

## Two Orphan Seven-Transmembrane Segment Receptors Which Are Expressed in CD4-positive Cells Support Simian Immunodeficiency Virus Infection

By Michael Farzan,\* Hyeryun Choe,\* Kathleen Martin,<sup>§</sup> Luisa Marcon,\*<sup>||</sup> Wolfgang Hofmann,\* Gunilla Karlsson,\* Ying Sun,\* Peter Barrett,<sup>§</sup> Nathalie Marchand,\* Nancy Sullivan,\* Norma Gerard,<sup>§</sup> Craig Gerard,<sup>§</sup> and Joseph Sodroski\*<sup>‡</sup>

From the \*Division of Human Retrovirology, Dana-Farber Cancer Institute, Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115; <sup>‡</sup>Department of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts 02115; <sup>§</sup>Perlmutter Laboratory, Children's Hospital, and Department of Medicine and Department of Pediatrics, Beth Israel Hospital and Harvard Medical School, Boston, Massachusetts 02115; and <sup>||</sup>Institute of Microbiology, University of Padua Medical School, Padua, Italy 35121

### Summary

Clinical isolates of primate immunodeficiency viruses, including human immunodeficiency virus type 1 (HIV-1), enter target cells by sequential binding to CD4 and the chemokine receptor CCR5, a member of the seven-transmembrane receptor family. HIV-1 variants which use additional chemokine receptors are present in the central nervous system or emerge during the course of infection. Simian immunodeficiency viruses (SIV) have been shown to use CCR5 as a coreceptor, but no other receptors for these viruses have been identified. Here we show that two orphan seven-transmembrane segment receptors, gpr1 and gpr15, serve as coreceptors for SIV, and are expressed in human alveolar macrophages. The more efficient of these, gpr15, is also expressed in human CD4<sup>+</sup> T lymphocytes and activated rhesus macaque peripheral blood mononuclear cells. The gpr15 and gpr1 proteins lack several hallmarks of chemokine receptors, but share with CCR5 an amino-terminal motif rich in tyrosine residues. These results underscore the potential diversity of seven-transmembrane segment receptors used as entry cofactors by primate immunodeficiency viruses, and may contribute to an understanding of viral variation and pathogenesis.

The human immunodeficiency viruses, HIV-1 and HIV-2, induce acquired immunodeficiency syndrome (AIDS) in humans, and simian immunodeficiency viruses (SIV)<sup>1</sup> can induce AIDS-like illness in Old World monkeys (1–5). Isolates of HIV-1, the major cause of AIDS in humans, have been phylogenetically segregated into groups M and O (6). HIV-2 and SIV form a distinct group of phylogenetically and antigenically related viruses (2, 3, 6–8).

AIDS induced by HIV-1 or HIV-2 in humans or by SIV in monkeys is characterized by the depletion of CD4<sup>+</sup> T lymphocytes, which represent a major target of viral infection in vivo (9). Infection of other CD4<sup>+</sup> cell types, such as

monocytes in the blood, tissue macrophages, and microglial cells in the brain, has been suggested to be important for the pathogenesis of primate immunodeficiency viruses in the central nervous system and in the lungs (10–14). Certain populations of dendritic cells in the blood and tissues may also be infected by these viruses (15, 16).

The tropism of primate immunodeficiency viruses for CD4<sup>+</sup> cells is explained by the use of the CD4 glycoprotein as a primary receptor for virus entry into the cell (17–19). The viral envelope glycoproteins, which mediate virus entry, consist of the gp120 exterior envelope glycoprotein and the gp41 transmembrane glycoprotein (20, 21). The gp120 glycoprotein binds the CD4 molecule, following which the gp120–CD4 complex binds one of the members of the chemokine receptor subgroup of seven-transmembrane segment (7-TMS) receptors (22–24). This binding is believed to promote conformational changes in the gp120 and gp41 glycoproteins which result in the fusion of viral and cellular membranes (25–27).

<sup>1</sup>Abbreviations used in this paper: 7-TMS, seven-transmembrane segment; CAT, chloramphenicol acetyltransferase; SIV, simian immunodeficiency virus.

M. Farzan and H. Choe contributed equally to this work.

Viral variation, particularly that found in the gp120 glycoprotein sequences (28, 29), dictates the specific chemokine receptor which can be used as an entry cofactor. M-tropic HIV-1 variants which use the chemokine receptor CCR5 as a coreceptor predominate during the asymptomatic stages of infection (30–35). CCR5 is expressed on T lymphocytes, monocytes/macrophages, brain microglia, and dendritic cells (36–39). Individuals with defects in CCR5 expression are relatively resistant to HIV-1 infection (40–42), indicating the critical contribution of this chemokine receptor to virus transmission. Some M-tropic brain isolates of HIV-1 also use the chemokine receptor CCR3 as a coreceptor, consistent with the expression of CCR3 in brain microglia (39). Later in the course of infection, T-tropic HIV-1 variants emerge which can use chemokine receptors, especially CXCR4, but also CCR3 and CCR2b, in addition to CCR5 (34, 35, 43–45). The emergence of these viruses has been suggested to coincide with a less favorable clinical prognosis (45), perhaps through an expansion of the range of infectable CD4<sup>+</sup> T cell subsets (46).

