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Differential Utilization of CCR5 Molecules from Three East African Primate Species by the HIV-1 Envelope Glycoprotein

David N. Mburu

Department of Biochemistry and Biotechnology,
School of Pure and Applied Sciences, Kenyatta University,
P. O. Box 43844 - 0100, Nairobi, Kenya
Email: nmburu01@gmail.com

Abstract

This study reports the cloning, sequencing, and functional characterization of novel simian homologs of CCR5 from three species of primates (*Papio anubis anubis*; *Colobus guereza* and *Cercopithecus neglectus*) which are indigenous to East Africa. Identity at the amino acid level to human CCR5 was 97% for molecules from all three primate species. Functional characterization of these coreceptors was performed using a sensitive gene reporter-based cell-cell fusion assay. Despite a high degree of sequence similarity, significant differences in the degree of M-tropic HIV envelope-specific fusion generated by individual coreceptor molecules were demonstrated. Remarkably, two simian CCR5 molecules (*Colobus guereza* and *Cercopithecus neglectus*) demonstrated an increased efficiency of HIV-mediated fusion as compared with the human CCR5 molecule. These results provide further evidence that simian CCR5 molecules differ in their ability to facilitate HIV-1 entry, and may have bearing on the investigation of new simian models for HIV-1 pathogenesis.

Key words: chemokine receptor, olive baboon, colobus monkey, de Brazza monkey, cell fusion assay

Introduction

Human immunodeficiency type 1 (HIV-1) gains entry to cells through fusion of its lipid envelope with the plasma membrane of target cells. This process requires the presence on the target cell membrane of CD4 and coreceptor molecules of the

chemokine receptor family. Chemokines and their G-coupled receptors are not only recognized as key mediators for HIV infection but they also make important contribution in the pathogenesis of other disease conditions including those of the cardiovascular system [1]. CXCR4 and CCR5 are the major coreceptors used *in vivo* by HIV-1. CXCR4 supports infection by T-cell-tropic (T-tropic or X4) viruses, while CCR5 supports infection by macrophage-tropic (M-tropic or R5) viruses. Additional coreceptors for HIV-1 and for SIV have also been identified, including BOB/GPR-15, STRL33/BONZO, CCR3, GPR-1, CCR8, CCR9, and CCR2b [2].

HIV-1 transmission in human populations is primarily mediated through the CCR5 molecule and genetic variation in CCR5 has been associated with susceptibility to HIV-1 infection, possibly by affecting the rate at which HIV-1 enters leukocytes [3, 4]. It has actually been shown that individuals homozygous for a 32-base pair deletion in this gene are protected from infection by HIV-1 [5, 6]. The same deletion also plays a role in protecting patients with *Schistosoma mansoni* infection against hepatitis C viral infection or progression [7]. A revolutionary HIV Mirror^{TM} scientific test has been developed for diagnosing the CCR5 delta 32 gene, however, this test is only available to participants in research studies [8]. Further evidence of the primacy of CCR5 in HIV-1 transmission in humans comes from the finding that viruses isolated from infected individuals early in the course of infection are generally CCR5-specific, while viruses capable of utilizing additional coreceptors are detected later [9, 10].

The SIV isolates tested to date have been shown capable of utilizing human CCR5 for cell entry. In a similar manner, M-tropic strains of HIV-1 can utilize simian CCR5 molecules for entry. M-tropic HIV-1 utilization of primate CCR5 molecules has been reported [11, 12] for baboon (*Papio anubis hamadryas*), chimpanzee (*Pan troglodytes*), gorilla (*Gorilla gorilla*), rhesus macaque (*Macacca mulatta*), pigtail macaque (*Macacca nemestrina*), cynomolgous macaque (*Macacca fasciculara*), and two subspecies of African green monkey (*Cercopithecus aethiops*). Despite a high degree of amino acid sequence identity (>97%), polymorphisms in CCR5 genes from African green monkeys have been shown to dramatically alter the ability of HIV-1 to use the coreceptor molecule for fusion [13]. In this work, amino acid polymorphisms in the N-terminal domain and within multiple extracellular loops of CCR5 were found to alter the competency of the simian CCR5 molecules to mediate fusion. In support of this finding, studies of human and mouse chimeric CCR5 molecules have indicated that both the N-terminal domain and elements in the extracellular loops are important to the coreceptor activity of CCR5 [11, 14, 15, 16, 17]. Furthermore, point mutations in a number of residues, while exhibiting no effect individually, may allow a synergistic inhibitory effect on HIV envelope-mediated fusion [18].

