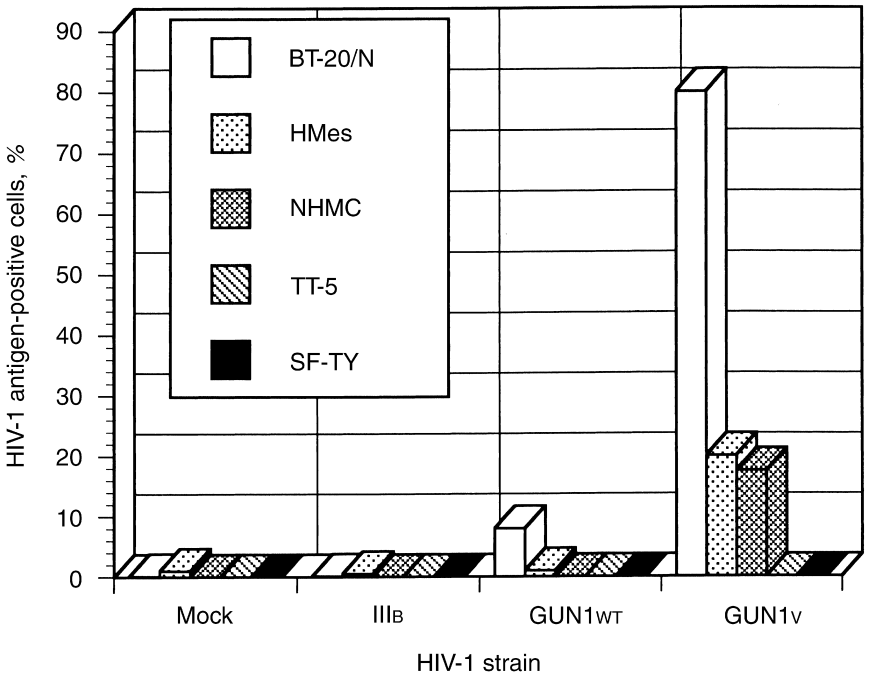

Fig. 1. Susceptibility of mesangial cells to HIV-1 strains with different cell tropisms. Mesangial cells, HMes and NHMC, primary brain-derived fibroblast-like cells, BT-20/N, and skin fibroblast strains, TT-5 and SF-TY, were infected with three HIV-1 strains, IIIB, GUN1WT and GUN1V, and examined for expression of HIV-1 antigen by IFA 7 days after infection. Amounts of HIV-1 inoculated were adjusted and corresponded to 1×10^5 cpm of reverse transcriptase (RT) activity as described in the **Methods** section.

through 1% (wt/vol) agarose gel electrophoresis. To estimate efficiencies of PCR for CXCR4 and GPR1, plasmid DNA containing CXCR4 or GPR1 was serially diluted and detected by PCR. PCR bands with similar intensities were detected at the same concentrations of the templates, that is, CXCR4- or GPR1-containing plasmid DNA (data not shown). The sensitivity of RT-PCR assays for CXCR4 or GPR1 was determined to be sufficient to allow the detection of a few copies of RNA per cell (data not shown).

RESULTS

Infection of mesangial cells with HIV-1

HMes and NHMC mesangial cells and BT-20/N brain-derived cells were infected with HIV-1 strains with different cell tropisms (Fig. 1). HIV-1 antigens were detected seven days after infection by IFA using human serum positive for HIV-1 antibodies. The mesangial cells as well as BT-20/N cells were clearly susceptible to the GUN1v variant strain. When the mesangial cells were inoculated with GUN1v, 20 to 30% cells became HIV-1 antigen-positive. BT-20/N cells were highly susceptible to GUN1v but not to IIIB or GUN1WT strain as previously reported [21, 28–30, 33]. Results of IFA using mAb against HIV-1 protein p24 were similar to those described above (data not shown). Morphological changes of mesangial cells infected with GUN1v were hardly noticed. Unlike BT-20/N cells, these infected mesangial cells formed few syncytia (data not shown). In contrast, the mesangial cells showed resistance to T-tropic IIIB strain and dual-tropic (T- and M-tropic) GUN1WT strain (Fig. 1). The mesangial cells and BT-20/N cells showed similar patterns of susceptibility to three HIV-1 strains, although there was marked difference in their susceptibility to GUN1v. Human skin fibroblast strains, TT-5 and SF-TY, were resistant to all of the HIV-1 strains examined (Fig. 1).