Primary isolates of HIV-2 and SIV have been shown to use rhesus macaque or human CCR5 as a coreceptor (47, 48) and are inhibited by the natural CCR5 ligands, MIP-1 $\alpha$ , MIP-1 $\beta$ , and RANTES (49). None of the other known HIV-1 coreceptors has been shown to be used by SIV, whereas some isolates of HIV-2 can use CXCR4 for entry into CD4<sup>+</sup> cells (50). Several lines of evidence have suggested the existence of at least one other coreceptor for SIV. A human B cell/T cell hybrid, CEM $\times$ 174, supports SIV entry, but lacks CCR5 and does not support efficient entry of HIV-1 viruses using CCR5 (48). A neuroglioma cell line, U87, stably transfected with CD4, similarly supports entry of SIV<sub>mac239</sub> but does not allow for efficient entry of any known HIV-1 virus (51). Finally, PBMCs from humans lacking a functional CCR5 receptor can nonetheless be infected with SIV (48). Here we identify two additional SIV coreceptors, gpr1 and gpr15, which are expressed in U87 and CEM $\times$ 174 cells, respectively. Both proteins are expressed in human alveolar macrophages, and the gpr15 protein is also expressed in CD4<sup>+</sup> T lymphocytes.

## Materials and Methods

**Preparation of cDNA Libraries and cDNA.** Messenger RNA was isolated using the CsCl method and selection on magnetic beads with oligo-dT (Dynabeads; DYNAL, Inc., Lake Success, NY). RNA was obtained from purified human CD4<sup>+</sup> peripheral blood T cells (gift of Dr. Linda Clayton, Dana-Farber Cancer Institutes, Boston, MA), human alveolar macrophages (gift of Dr. Hal Chapman, Brigham and Women's Hospital, Boston, MA), CEM $\times$ 174 cells, and U87 neuroglioma cells. RNA was also isolated from phytohemagglutinin-treated, interleukin-2-stimulated PBMCs from a healthy rhesus macaque (New England Regional Primate Research Center, Foxboro, MA). The cDNA libraries from the U87 and CEM $\times$ 174 cell lines were made by reverse transcription (Superscript; GIBCO BRL, Gaithersburg, MD) using a unidirectional primer supplied by the manufacturer. Size-selected cDNAs were cloned into a BstXI/NotI-digested pcDNA3.1 vector (In-

vitrogen, Carlsbad, CA). The human alveolar macrophage library was prepared by Invitrogen in pcDNA1. Double-stranded CD4<sup>+</sup> T cell cDNA was synthesized using a kit from Boehringer Mannheim (Indianapolis, IN).

**Cloning of cDNAs for 7-TMS Proteins.** The expression plasmids for rdc1, ebi2, gpr1, gpr15, and dez were prepared by PCR amplification of a cDNA library made from either CEM $\times$ 174 or U87 cells, as described above. The amplified fragments were cloned into the pcDNA3 plasmid for expression. Expression plasmids for other chemokine receptors were generously supplied by Drs. Paul Ponath and Walter Newman (LeukoSite, Inc., Cambridge, MA) (v28, CXCR1, CXCR2), Dr. Elliot Kieff (Harvard Medical School, Boston, MA) (ebi1), and Dr. Monica Napolitano (Regina Elena Cancer Institut, Rome, Italy) (ter1).

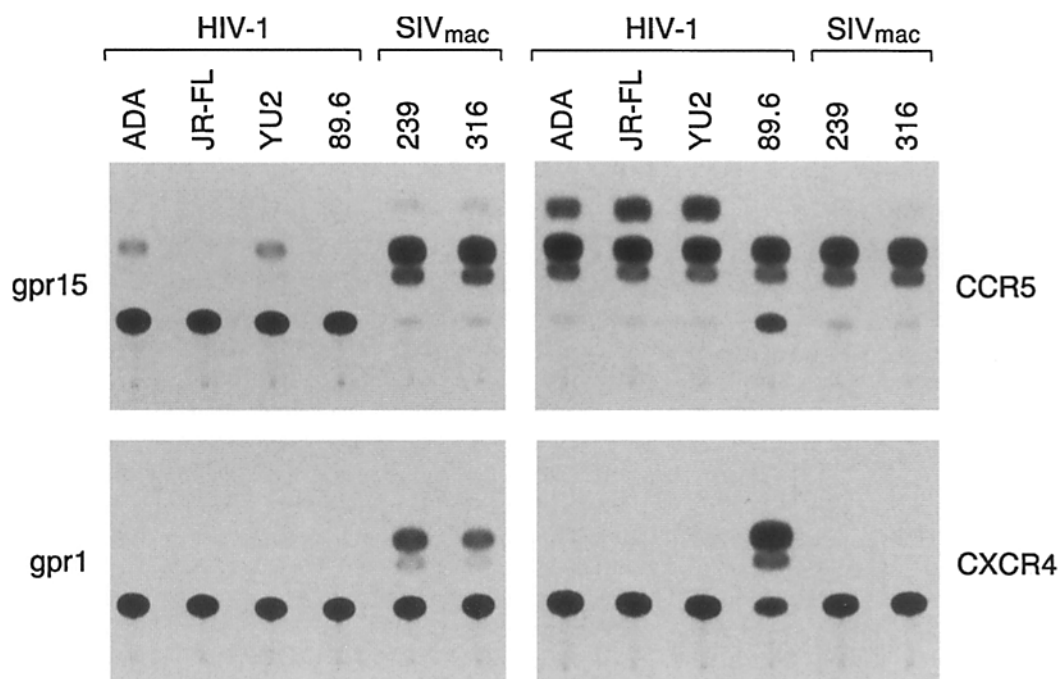
**Testing SIV Coreceptor Activity.** A previously described *env*-complementation method (27, 28, 47) was used to produce recombinant HIV-1 viruses which contained the SIV envelope glycoproteins and were capable of encoding chloramphenicol acetyltransferase (CAT) in target cells. Briefly, recombinant virus was incubated with C2Th cells transfected with plasmids expressing human CD4 and candidate coreceptors. Cells were har-