This study reports the sequencing and HIV coreceptor analysis of novel CCR5 molecules from three primate species native to East Africa: the olive baboon (*Papio anubis anubis*), colobus monkey (*Colobus guereza*), and de Brazza monkey (*Cercopithecus neglectus*). This study was initiated to expand knowledge of simian coreceptors and as part of efforts to evaluate East African primate species as candidates for new models of HIV-1 pathogenesis. Consistent with reports from other primate species, the CCR5 molecules from these three species were 97% identical to

human CCR5. Using a sensitive reporter gene-based cell-cell fusion assay, all three molecules were shown to be competent to mediate HIV-1 entry. Surprisingly, however, a small number of amino acid differences between the three molecules led to marked differences in the efficiency of their utilization as coreceptors by M-tropic HIV-1 envelopes. The identification of African primate species which express CCR5 molecules which are highly permissive for HIV entry may facilitate efforts to develop new primate models for studying HIV-1 pathogenesis and vaccines.

Materials and Methods

Primary cells and cell lines

Blood samples were obtained from olive baboons, colobus monkeys, and de Brazza monkeys maintained in colonies at the Institute of Primate Research (IPR) in Nairobi, Kenya, and the study was approved by the Institutional Ethical Review Committee. The three simian species used in this study are indigenous to East Africa and the colonies were previously established at IPR from existing native populations. Peripheral blood mononuclear cells (PBMCs) utilized in RT-PCR reactions were purified by density gradient centrifugation on Ficoll-Paque (Amersham Pharmacia Biotech, Piscataway, NJ) according to the manufacturer's instructions. The human cervical carcinoma cell line HeLa and the human kidney cell line 293T were maintained in Dulbecco modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum and penicillin-streptomycin.

Reverse transcriptase-PCR (RT-PCR)

Total RNA was isolated from 2 x 10^7 simian PBMCs by using Trizol (Gibco BRL, Gaithersburg, MD) as recommended by the manufacturer. The 5' and 3' primers were designed from the published human CCR5 sequence (Gene Bank accession number HSU 54994). The sense and antisense primers were CCCAAGCTTGGTGGAACAAGATGGATTAT and GGCGAGCTCGTCGACA TGTGCACAACTCTGACTG, respectively. Reagents for RT-PCR were obtained from the Gene Amp RNA PCR Kit (Perkin-Elmer, Norwalk, CT). Single stranded cDNA was prepared from 1 µg of total RNA in a 20-µl reaction containing 5 mM $MgCl_2$, 1x PCR buffer, 1 mM dNTPs, 1 U/µl Rnase inhibitor, 2.5 U/µl MuLV reverse transcriptase and 2.5 µM random hexamers. The reaction was then subjected to one cycle of PCR (42^{o}C, 15 min; 99 ^{o}C, 5 min; and 5 ^{o}C, 5 min) followed by another 30 cycles (92 ^{o}C, 40 s; 55 ^{o}C, 80 s; and 75 ^{o}C, 90 s) in 2 mM $MgCl_2$, 1x PCR buffer, 2.5U AmpliTaq DNA polymerase, and 0.15 µM sense and antisense primers. PCR products were then visualized on 1% agarose gel prior to capture.

Cloning and sequencing of simian CCR5 homologs

The PCR products were ligated into the cloning vector pCR 2.1 using a TA cloning Kit (Invitrogen, Carlsbad, CA). Miniprep DNAs that yielded an insert of the expected size as judged by restriction digest analysis were subjected to DNA sequencing. Plasmid DNA was extracted by alkaline lysis mini-prep and purified by PEG precipitation. The forward and reverse strands of the cDNAs were sequenced by

dideoxynucleotide termination method using a Fluorescent-Dye Terminator Kit (PE Applied Biosystems, Foster City, CA) with AmpliTaq DNA polymerase. Initial cycle sequencing reactions were set using M13 forward and reverse primers that are complimentary to the pCR 2.1 vector and complete sequence of the insert was determined by synthesizing additional internal primers. Sequencing was then performed using an ABI Prism 377 automated sequencer. The MacVector sequence analysis software package (Oxford Molecular Group) was used to construct a consensus sequence for each clone using the overlapping sequences that were generated by sequencing the plus and minus strands.