The susceptibility of mesangial cells to the GUN1v strain was confirmed by detection of HIV-1 p24 in the culture supernatants of mesangial cells infected with HIV-1 strains (Fig. 2). Mesangial cells, HMes and NHMC, were markedly more susceptible to GUN1v than to GUN1WT. Infection of mesangial cells and BT-20/N cells with GUN1v was almost completely inhibited by pretreatment of the cells with mAb against CD4 (Fig. 2A).

Expression of CD4 and GPR1 mRNA in the mesangial cells

HIV-1 entry into target cells is mediated by CD4 molecules as the first receptor and one of several GPCRs as the coreceptor. We examined the expression of CD4 mRNA in mesangial cells by RT-PCR. As shown in Figure 3A, expression of CD4 mRNA was detected in HMes and NHMC cells as well as in C8166 T cells, suggesting that these mesangial cells would be susceptible to HIV-1 if some HIV/SIV coreceptors were expressed on their cell surface. The skin fibroblast cell strains TT-5 and SF-TY were negative for CD4 mRNA (data not shown). The cDNA used in RT-PCR experiments was serially diluted tenfold and the expression level of CD4 mRNA was determined by RT-PCR (Fig. 3B). HMes and NHMC cells were estimated to express

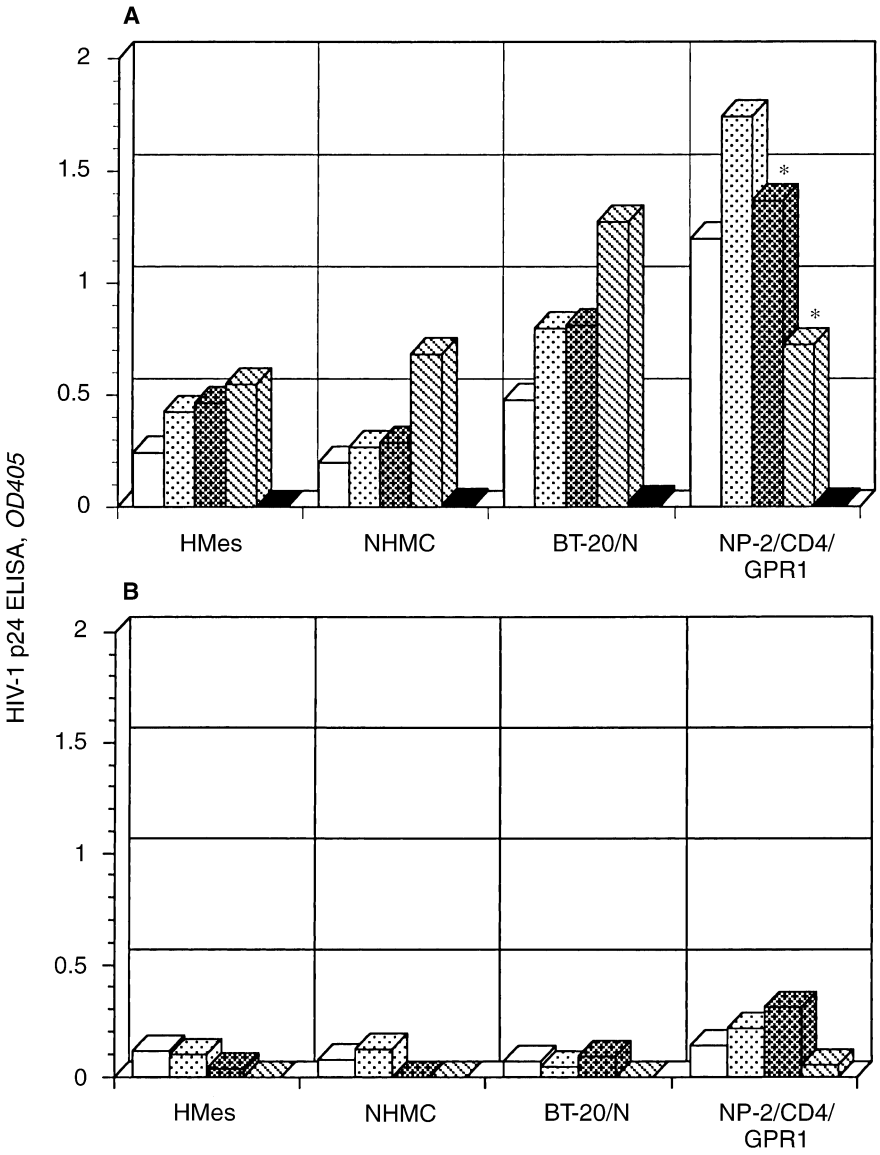

Fig. 2. Detection of HIV-1 protein p24 after infection. HMes, NHMC, BT-20/N and NP-2/CD4/GPR1 cells were treated with anti-CD4 mAb and infected with GUN1v (*A*) or GUN1WT (*B*) strain. The cells were passaged on day 6. Culture supernatants were harvested on days 2 (□), 4 (▨), 6 (▩) or 14 (▧) after infection and examined by ELISA for p24. Symbol (■) is anti-CD4 (a). The culture supernatants of mAb-treated cells were harvested on day 6 (A). The GUN1v strain severely damaged NP-2/CD4/GPR1 cells 6 days after infection (*).