**Table 1.** Expression of 7-TMS Proteins and Activity as an SIV<sub>mac239</sub> Coreceptor

7-TMS protein	Reference	Expression in:			SIV <sub>mac239</sub> coreceptor activity
		CEM $\times$ 174	U87	Primary CD4 <sup>+</sup> T cells	
apj	55	–	–	–	ND
blr1	56	+	–	ND	–
CCR5	38, 57	–	–	+	+
CXCR1	58	ND	ND	ND	–
CXCR2	59	ND	ND	ND	–
CXCR3	60	ND	ND	ND	–
CXCR4	61	+	+	+	–
dez	62	–	+	+	–
ebi1	63	+	+	+	–
ebi2	63	+	+	+	–
gc96	*	–	–	+	ND
gcy4	64	–	–	+	ND
gpr1	53	–	+	–	+
gpr2	53	–	–	–	ND
gpr4	65	–	–	+	ND
gpr5	65	–	–	–	ND
gpr15	54	+	–	+	+
rdc1	66	+	+	+	–
ter1	67	+	–	+	–
v28	68	ND	ND	ND	–

Positive expression values indicate the detection of a PCR product of expected size and restriction map amplified from the indicated cDNA source. Coreceptor activity for viruses with SIV<sub>mac239</sub> envelope glycoproteins was determined as described in Materials and Methods.

\*These sequence data are available from EMBL/GenBank/DBJ under accession number U45982.



**Figure 1.** CAT activity in C2Th cells expressing CD4 alone or together with gpr1, gpr15, CCR5, or CXCR4 after incubation with HIV-1 recombinant viruses carrying the SIV<sub>mac</sub>239, SIV<sub>mac</sub>316, or HIV-1 (YU2, HXBc2, 89.6, or ADA) envelope glycoproteins. A representative experiment is shown. The amount of target cell lysate used was equivalent for all the experiments shown. CAT activity was determined by calculating the percentage of chloramphenicol present in acetylated forms (three uppermost spots) to the total amount of chloramphenicol. The nonacetylated form of chloramphenicol is present in the spot closest to the origin, which is near the bottom of the figure.

vested and assayed for CAT activity, which was determined by measuring the conversion of chloramphenicol to acetylated forms of chloramphenicol. The SIV<sub>mac</sub>239 and SIV<sub>mac</sub>316 envelope glycoproteins were expressed from the previously described pSIVΔgpv plasmid (47). The HIV-1 envelope glycoproteins were expressed as previously described (27, 28).

**Analysis of Expression of 7-TMS Proteins in Cells and Tissues.** The expression of 7-TMS protein messenger RNA in CEM×174 and U87 cells and primary human CD4<sup>+</sup> T lymphocytes and alveolar macrophages was examined by synthesis of cDNA from polyadenylated RNA prepared from these cells, as described above. Primers corresponding to the nucleotide sequences encoding the first and third extracellular loops of the proteins were used for amplification by PCR. The identity of amplified fragments was confirmed by restriction enzyme digestion.