Generation of CCR5 expression constructs incorporating the FLAG epitope tag

Human and simian CCR5 genes were cloned into the pCDNA 3.1 plasmid (Invitrogen) for expression in mammalian cell lines. To facilitate detection of cell-surface expression, the FLAG epitope (DYKDDDDK) was placed at the N-terminus of each coreceptor cDNA by PCR cloning. The following primers were utilized to PCR amplify simian and human CCR5 molecules using Pfu polymerase: FLAG-CCR5 (forward primer), CGCGGATCCGCGGATGGACTACAAGG ACGACGATGACAAGATGGATTATCAAGTGTCA; *Xho*1-CCR5 (reverse primer), CCGCTCGAGCGGGTCACAAGCCCACAGATATTTC. The PCR products were then ligated into the *Bam*H1 and *Xho*1 sites of pCDNA 3.1.

Recombinant Vaccinia Viruses

The recombinant vaccinia viruses were obtained through the NIH/NIAID AIDS Research program and were employed in experiments described below as a source of CD4 (vCB-3), a nonfusogenic HIV-1 envelope protein (vCB-16), beta-galactosidase reporter gene expression (vCB21R-LacZ), JR-FL envelope protein (vCB-28), ADA envelope protein (vCB-39), and T7 polymerase (vp11gene1).

Gene reporter cell-cell fusion assay

To quantitate cell-cell fusion events of the CCR5 co-receptors derived from human, baboon, colobus monkey, and De Brazza monkey, a gene reporter assay was utilized [19, 20]. Briefly, target HeLa cells were transfected in 100 mm plates by transfection of 20 µg of each co-receptor expression construct using LipofectAmine (Gibco BRL). The transfectant was removed after 5 hours, and cells were infected with the recombinant vaccinia viruses vCB-3 and vCB21R-lacZ. T7 RNA polymerase and Env proteins were introduced into the HeLa effector cells by infection with vp11gene1 and either vCB-16, vCB-28, or vCB-39. Infection with recombinant vaccinia viruses was performed at a multiplicity of infection of 10 for 45 min at 37^{o}C. After infection, target and effector cells were trypsinized, washed with PBS, resuspended in medium containing 100 µg/ml of rifampicin, and incubated overnight at 32^{o}C. Following overnight incubation, the cells were washed twice with cold PBS and resuspended to a final concentration of 1 x 10^{6}/ml in medium containing 100 µg/ml rifampicin and 10 µM AraC. To initiate fusion 1 x 10^{5} cells of each population were dispensed in triplicates into individual well of a 96-well flat-bottomed tissue culture plate and incubated at 37^{o}C. After 3 h, 5 X 10^{4} cells were lysed in 5 µl of 20% NP-40 in PBS.

β-galactosidase activity was assayed by kinetic ELISA using a Thermomax microplate reader (Molecular Devices, Sunnyvale, CA) at 590 nm for 5 min, and results recorded in mOD/minute. Alternatively, β-galactosidase was detected by in situ staining after fixing the cells in a fixative solution (20% Formaldehyde, 2% glutaraldehyde) at 4°C for 5 minutes. 150 µl of the media was replaced with the X-gal staining solution and incubated at 37°C. Photomicrographs were taken 3 h after the addition of the substrate. To confirm the results, this assay was repeated on three different occasions.