about one thousandfold less CD4 mRNA than C8166 cells, which are known to be highly sensitive to HIV-1. Next, CD4 molecules expressed on the surface of HMes or NHMC cells were detected by flow cytometric analyses. However, neither mesangial cell strain gave clearly positive results: only 2 to 7% of the mesangial cells were estimated to be CD4-positive (data not shown). Expression of CD4 in cultured mesangial cells were examined by Western blotting using the mAb against CD4. About 2 to 5% of CD4 protein detected in human C8166 T cells was estimated to be present in the mesangial cell lysates (Fig. 4).

We examined the expression of HIV/SIV coreceptors, such as CCR2b, CCR3, CCR5, CXCR4, CCR8, STRL33/Bonzo, US28, v28, GPR1 and GPR15, in the mesangial cells by RT-PCR using specific primer pairs for each GPCR (Fig. 5). The mRNAs for CCR2b and GPR1 were regularly detected in HMes and NHMC cells, although CCR2b bands were much fainter than those of GPR1. Expression of GPR1 RNA was also detected in TT-5 and SF-TY fibroblasts (data not shown). CCR2b has been shown to act as a coreceptor for M-tropic HIV-1 strains and several SIV strains [15]. We noticed that GPR1 is specifically used by HIV-1 variants and several HIV-2 strains that infect brain-derived cells, such as BT-20/N, BT-3 or U87/CD4 [30, 35, 40, unpublished data]. These findings suggest that infection of mesangial cells with HIV-1 is mediated by GPR1 as a coreceptor.

The relative amounts of GPR1 mRNA expressed in HMes, NHMC, BT-20/N, BT-3, U87/CD4 and C8166 cells were examined by RT-PCR (Fig. 6). The relative amount of CXCR4 mRNA expressed in C8166 cells was