## Results

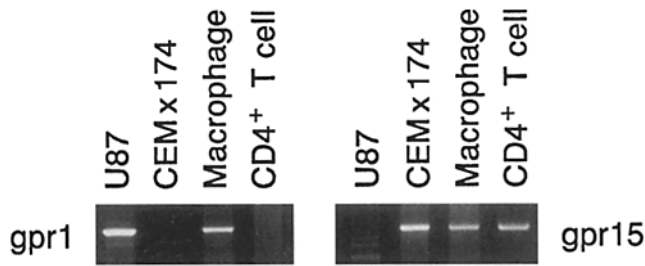
We had previously tested a number of human chemokine receptors (CCR1–CCR5, as well as CXCR4) and found that of these only CCR5 could support entry of an HIV-1 virus pseudotyped with the envelope glycoproteins of a pathogenic, molecularly cloned SIV, SIV<sub>mac</sub>239 (47). To identify additional coreceptors which might be used by SIV, we screened cDNA libraries from SIV-infectable cells, CEM×174 and U87, for the expression of mRNA encoding known 7-TMS proteins exhibiting some sequence similarity to chemokine receptors. The cDNAs which were shown to be expressed in either cell line were tested for the ability to support SIV and HIV-1 entry. Recombinant HIV-1 viruses which contained either HIV-1 or SIV enve-

lope glycoproteins and expressed CAT were incubated with C2Th canine thymocytes transfected with plasmids expressing human CD4 and the 7-TMS proteins. Table 1 lists the 7-TMS proteins tested, summarizes their expression in CEM×174, U87, and human CD4<sup>+</sup> T cells, and indicates coreceptor activity for viruses with the SIV<sub>mac</sub>239 envelope glycoproteins. Of the 7-TMS proteins tested, only gpr1, gpr15, and CCR5 supported the entry of viruses with the

**Table 2.** CAT Activity in C2Th Cells Expressing CD4 and 7-TMS Proteins following Incubation with Viruses Containing Different Envelope Glycoproteins

7-TMS protein	Viral envelope glycoproteins					
	SIV		HIV-1			
	SIV <sub>mac</sub> 239	SIV <sub>mac</sub> 316	ADA	YU2	JR-FL	89.6
CXCR4	<0.1	<0.1	<0.1	<0.1	<0.1	9.3
CCR5	19.0	12.1	113.9	290.8	203.7	9.4
gpr1	7.0	2.7	<0.1	<0.1	<0.1	<0.1
gpr15	30.3	30.5	0.7	0.9	<0.1	<0.1

The percent conversion of chloramphenicol to acetylated forms is shown following incubation of comparable amounts of lysates derived from C2Th cells exposed to recombinant viruses. The CAT activity was calculated as described in the legend to Fig. 1. In some cases, dilutions of the lysates were tested to bring the assay within the linear range and, thus, the reported values exceed 100%.



**Figure 2.** Expression of *gpr1* and *gpr15* RNA in cells. The cDNA libraries from U87 and CEM×174 cells and from human alveolar macrophages, as well as cDNA prepared from human CD4<sup>+</sup> T lymphocytes, were PCR amplified using *gpr1*- or *gpr15*-specific primers.

SIV<sub>mac</sub>239 envelope glycoproteins. These three 7-TMS proteins also supported the entry of viruses with the macrophage-tropic SIV<sub>mac</sub>316 envelope glycoproteins (Fig. 1). The SIV coreceptor activity exhibited by the *gpr15* protein was greater than that of CCR5, whereas the coreceptor activity of *gpr1* was ~30% that of CCR5 (Table 2). Most of the viruses with HIV-1 envelope glycoproteins (HXBc2, JR-FL, 89.6) did not infect Cf2Th cells expressing CD4 and *gpr15*, although the viruses with the M-tropic HIV-1 ADA and YU2 envelope glycoproteins demonstrated a low but reproducible signal in these cells (Fig. 1 and Table 2). Following incubation with the ADA and YU2 viruses, the CAT conversion in the CD4<sup>+</sup>, *gpr15*<sup>+</sup> Cf2Th cells was <1% of that seen in the CD4<sup>+</sup>, CCR5<sup>+</sup> control cells (Table 2). Cf2Th cells expressing CD4 and *gpr1* were not infected by viruses containing any of the HIV-1 envelope glycoproteins tested (Table 2).

The expression of *gpr15* and *gpr1* in different cell types was examined. Since specific reagents to detect these proteins were not available, expression was examined by RNA analysis. A cDNA for *gpr15* was readily detected in human CD4<sup>+</sup> T lymphocytes, in human alveolar macrophages, in activated rhesus macaque PBMCs, and in CEM×174 cells, but not in U87 cells (Table 1, Fig. 2, and data not shown). By contrast, a cDNA for *gpr1* could not be detected in primary human T lymphocytes, activated rhesus macaque PBMCs, or CEM×174 cells, but was detected in U87 cells and human alveolar macrophages.

## Discussion

HIV-1, HIV-2, and SIV all use CCR5 as a coreceptor, indicating the importance of this protein in primate immunodeficiency virus pathogenesis (28, 30–33, 47, 48). The use of other chemokine receptors by HIV-1 has been suggested to be important for infection of anatomical compartments such as the brain or for more efficient T cell depletion (39, 45, 46). The identification of *gpr1* and *gpr15* as additional SIV coreceptors should assist efforts to understand the consequences of the use of coreceptors other than CCR5 in primate models of AIDS. While the *in vivo* contribution of *gpr15* to SIV replication and pathogenesis requires further investigation, several lines of evidence indi-

```

gpr15      MDPEETSVYLDYYYATSPN
gpr1       MEDLEETLFEEFENYSYDLDYYSLESD
rCCR5      MDYQVSSPTYDIDYYTSEPC
CCR5       MDYQVSSPIYDINYYTSEPC