Cell surface expression of coreceptor molecules

For analysis of cell surface expression of coreceptors, 293T cells were transiently transfected with 20 µg FLAG-CCR5 using LipofectAMINE (Gibco). After 5 hours, an equal volume of DMEM containing twice the normal concentration of the serum was added without removing the transfectant mixture. Following overnight incubation the medium was replaced with fresh complete medium. After 24 h, cells were lifted using 1 mM EDTA in PBS and washed once with PBS. About 3 x 10^5 cells were then resuspended in 50 µl PBS containing 2% FBS in presence of anti-FLAG monoclonal antibody (IBI, Kodak, Rochester, NY), or an IgG_1 isotype control (R & D Systems, Minneapolis, MN), and incubated for 30 min at 4°C. Primary antibodies were used at a final concentration of 1 and 10 µg/ml. Cells were then washed twice in PBS-FBS and then resuspended in 50 µl PBS-FBS in the presence of 1:100 dilution of PE-conjugated affinity purified $F(ab')_2$ goat anti-mouse IgG (Immunotech, Westbrook, ME). Cells were incubated for 30 min at 4°C, washed twice with PBS-FBS and resuspended in 400 µl PBS-FBS. Cells were analyzed on a FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ) using a 585 nm bandpass filter. Live cells were gated and analyzed using CellQuest 3.1 software.

Results

CCR5 genes were cloned from PBMCs obtained from olive baboon, colobus monkey, and de Brazza monkey and sequenced by the dideoxynucleotide termination method. The deduced amino acid sequences were compared with human CCR5, revealing a high degree of inter-species identity (Fig.1). However, some point mutation differences of potential significance were identified. Baboon, colobus and de Brazza CCR5 differed from the human CCR5 by 8, 9, and 10 amino acids respectively, giving them a 97.7%, 97.4%, and 97.2% sequence identity. Six of the changes from the human sequence were common to all three simian CCR5 molecules, and are identical to residues reported for simian CCR5 molecules from rhesus, pig tail, and cynomolgous macaque (T9, D13, I49,V52, I130, and R171). Additional individual amino acid differences from the human sequence were found for each simian molecule. Of note, some of these changes occurred in the N-terminal domain (I23V, colobus) or within or immediately adjacent to the third extracellular loop of the molecule (L174F and I198T, De Brazza). Sequences reported in this manuscript have already been submitted to Genbank and are available under the following accession numbers: AF141639, AF141640 and AF141641.

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BABOON          MDYQVSSPTYDIDYYTSEPCQKINVKQIAARLLPPLYSLVFIFGFVGNILVVLILINCKR 60
DEBRAZZA        MDYQVSSPTYDIDYYTSEPCQKINVKQIAARLLPPLYSLVFIFGFVGNILVVLILINCKR 60
COLOBUS         MDYQVSSPTYDIDYYTSEPCQKVNVKQIAARLLPPLYSLVFIFGFVGNILVVLILINCKR 60
HUMAN           MDYQVSSPIYDINYYTSEPCQKINVKQIAARLLPPLYSLVFIFGFVGNMLVILILINCKR 60
                ******** *** ********* ************************** ** ********

BABOON          LKSMTDNYLLNLAISDLLFLLTVPFWAHYAAAQWDFGNTMCQLLTGLYFIGFFSGIFFII 120
DEBRAZZA        LKSMTDIYLLNLAISDLLFLLTVPFWAHYAAAQWDFGNTMCQLLTGLYFIGFFSGIFFII 120
COLOBUS         LKSMTDIYLLNLAISDLFFLLTVPFWAHYAAAQWDFGNTMCQLLTGLYFIGFFSGIFFII 120
HUMAN           LKSMTDIYLLNLAISDLFFLLTVPFWAHYAAAQWDFGNTMCQLLTGLYFIGFFSGIFFII 120
                ****** ********** ********************************************

BABOON          LLTIDRYLAIVHAVFALKARTVTFGVVTSVITWVVAVFASLPGIIFTRSQREGLHYTCSS 180
DEBRAZZA        LLTIDRYLAIVHAVFALKARTVTFGVVTSVITWVVAVFASLPGIIFTRSQREGFHYTCSS 180
COLOBUS         LLTIDRYLAIVHAVFALKARTATFGVVTSVITWVVAVFASLPGIIFTRSQREGLHYTCSS 180
HUMAN           LLTIDRYLAVVHAVFALKARTVTFGVVTSVITWVVAVFASLPGIIFTRSQKEGLHYTCSS 180
                ********* ********** ***************************** ** ******