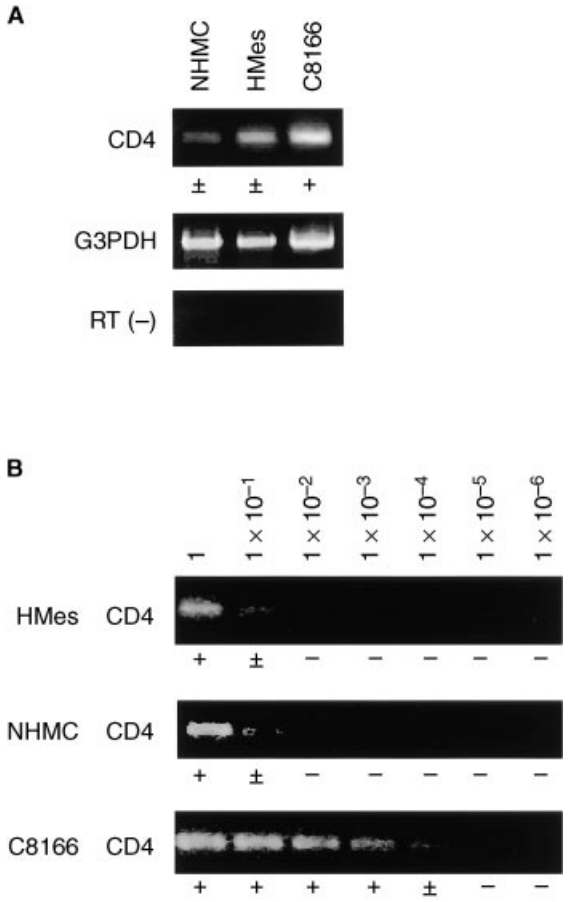

Fig. 3. Detection of CD4 mRNA in mesangial cells. (*A*) Detection of CD4 mRNA by RT-PCR. Cellular RNAs extracted from NHMC or HMes mesangial cells or C8166 T cells were reverse-transcribed, and CD4 or G3PDH cDNA was detected by PCR (RT-PCR). RT-PCR assay was also done using cellular RNA without reverse transcription, RT (−). (*B*) Quantification of CD4 RNA expressed in mesangial cells. cDNA made from RNA extracted from HMes, NHMC or C8166 cells was serially tenfold diluted and used for PCR to detect CD4 DNA. Intensities of PCR bands are: +, clearly detected; ±, faintly detected; −, not detected.

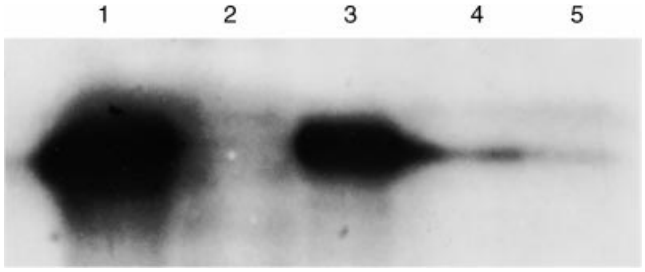

Fig. 4. Detection of CD4 by Western blotting. Cell lysates of human origins were electrophoresed, and were examined by mAb against CD4 using the ECL detection system after Western blotting. C8166 T cells are known to highly express CD4, while K562 erythroleukemia cells are negative for CD4. Lanes: 1, C8166; 2, K562; 3, BT-20/N; 4, HMes; 5, NHMC.

also determined by RT-PCR as a control. C8166 human T cells are highly susceptible to T-tropic HIV-1 strains, and this infection is mediated by a major coreceptor, CXCR4. As shown in Figure 6, CXCR4 mRNA was detected in ten thousandfold diluted cDNA of C8166 cells. Using the results of the control experiments described in the **Methods** section we estimated that ten thousand copies of CXCR4 mRNA were expressed in a single C8166 cell. Next, the expression levels of GPR1 mRNA in various cells were determined as described previously [30]. End points of cDNA dilutions that gave positive bands for GPR1 by RT-PCR were as follows: NHMC, ×10; HMes, ×1; BT-20/N, ×100; BT-3, ×100; U87/CD4, ×1000; and C8166, ×1. The levels of GPR1 mRNA expressed in mesangial cells, NHMC and HMes, were tenfold to one hundredfold lower than those of brain-derived cells such as BT-20/N, BT-3, and U87/CD4 cells. About one to ten copies of the GPR1 mRNA were estimated to be expressed in a single mesangial cell in average.

Infection of mesangial cells with the single-point mutants of the V3 tip of HIV-1 *Env* protein

If infection of mesangial cells with HIV-1 is mediated through GPR1, mesangial cells and GPR1-transduced cells should show similar patterns of susceptibility to various HIV/SIV strains. We examined this possibility. NP-2/CD4 cells, which were not transduced with any GPCRs, showed strong resistance to all of the HIV-1, HIV-2 or SIV strains examined thus far in our assay conditions as described elsewhere [21, 30, 35, 40], although they abundantly expressed CD4 molecules on their cell surface. NP-2/CD4 cells were transduced with GPR1 and CXCR4 and the resultant cells were designated NP-2/CD4/GPR1 and NP-2/CD4/CXCR4, respectively. NHMC, NP-2/CD4/GPR1, NP-2/CD4 and NP-2/CD4/CXCR4 cells were infected with GUN1 mutants. NP-2/CD4/GPR1 cells were highly susceptible to the variant type, BT-tropic viruses (GUN1v, GUN1/A, GUN1/T and GUN1/S), but retained their strong resistance to the T-tropic (X4) IIIB strain and dual-tropic (T- and M-tropic) (X4R5) strains (GUN1WT, GUN1/L and GUN1/R; Fig. 7) as previously reported [30, 35, 40]. Next, the susceptibilities of the mesangial cells and NP-2/CD4/GPR1 cells were compared to these HIV-1 mutants. As shown in Figure 7, NHMC cells were susceptible to GUN1v, GUN1/A, GUN1/S and GUN/T, and thus the patterns of susceptibility of NHMC cells to the GUN1v variant and GUN1 mutants were quite similar to those of NP-2/CD4/GPR1 cells. NP-2/CD4/CXCR4 cells were highly susceptible to all of the HIV-1 strains shown in Figure 7 (data not shown).