```

**Figure 3.** An alignment of human *gpr1*, human *gpr15*, rhesus CCR5 (rccr5), and human CCR5 from the NH<sub>2</sub>-terminus through the first cysteine of CCR5 is shown. Tyrosines shown to be important for HIV-1 and SIV<sub>mac</sub>239 entry are shown in bold. Other residues similarly positioned in these proteins are underlined. Sequences for *gpr1* and *gpr15* are provided in references 53 and 54, respectively.

cate that *gpr15* is an important SIV coreceptor. The *gpr15* protein is expressed on CD4<sup>+</sup> T lymphocytes, a major target cell for SIV infection *in vivo* (10), and on alveolar macrophages. The *gpr15* protein is also expressed on CEM×174 cells, which are routinely used to passage SIV obtained from monkey PBMCs (52). The rapid outgrowth of SIV viruses with minimal sequence changes on CEM×174 cells suggests that these cells express a receptor used by primary SIV viruses. The weak use of *gpr15* by the ADA and YU2 HIV-1 viruses may be an inadvertent consequence of similarities in the amino-terminal regions of *gpr15* and CCR5, or may indicate that adaptation to these receptors or to a related receptor occurs in some subsets of HIV-1.

The *in vivo* contribution of *gpr1* to primate immunodeficiency virus infection is also unresolved. The *gpr1* protein weakly supported SIV infection in our studies. Whether this inefficient coreceptor activity is an intrinsic property of *gpr1* or merely reflects low cell surface expression of *gpr1* requires further investigation. While *gpr1* is not apparently expressed on primary CD4<sup>+</sup> lymphocytes, it is expressed on tissue macrophages and in the brain (53) and thus may play a role in SIV infection of particular nonlymphoid target cells.

In primary structure, *gpr1* and *gpr15* resemble the angiotensin II receptor and the orphan receptors *dez* and *apj* more than they do any of the known chemokine receptors (53, 54). *Gpr15*, like *dez* and *gpr1*, lacks the cysteines in the NH<sub>2</sub>-terminal region and the third extracellular loop which, in the chemokine receptors, are thought to be disulfide linked. It is interesting that despite the general sequence divergence of *gpr15/gpr1* and other identified primate immunodeficiency virus coreceptors the *gpr15* and *gpr1* amino termini contain three tyrosines which align with similarly positioned tyrosines in CCR5 (Fig. 3). Alteration of these tyrosines has been shown to decrease the efficiency with which CCR5 supports the entry of SIV and M-tropic HIV-1 isolates (Farzan, M., H. Choe, and J. Sodroski, unpublished observations). The identification of *gpr15* and *gpr1* as SIV coreceptors suggests a greater range and complexity of coreceptors for the primate immunodeficiency viruses than previously described. Comparative studies of these divergent coreceptors with the known coreceptors for these viruses should assist in the identification of common structural elements in 7-TMS proteins which serve as viral entry cofactors.

We thank Dr. Ronald Desrosiers for the gift of the SIV<sub>mac239</sub> and SIV<sub>mac316</sub> infectious proviral clones and Drs. Paul Ponath, Walter Newman, Elliot Kieff, and Monica Napolitano for plasmids expressing chemokine receptors. We thank Ms. Lorraine Rabb and Ms. Yvette McLaughlin for manuscript preparation.

This work was supported by a grant to Joseph Sodroski from the National Institutes of Health (AI-24755) and by a Center for AIDS Research grant to the Dana-Farber Cancer Institute (AI-28691). Dana-Farber Cancer Institute is also the recipient of a Cancer Center grant from the National Institutes of Health (CA-06516). Luisa Marcon was supported by National Cancer Institute National Research Science Award Training Grant (CA-09382) and by an award from Istituto Superiore di Sanità and by the University of Padua. Craig Gerard was supported by National Institutes of Health grants HL-51366 and AI-36162 as well as by the Rubenstein/Cable Fund at the Perlmutter Laboratory. This work was made possible by gifts from the late William McCarty-Cooper, from the G. Harold and Leila Y. Mathers Charitable Foundation, and from the Friends 10.

Address correspondence to Dr. Joseph Sodroski, JFB 824, Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115. Phone: 617-632-3371; FAX: 617-632-4338; E-mail: Joseph\_Sodroski@DFCI.harvard.edu or Dr. Craig Gerard, Perlmutter Laboratory, Children's Hospital, Hunnewell, 300 Longwood Avenue, Boston, MA 02115. Phone: 617-735-6174; FAX: 617-730-0422; E-mail: gerard\_c@a1.tch.harvard.edu

Received for publication 7 May 1997 and in revised form 9 June 1997.