BABOON          HFPYSQYQFWKNFQTLKIVILGLVLPLLVMVICYSGILKTLLRCRNEKKRHRAVRLIFTI 240
DEBRAZZA        HFPYSQYQFWKNFQTLKTVILGLVLPLLIMVICYSGILKTLLRCRNEKKRHRAVRLIFTI 240
COLOBUS         HFPYSQYQFWKNFQTLKIVILGLVLPLLVMVICYSGILKTLLRCRNEKKRHRAVRLIFTI 240
HUMAN           HFPYSQYQFWKNFQTLKIVILGLVLPLLVMVICYSGILKTLLRCRNEKKRHRAVRLIFTI 240
                ***************** ********** ******************************

BABOON          MIVYFLFWAPYNIVLLLNTFQEFFGLNNCSSSNRLDQAMQVTETLGMTHCCINPIIYAFV 300
DEBRAZZA        MIVYFLFWAPYNIVLLLNTFQEFFGLNNCSSSNRLDQAMQVTETLGMTHCCINPIIYAFV 300
COLOBUS         MIVYFLFWAPYNIVLLLNTFQEFFGLNNCSSSNRLDQAMQVTETLGMTHCCINPIIYAFV 300
HUMAN           MIVYFLFWAPYNIVLLLNTFQEFFGLNNCSSSNRLDQAMQVTETLGMTHCCINPIIYAFV 300
                ************************************************************

BABOON          GEKFRNYLLVFFQKHIAKRFCKCCSIFQQEAPERASSVYTRSTGEQEISVGL 352
DEBRAZZA        GEKFRNYLLVFFQKHIAKRFCKCCSIFQQEAPERASSVYTRSTGEQEISVGL 352
COLOBUS         GEKFRNYLLVFFQKHIAKRFCKCCRIFQQEAPERASSVYTRSTGEQEISVGL 352
HUMAN           GEKFRNYLLVFFQKHIAKRFCKCCSIFQQEAPERASSVYTRSTGEQEISVGL 352
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Fig. 1. Sequence comparison of human, colobus, de Brazza, and olive baboon CCR5. Asterisk indicates positions which have a single, fully conserved residue.

Human CCR5 is the major coreceptor for M-tropic HIV-1 strains *in vivo*. To analyze the extent to which HIV envelope proteins could mediate fusion through interactions with CD4 and CCR5 derived from human, olive baboon, colobus monkey, and de Brazza monkey, a previously described gene reporter cell-cell fusion assay [18, 19] was utilized. In this assay, HeLa target cells were transiently transfected with the coreceptor gene of interest. Recombinant vaccinia viruses were used to provide target cells with human CD4 and with a β-galactosidase reporter gene under the control of the T7 polymerase promoter. M-tropic HIV envelopes were expressed in effector cell populations together with T7 polymerase, using recombinant vaccinia viruses. After overnight expression, target and the effector were mixed and fusion allowed to proceed for 3 h. Fusion was monitored by scoring for syncytium formation with in situ β-galactosidase staining and also by measuring β-galactosidase activity by quantitative colorimetric assay of cell lysates.

Figure 2 shows *in situ* staining for β-galactosidase activity performed to visualize syncytia formation. Fusion did not occur, as indicated by the presence of only rare blue cells, when a non-fusogenic HIV-1 env (vCB16), rendered inactive by deletion of the gp120/gp41 cleavage site, was employed. In contrast, large blue syncytia were