Susceptibilities of mesangial cells to HIV-2 and SIVs

We noticed that the brain-derived cells as well as NP-2/CD4/GPR1 cells are susceptible to some HIV-2 and SIV

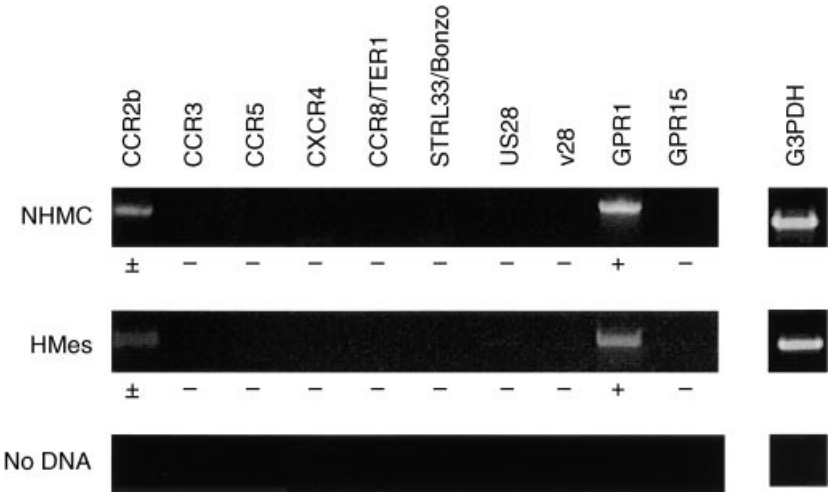

Fig. 5. Expression of HIV/SIV coreceptor RNA detected by RT-PCR. Cellular RNAs were extracted and examined by RT-PCR using specific PCR primer pairs for HIV/SIV coreceptors. G3PDH RNA was detected as a control. No DNA represents PCR controls lacking a template for cDNA.