## References

1. Barre-Sinoussi, F., J.C. Chermann, F. Rey, M.T. Nugeyre, S. Charnaret, J. Gruest, C. Dautet, C. Axler-Bin, F. Vezinet-Brun, C. Rouzioux, et al. 1983. Isolation of a T-lymphocyte retrovirus from a patient at risk for acquired immunodeficiency syndrome (AIDS). *Science (Wash. DC)*. 220:868-871.
2. Clavel, F. 1987. HIV-2, the West African AIDS virus. *AIDS (Lond.)*. 1:135-140.
3. Desrosiers, R.C. 1990. The simian immunodeficiency viruses. *Annu. Rev. Immunol.* 8:557-578.
4. Gallo, R.C., S.Z. Salahuddin, M. Popovic, G.M. Shearer, M. Kaplan, B.F. Haynes, T.J. Palker, R. Redfield, J. Oleske, B. Safai, et al. 1984. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. *Science (Wash. DC)*. 212:872-877.
5. Letvin, N.L., M.D. Daniel, P.K. Sehgal, R.C. Desrosiers, R.D. Hunt, L.M. Waldron, J.J. MacKey, D.K. Schmidt, L.V. Chalifoux, and N.W. King. 1985. Induction of AIDS-like disease in macaque monkeys with T-cell tropic retrovirus STLV-III. *Science (Wash. DC)*. 230:71-73.
6. Myers, G., S. Wain-Hobson, L. Henderson, B. Korber, K.-T. Jeang, and G. Pavlakis. 1994. Human Retroviruses and AIDS: A Compilation and Analysis of Nucleic Acid and Amino Acid Sequences. Los Alamos National Laboratory, Los Alamos, New Mexico. Section III:23-31.
7. Kanki, P., M. McLane, N. King, and M. Essex. 1985. Serological identification and characterization of a macaque T-lymphocytic retrovirus closely related to HTLV-III. *Science (Wash. DC)*. 228:1199-1201.
8. Weiss, R., P. Clapham, J. Weber, A. Dalgleish, L. Lasky, and P. Berman. 1986. Variable and conserved neutralization antigens of human immunodeficiency virus. *Nature (Lond.)*. 324:572-575.
9. Fauci, A., A. Macher, D. Longo, H.C. Lane, A. Rook, H. Masur, and E. Gelmann. 1984. Acquired immunodeficiency syndrome: epidemiologic, clinical, immunologic, and therapeutic considerations. *Ann. Intern. Med.* 100:92-106.
10. Desrosiers, R.C., A. Hansen-Moosa, K. Mori, D.P. Bouvier, N.W. King, M.D. Daniel, and D.J. Ringler. 1991. Macrophage-tropic variants of SIV are associated with specific AIDS-related lesions but are not essential for the development of AIDS. *Am. J. Pathol.* 139:29-35.
11. Gartner, S., P. Markovits, D. Markovitz, M.H. Kaplan, R.C. Gallo, and M. Popovic. 1986. The role of mononuclear phagocytes in HTLV-III/LAV infection. *Science (Wash. DC)*. 233:215-219.
12. Gendelman, H., J. Orenstein, M. Martin, C. Ferrva, R. Mitra, T. Phipps, L. Wahl, C. Lane, A. Fauci, and D. Burke. 1988. Efficient isolation and propagation of human immunodeficiency virus on recombinant colony-stimulating factor 1-treated monocytes. *J. Exp. Med.* 167:1428-1441.
13. Koenig, S., H.E. Gendelman, J.M. Orenstein, M.C. Dal Canto, G.H. Pezeshkpour, M. Yungbluth, F. Janotta, A. Aksamit, M.A. Martin, and A.S. Fauci. 1986. Detection of AIDS virus in macrophages in brain tissue from AIDS patients with encephalopathy. *Science (Wash. DC)*. 233:1089-1093.
14. Ringler, D.J., M.S. Wyand, D.G. Walsh, J.J. MacKey, P.K. Sehgal, M.D. Daniel, R.C. Desrosiers, and N.W. King. 1989. The productive infection of alveolar macrophages by simian immunodeficiency virus. *J. Med. Primatol.* 18:217-226.
15. Pope, M., M. Betjes, N. Romani, H. Hirmand, P. Cameron, L. Hoffman, S. Gezelter, G. Schuler, and R. Steinman. 1994. Conjugates of dendritic cells and memory T lymphocytes from skin facilitate productive infection with HIV-1. *Cell*. 78:389-398.
16. Weissman, D., Y. Li, J. Ananworanich, L.-J. Zhou, J. Adelsberger, T. Tedder, M. Baseler, and A. Fauci. 1995. Three populations of cells with dendritic morphology exist in peripheral blood only one of which is infectable with human immunodeficiency virus type 1. *Proc. Natl. Acad. Sci. USA*. 92:826-830.
17. Dalgleish, A.G., P.C.L. Beverley, P.R. Clapham, D.H. Crawford, M.F. Greaves, and R.A. Weiss. 1984. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. *Nature (Lond.)*. 312:763-767.
18. Klatzmann, D., E. Champagne, S. Charnaret, J. Gruest, D.