observed when HeLa cells expressing a functional HIV Env (JR-FL or ADA) were incubated with target cells expressing CD4 and CCR5 derived from human, olive baboon, colobus monkey, and de Brazza monkey. Fusion was noted to be greatest when colobus (Fig. 2B) or de Brazza (Fig. 2C) CCR5 molecules were employed in this assay, and least upon use of the olive baboon CCR5 (Fig. 2D). To quantitate the extension of fusion, cells were lysed with the detergent NP-40 and the lysate assayed for ß-galactosidase activity by colorimetry. This assay was repeated on three different occasions in triplicate wells. Expression of CD4 without coreceptor led to minimal (background) β-galactosidase activity in this assay (Fig. 3). Similarly, when a fusion-incompetent envelope was expressed in the effector cell population, little fusion was noted. As expected, expression of human CCR5 led to fusion which was reproducibly detected in this assay and well above background. Next, there was quantification of the ability of the olive baboon, colobus and de Brazza CCR5 molecules to serve as coreceptors for HIV-1. In a manner consistent with the data presented in Figure 2, it was detected that there were differences in the ability of these three simian coreceptor homologs to mediate fusion. Fusion mediated by the de Brazza and colobus molecules was markedly greater than that of the olive baboon, and exceeded that of the human molecule (Fig. 3). Although the degree of fusion detected in this assay was somewhat greater when the ADA envelope was used than that seen for JR-FL, the relative differences in the ability of the three simian coreceptors to mediate fusion remained the same.

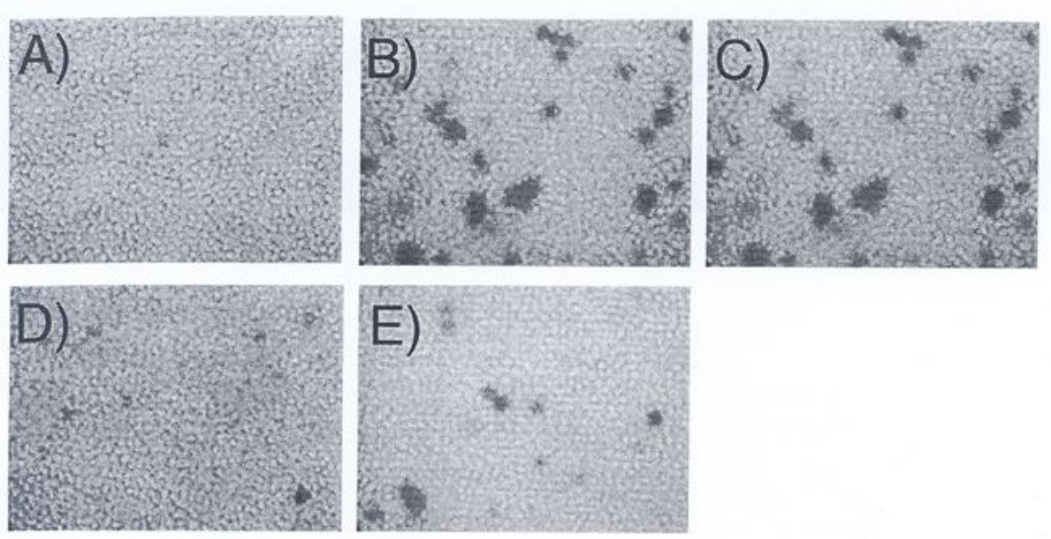

Fig. 2. Differential use of CCR5 homologs by HIV-1 Env as analyzed by *in situ* staining. HeLa target cells were transiently transfected with the coreceptor genes of interest, infected with a recombinant vaccinia virus encoding a T7/beta galactosidase reporter gene, and incubated with effector cells expressing HIV envelope and T7 polymerase. A) Control reaction with non-fusogenic envelope (vCB16), B) colobus CCR5, C) de Brazza CCR5, D) olive baboon CCR5, E) human CCR5.