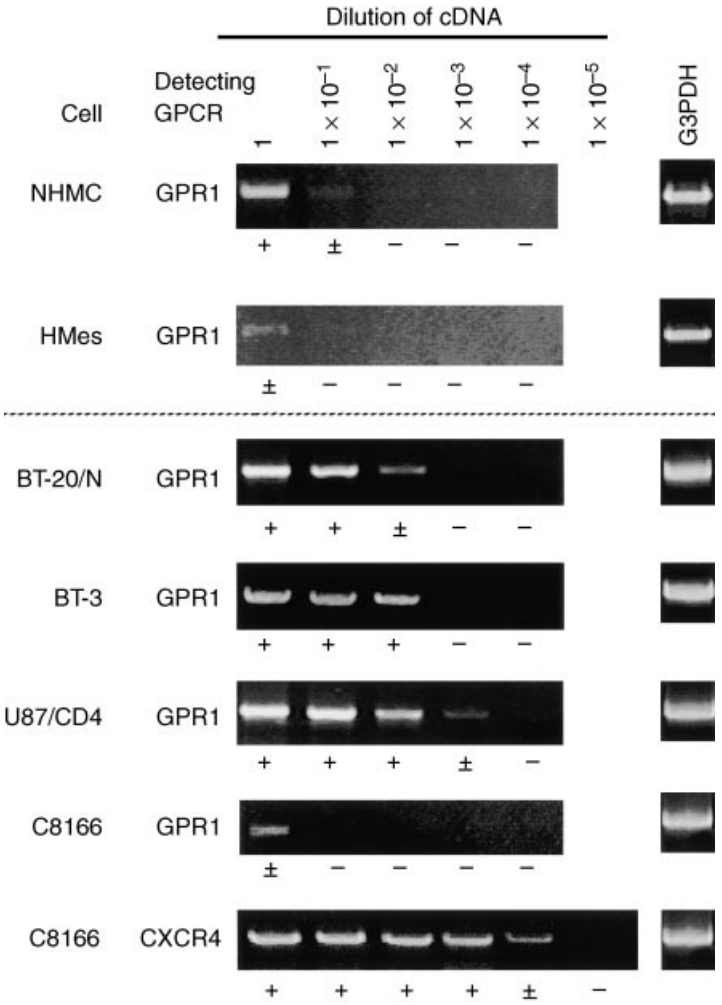

Fig. 6. Quantification of GPR1 and CXCR4 cDNA. RNAs extracted from NHMC and HMes mesangial cells, BT-20/N, BT-3 and U87/CD4 brain-derived cells, and C8166 T cells were reverse transcribed, serially tenfold diluted and used for PCR. Amounts of cDNA used were adjusted by results of PCR for G3PDH.

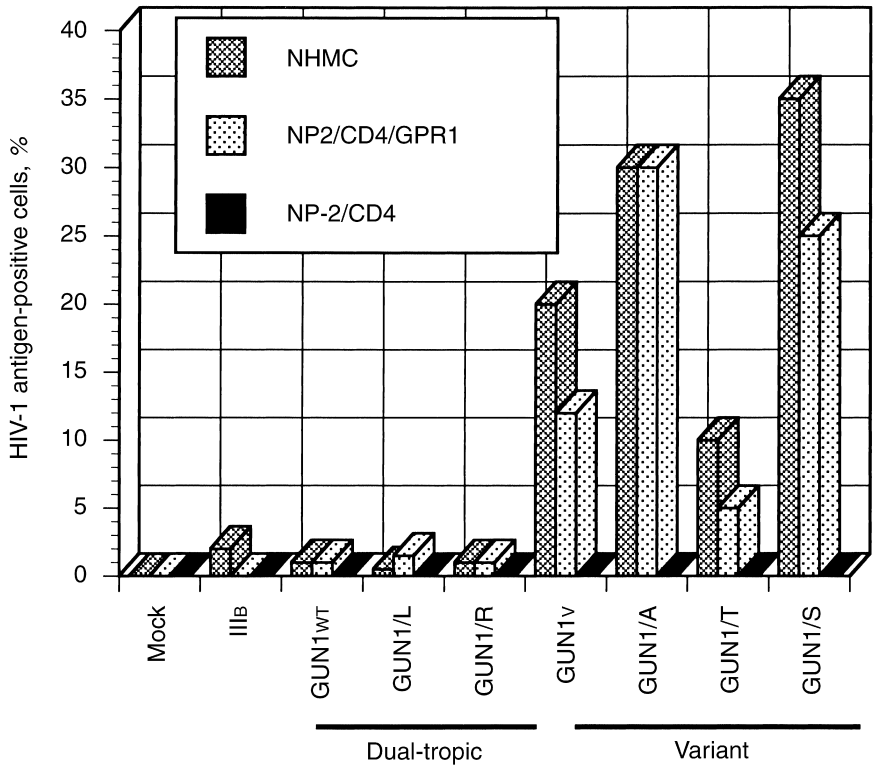

Fig. 7. Susceptibility of mesangial cells to HIV-1 mutants. NHMC cells (RT, 1×10^5 cpm) and NP-2/CD4/GPR1 and NP-2/CD4 cells (RT, 1×10^4 cpm) were infected with indicated RT amounts of HIV-1. HIV-1 antigen-positive cells were detected by IFA 7 days (NHMC cells) or 5 days (NP-2/CD4/GPR1 and NP-2/CD4 cells) after infection.

strains in addition to BT-tropic HIV-1 variants [31, 36, unpublished data]. Therefore, we examined the susceptibility of mesangial cells to HIV-2 and SIV. HMes and NHMC mesangial cells were inoculated with several HIV-2 and SIV strains, and they were examined for expression of HIV-2 or SIV antigens 10 days after infection (Fig. 8). Among the HIV-2 strains, CBL23 and ROD/B but not CBL20 or CBL21 plated onto these cells. The T-tropic IIIB or M-tropic BaL or SF162 HIV-1 strain was not infectious to these cells. As for SIVs, SIVmac, SIVmnd (Fig. 8) and SIVagm (data not shown) plated onto these mesangial cells, and 5 to 10% of the cells became SIV antigen-positive. NP-2/CD4/GPR1 cells were also susceptible to the CBL23 and ROD/B, but not to CBL20 or CBL21, HIV-2 strains and to these three SIV strains [30, 35, data not shown]. These findings also suggest that GPR1 functions as an HIV/SIV coreceptor in the mesangial cells.