- Guétard, T. Hercend, J.C. Gluckman, and L. Montagnier. 1984. T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. *Nature (Lond.)*. 312:767–768.
19. Maddon, P.J., A.G. Dalgleish, J.S. McDougal, P.R. Clapham, R.A. Weiss, and R. Axel. 1986. The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. *Cell*. 47:333–348.
  20. Allan, J., T.H. Lee, M.F. McLane, J. Sodroski, W. Haseltine, and M. Essex. 1983. Identification of the major envelope glycoprotein product of HTLV-III. *Science (Wash. DC)*. 228:1091–1094.
  21. Robey, W.G., B. Safai, S. Oroszlan, L. Arthur, M. Gonda, R. Gallo, and P.J. Fischinger. 1985. Characterization of envelope and core structural gene products of HTLV-III with sera from AIDS patients. *Science (Wash. DC)*. 228:593–595.
  22. Lapham, C., J. Ouyang, B. Chandraseklar, N. Nguyen, D. Dimitrov, and H. Golding. 1996. Evidence for cell-surface association between fusin and the CD4-gp120 complex in human cell lines. *Science (Wash. DC)*. 274:602–605.
  23. Wu, L., N. Gerard, R. Wyatt, H. Choe, C. Parolin, N. Ruffing, A. Borsetti, A. Cardoso, E. Desjardin, W. Newman, et al. 1996. CD4-induced interaction of primary HIV-1 gp120 glycoproteins with the chemokine receptor CCR5. *Nature (Lond.)*. 384:179–183.
  24. Trkola, A., T. Dragic, J. Arthos, J.M. Binley, W.C. Olson, G.P. Allaway, C. Cheng-Mayer, J. Robinson, P.J. Maddon, and J.P. Moore. 1996. CD4-dependent, antibody-sensitive interactions between HIV-1 and its co-receptor CCR-5. *Nature (Lond.)*. 384:184–187.
  25. Stein, B., S. Gouda, J. Lifson, R. Penhallow, K. Bensch, and E. Engelman. 1987. pH-independent HIV entry into CD4-positive T cells via virus envelope fusion to the plasma membrane. *Cell*. 49:659–668.
  26. Kowalski, M., J. Potz, L. Basiripour, T. Dorfman, W.G. Goh, E. Terwilliger, A. Dayton, C. Rosen, W. Haseltine, and J. Sodroski. 1987. Functional regions of the human immunodeficiency virus envelope glycoproteins. *Science (Wash. DC)*. 237:1351–1355.
  27. Helseth, E., M. Kowalski, D. Gabuzda, U. Olshevsky, W. Haseltine, and J. Sodroski. 1990. Rapid complementation assays measuring replicative potential of HIV-1 envelope glycoprotein mutants. *J. Virol.* 64:2416–2420.
  28. Choe, H., M. Farzan, Y. Sun, N. Sullivan, B. Rollins, P.D. Ponath, L. Wu, C.R. Mackay, G. LaRosa, W. Newman, et al. 1996. The  $\beta$ -chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. *Cell*. 85:1135–1148.
  29. Cocchi, F., A. DeVico, A. Garzino-Demo, A. Cara, R.C. Gallo, and P. Lusso. 1996. The V3 domain of the HIV-1 gp120 glycoprotein is critical for chemokine-mediated blockade of infection. *Nat. Med.* 2:1244–1247.
  30. Alkhatib, G., C. Combadiere, C.C. Broder, Y. Feng, P.M. Murphy, and E. Berger. 1996. CC-CKR5: a RANTES, MIP-1 $\alpha$ , MIP-1 $\beta$  receptor as a fusion cofactor for macrophage-tropic HIV-1. *Science (Wash. DC)*. 272:1955–1958.
  31. Deng, H.K., S. Choe, W. Ellmeier, R. Liu, D. Unutmaz, M. Burkhart, P. di Marzio, S. Marmon, R.E. Sutton, C.M. Hill, et al. 1996. Identification of a major co-receptor for primary isolates of HIV-1. *Nature (Lond.)*. 381:661–666.
  32. Doranz, B., J. Rucker, Y. Yi, R. Smyth, M. Samson, S. Peiper, M. Parmentier, R. Collman, and R. Doms. 1996. A dual-tropic primary HIV-1 isolate that uses fusin and the  $\beta$ -chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. *Cell*. 85:1149–1158.
  33. Dragic, T., V. Litwin, G.P. Allaway, S. Martin, Y. Huang, K.A. Nagashima, C. Cayanan, P.J. Maddon, R.A. Koup, J.P. Moore, and W.A. Paxton. 1996. HIV-1 entry into CD4<sup>+</sup> cells is mediated by the chemokine receptor CC-CKR-5. *Nature (Lond.)*. 381:667–673.
  34. Zhang, L., Y. Hsung, T. He, Y. Cao, and D.D. Ho. 1996. HIV-1 subtype and second-receptor use. *Nature (Lond.)*. 383:768.
  35. Connor, R.I., K.E. Sheridan, D. Ceradini, S. Choe, and N.R. Landau. 1997. Change in coreceptor use correlates with disease progression in HIV-1 infected individuals. *J. Exp. Med.* 185:621–628.
  36. Wu, L., W.A. Paxton, N. Kassam, N. Ruffing, J.B. Rottman, N. Sullivan, H. Choe, J. Sodroski, W. Newman, R.A. Koup, and C.R. Mackay. 1997. CCR5 levels and expression pattern correlate with infectability by macrophage-tropic HIV-1 in vitro. *J. Exp. Med.* 185:1681–1691.
  37. Granelli-Piperno, A., B. Moser, M. Pope, D. Chen, Y. Wei, F. Isdell, U. O'Doherty, W. Paxton, R. Koup, S. Mojssov, et al. 1996. Efficient interaction of HIV-1 with purified dendritic cells via multiple chemokine coreceptors. *J. Exp. Med.* 184:2433–2438.
  38. Raport, C.J., J. Gosling, V.L. Schweickart, P.W. Gray, and I.F. Charo. 1996. Molecular cloning and functional characterization of a novel human CC chemokine receptor (CCR5) for RANTES, MIP-1 $\beta$ , and MIP-1 $\alpha$ . *J. Biol. Chem.* 271:17161–17166.
  39. He, J., Y. Chen, M. Farzan, H. Choe, A. Ohagen, S. Gartner, J. Busciglio, X. Yang, W. Hofmann, W. Newman, et al. 1997. CCR3 and CCR5 are co-receptors for HIV-1 infection of microglia. *Nature (Lond.)*. 385:645–649.
  40. Liu, R., W. Paxton, S. Choe, D. Ceradini, S. Martin, R. Horuk, M. MacDonald, H. Stuhlmann, R. Koup, and N. Landau. 1996. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell*. 86:367–378.
  41. Dean, M., M. Carrington, C. Winkler, G.A. Huttley, M.W. Smith, R. Allikmets, J.J. Goedert, S.P. Buchbinder, E. Vittinghoff, E. Gomperts, et al. 1996. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. Hemophilia Growth and Development Study, Multicenter AIDS Cohort Study, Multicenter Hemophilia Cohort Study, San Francisco City Cohort, ALIVE Study. *Science (Wash. DC)*. 273:1856–1862.
  42. Samson, M., F. Libert, B. Doranz, B. Rucker, C. Liesnard, C. Farber, S. Saragosti, C. Lapoumeroulie, J. Cognaux, C. Forceille, et al. 1996. Resistance to HIV-1 infection of Caucasian individuals bearing a mutant allele of the CCR5 chemokine receptor gene. *Nature (Lond.)*. 382:722–725.
  43. Simmons, G., D. Wilkinson, J.D. Reeves, M.T. Dittmar, S. Beddows, J. Weber, G. Carnegie, U. Desselberger, P.W. Gray, R.A. Weiss, and P.R. Clapham. 1996. Primary, syncytium-inducing human immunodeficiency virus type 1 isolates are dual-tropic and most can use either LESTR or CCR5 as coreceptors for virus entry. *J. Virol.* 70:8355–8360.
  44. Feng, Y., C.C. Broder, P.E. Kennedy, and E.A. Berger. 1996. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science (Wash. DC)*. 272:872–877.
  45. Schuitemaker, H., M. Koot, N.A. Koostera, M.W. Dercksen, R.E.Y. deGoede, R.P. van Steenwijk, J.M.A. Lange, J.K.M. Eeftink-Schattenkert, F. Miedema, and M. Tersmette. 1992.