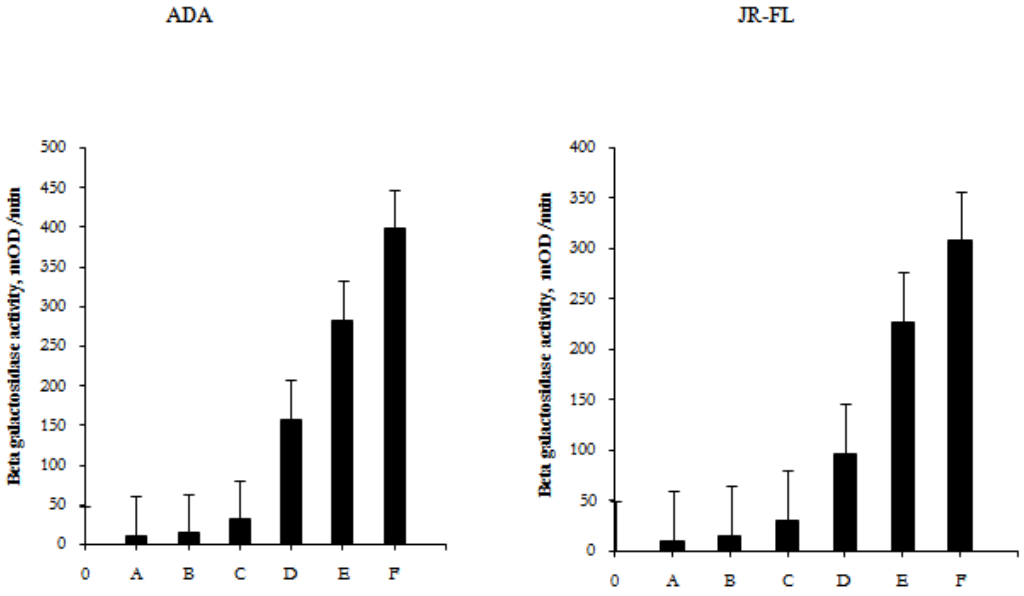

Fig. 3. Differential use of CCR5 homologs by HIV-1 Envelop as analyzed by quantitative colorimetry. HeLa target cells were transiently transfected with the coreceptor genes of interest, and the cell-cell fusion assay performed as described in Materials and Methods. Data is presented as mean ± standard error of the mean from three independent experiments. A = non-fusogenic, B = CD4 only, C = baboon, D = human, E = colobus F = de Brazza.

The differences in cell-cell fusion described above could have been due to coreceptor function or to differences in cell surface expression. To quantitate the cell surface expression of the simian and human molecules utilized in this study, 293T cells were transiently transfected with the expression plasmids encoding FLAG-tagged human, olive baboon, colobus, and de Brazza monkey CCR5 molecules. An anti-FLAG monoclonal antibody was then used to measure cell surface expression levels of each CCR5 by flow cytometry. The Mean Fluorescence Chamber (MCF) intensities when 1 µg/ml of anti-FLAG monoclonal antibody was used as the primary antibody for coreceptor detection, shows that human CCR5 was expressed at levels 0.9, 1.4 and 1.6 fold that of olive baboon, colobus, and De Brazza CCR5. Results obtained when 10 µg/ ml of the primary antibody was utilized were similar, with ratios of human to baboon, colobus and De Brazza of 0.9, 1.2 and 1.3 fold, respectively. Thus, all coreceptor molecules examined were expressed at the cell surface and without substantial differences in expression level.

Discussion

The predominant coreceptor involved in human-to-human spread of HIV-1 is CCR5. Simian immunodeficiency viruses can utilize CCR5 for cell entry, but can also

efficiently utilize alternative coreceptor molecules such as include BOB/GPR-15 and STRL33/BONZO [21]. The relevant coreceptor involved in SIV transmission in the wild is not yet known. It will be important to develop a comprehensive understanding of primate coreceptor utilization by HIV and SIV for the following reasons. First, coreceptor utilization *in vivo* may correlate with pathogenesis. This may be especially relevant to understanding differences between viruses such as SIV_{agm} which replicate in their natural host without apparent adverse consequences [22], and HIV-1 replication in humans or SIV_{mac} replication in rhesus macaques, both of which can cause AIDS and death. Second, cross-species transmission events are thought to explain the origin of the AIDS epidemic, and continue to be a risk for the future. Understanding coreceptor utilization by SIV in human cells and HIV-1 on simian cells may provide clues to predicting or dealing with future cross-species transmission events. Third, the development of new animal models may be facilitated by discovery of simian cells that are highly permissive for HIV-1 infection. Finally, the basic understanding of envelope glycoprotein-coreceptor interactions may be increased through the sequencing and functional characterization of additional coreceptors from a variety of species.