DISCUSSION

Green, Resnick and Bourgoignie [8] and Kimmel et al [46] reported that HIV-1 can infect mesangial cells in vitro and that the glomerulus isolated from HIV-1-infected patients contains the HIV-1 genomic DNA, respectively. However, Alpers, McClure and Burstein reported that mesangial cells do not express CD4 molecules at a detectable level by FCM and HIV-1 cannot infect them in vitro [9]. Karlsson-Parra et al showed that mesangial cells express CD4 molecules at a detectable level by FCM [47]. To check these contradictory results, we tried to detect expression of CD4 on the surface of mesangial cells by RT-PCR, FCM and Western blotting. We noticed that HMes and NHMC mesangial cells showed, at most, only a marginal level of expression of CD4 on their surface: 2 to 7% of the mesangial cells were estimated to be positive under our assay conditions described previously [30, 35, 40]. Results of RT-PCR and Western blotting also showed that the mesangial cells expressed a small amount of CD4 (Figs. 3 and 4). One of the reasons for the low susceptibility of the mesangial cells to the HIV-1 variant and mutants observed in this study as compared with the brain-derived cells (Figs. 1, 7, and 8) is thought to be due to the low level of CD4 expression in them. Under similar assay conditions, these BT-tropic viruses plated much more efficiently onto BT-20/N or NP-2/CD4/GPR1 cells than onto the mesangial cells (Figs. 1, 2, and 7).

mRNAs for two HIV/SIV coreceptors, GPR1 and CCR2b, were expressed in the mesangial cell strains. GPR1 mRNA, which had been shown to act as a coreceptor for SIVmac strain [24], was detected in mesangial cells by RT-PCR (Figs. 5 and 6). The mRNA of CCR2b, which is a coreceptor for M-tropic or dual-tropic HIV-1 [20], was very weakly detected in mesangial cells (Fig. 5). Expression of the other known HIV/SIV coreceptors

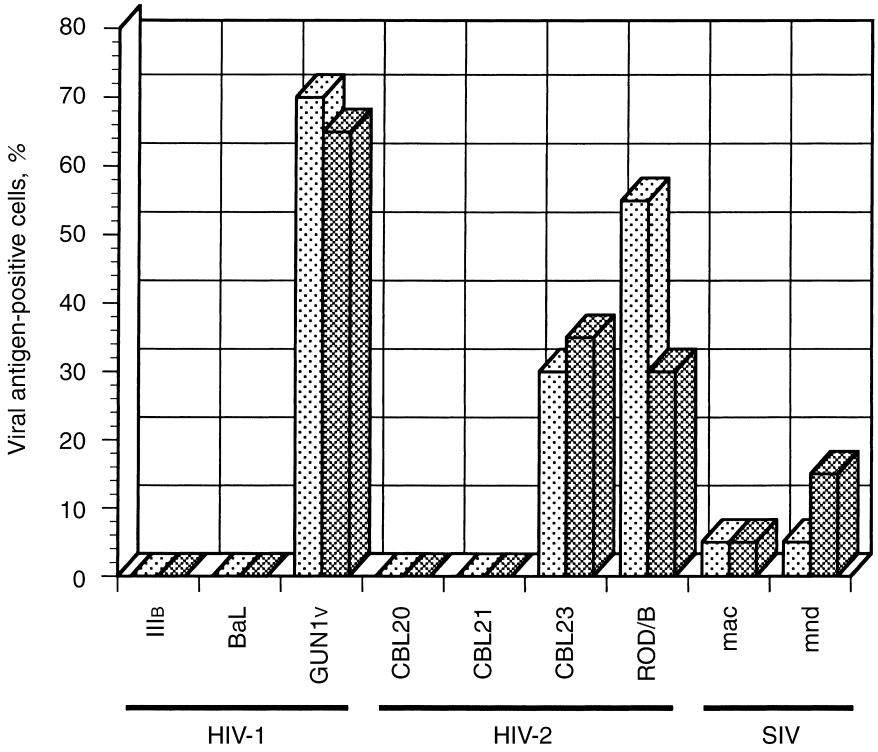

Fig. 8. Susceptibility of mesangial cells to HIV-2 and SIV. HMes (▨) and NHMC (▩) mesangial cells were infected with HIV-2 and SIV strains as well as HIV-1 strains (RT, 1×10^5 cpm). Viral antigen-positive cells were detected by IFA 10 days after infection.