Simian CCR5 homologs have been previously sequenced and characterized from African green monkey, gorilla, chimpanzee, baboon (*Papio hamadryas anubis*), macaques and sooty mangabeys [12, 23, 24]. In this study we extend information on simian CCR5 homologs using clones isolated from primary cells from the olive baboon (a separate species from *Papio anubis hamadryas*), colobus monkey, and de Brazza monkey. These three species are indigenous to East Africa, and are available for scientific inquiry in colonies maintained at the Institute for Primate Research in Nairobi, Kenya. It is important to note that SIV seropositive members of these three species have been detected, although viral isolates or sequences derived from these species are not yet available [25]. Although some seropositive animals are captive at IPR, the coreceptor data presented in this work was derived from PBMCs originating in SIV-seronegative animals.

The sequence of the three CCR5 clones reported here revealed a high degree of identity with previously reported simian CCR5 molecules. However, potentially significant point mutations were noted. For example, the phenylalanine at residue 174 found in the de Brazza isolate is unique among reported human or simian CCR5 molecules. The presence of this non-conserved change in the third extracellular loop may significantly alter the structure of this region, and could result in differences in envelope glycoprotein interaction. However, predicting the effects of an individual amino acid change upon the coreceptor function of CCR5 is difficult, as will be discussed further below. In order to determine the significance of the sequence differences reported, and to determine whether each simian coreceptor molecule was competent to serve as an HIV-1 coreceptor, a functional assessment using a sensitive cell-cell fusion assay was performed.

The CCR5 coreceptor homologs derived from the olive baboon, colobus and de Brazza monkeys all were competent to mediate cell-cell fusion in the presence of CD4 when the R5 HIV envelopes from isolates JR-FL or ADA were employed. Surprisingly, however, there were substantial differences in the efficiency of fusion

mediated by the CCR5 molecules from the three species. Two of the simian coreceptor molecules, those from the de Brazza and colobus monkeys, were found to be more efficient than human CCR5 in their ability to mediate CD4- and HIV envelope-dependent fusion. In contrast, the olive baboon CCR5 molecule was consistently poor in mediating fusion. The relative differences in fusogenicity of the simian coreceptors were demonstrated consistently in repeated experiments, and were demonstrted with both of the R5 envelope isolates tested. The differences highlighted in this report were not due to differences in cell surface expression of the coreceptor molecules, as determined by flow cytometric analysis. Thus, this study documents species-specific differences in the ability of simian CCR5 molecules to function as HIV-1 coreceptors. This finding is consistent with reports of naturally-occuring polymorphisms in SIV_{agm} CCR5 molecules which may have drastic effects upon the ability of the molecule to serve as an HIV coreceptor. However, the ability of the colobus and de Brazza CCR5 molecules to mediate fusion in a manner substantially greater than that of the human molecule is unique. While it is likely that some of the observed sequence differences between the three coreceptor molecules, such as the L174R change in de Brazza molecule, can account for the marked differences in fusion, it may not be possible to predict the influence of the individual point mutations without further study. The precise structural requirements of the extracellular regions of CCR5 in mediating fusion still remain unclear. Analysis of chimeric receptors based on human and murine CCR5 have demonstrated that no single CCR5 domain is both necessary and sufficient for coreceptor function. It has been clearly demonstrated that interactions between CCR5 and HIV envelope involve more than one region of the coreceptor [15, 16, 26], and that all four extracellular domains participate in the coreceptor activity to at least some degree [11]. Additional studies to determine which change or changes are responsible for the enhanced efficient fusion seen in two of the three molecules are needed.

The finding that some simian coreceptor molecules are more efficient in promoting HIV entry has potential relevance to animal models of HIV pathogenesis. At present, few simian animal models which allow the study of HIV-1 replication and pathogenesis are available. The chimpanzee model allows HIV-1 replication, has been quite useful for some vaccine studies, and has rarely led to disease in the animals [27, 28]. However, this model is limited due to the general lack of an AIDS-like illness in infected animals, difficulties and expense in acquiring and maintaining them, and resistance among investigators and animal rights advocacy groups to the use of chimpanzees for pathogenic challenge experiments. It will be important to perform studies of alternative primate models for HIV-1, and to determine if primate species other than the chimpanzee in which HIV entry can occur efficiently can also support the full replication cycle of HIV-1.

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