was not always detected in mesangial cells. These results suggest that HIV-1 might infect CD4-positive mesangial cells using GPR1 or CCR2b as a coreceptor. Immunohistochemical study [48] and in situ hybridization study [49] of renal tissues showed that CCR5 is not expressed in intrinsic renal cells. Our findings on CCR5 expression in cultured mesangial cells may be consistent with results of these reports.

Mesangial cells have been reported to have only slight, or no, susceptibility to either T- or M-tropic HIV-1 strains [8, 9, 46], and these seemingly contradictory results could possibly be explained by our findings that a major coreceptor for T-tropic HIV-1 strains, CXCR4, or those for M-tropic HIV-1 strains, CCR5 and CCR3, were not expressed in mesangial cells, but that GPR1 was expressed in them. NP-2/CD4/GPR1 cells were highly, but not completely, resistant to T- or dual-tropic HIV-1 strains as shown in Figures 2 and 7. A small number of cells could still be infected with T- or dual-tropic HIV-1 strains as reported [30, 35], if a large amount of HIV-1 were inoculated onto mesangial cells.

We have shown that the variant type of HIV-1 strains, such as GUN1v, GUN1/A, GUN1/S, and GUN1/T, infect the brain-derived cells BT-3, BT-20/N and U87/CD4, and this infection is mediated by GPR1 [30, 40]. In this study, we showed that the mesangial cells HMes and NHMC as well as the brain-derived cells BT-20/N or BT-3 were susceptible to BT-tropic GUN1v, GUN1/A, GUN1/S and GUN1/T strains, while both types of the cells were resistant to GUN1WT, GUN1/L and GUN1/R strains. Thus, the mesangial cells were susceptible to all of the HIV-1 variant and mutants, which have been shown to use GPR1 as their coreceptor, but not to T-, M- or dual-tropic (T- and M-) HIV-1 strains, which cannot use GPR1 as a coreceptor. The expression of GPR1 mRNA was also detected in skin fibroblast cells, TT-5 and SF-TY, although CD4 mRNA was not detected in these cells (data not shown). FSGS is induced in primates infected with SIVmac [14]. It remains to be determined whether simian mesangial cells express CD4 and GPR1 and whether simian GPR1 can function as a coreceptor for SIVmac.

We could isolate BT-tropic HIV-1 variants from other strains, GUN4 and GUN7, which had been passaged less than 10 times in tissue culture. These variants also have serine or threonine at the tip of the V3 region [29]. LaRosa et al reported nucleotide sequences of a large number of HIV-1 present in peripheral blood: a few percent of HIV-1 strains are expected to have serine, threonine or alanine at the tip of the V3 region [50]. These findings suggest that HIV-1 strains infectious to mesangial cells may be present in HIV-1-infected persons, although they may represent a very minor population.

If HIV-1 virions or Env proteins bind to mesangial cells, it is expected that HIV-1 or HIV-1 proteins may damage mesangial cells or affect functions of mesangial cells. Although mesangial cells have low positivity for CD4 and GPR1 (Figs. 2 to 4), T-, M- or dual-tropic (T-

and M-tropic) HIV-1 or Env proteins may still be able to bind them. Even if this binding may not lead to the entry of HIV-1 into these cells, the functions of the cells can be affected by HIV-1 proteins.

Kidney disorders such as FSGS are frequently observed in African American, but not Hispanic or Thai AIDS patients [2, 5, 6, 51, 52]. Although the reason for this is not clear, specific HIV-1 mutant strains may possibly play an important role in the development of HIVAN. Renal epithelial cells are specifically susceptible to HIV-1 strains isolated from children with HIVAN [10]. Mesangial cells can be a key component to initiate HIVAN, because damage to mesangial cells is critical for the development of FSGS in the primate model [14] and *HIV-1* gene product is responsible for the development of renal lesions in transgenic mice [11, 12]. Our results may help to elucidate a mechanism as to how HIV-1 can induce HIVAN in AIDS patients.

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