- Biological phenotype of human immunodeficiency virus type 1 clones at different stages of infection: progression of disease is associated with a shift from monocytotropic to T-cell-tropic virus populations. *J. Virol.* 65:356–363.
46. Bleul, C.C., L. Wu, J.A. Hoxie, T.A. Springer, and C.R. Mackay. 1996. The HIV coreceptors CXCR4 and CCR5 are differentially expressed and regulated on human T lymphocytes. *Proc. Natl. Acad. Sci. USA.* 94:1925–1930.
  47. Marcon, L., H. Choe, K.A. Martin, M. Farzan, P.D. Ponath, L. Wu, W. Newman, N. Gerard, C. Gerard, and J. Sodroski. 1997. Utilization of C-C chemokine receptor 5 by the envelope glycoproteins of a pathogenic simian immunodeficiency virus (SIV<sub>mac239</sub>). *J. Virol.* 71:2522–2527.
  48. Chen, Z., P. Zhou, D.D. Ho, N. Landau, and P. Marx. 1997. Genetically divergent strains of simian immunodeficiency virus use CCR5 as a coreceptor for entry. *J. Virol.* 71:2705–2714.
  49. Cocchi, F., A. DeVico, A. Garzino-Demo, S. Arya, R. Gallo, and P. Lusso. 1995. Identification of RANTES, MIP-1 $\alpha$ , and MIP-1 $\beta$  as the major HIV-suppressive factors produced by CD8+ T cells. *Science (Wash. DC).* 270:1811–1815.
  50. Endres, M., P. Clapham, M. Marsh, M. Ahuja, J. Turner, A. McKnight, J. Thomas, B. Stoebenau-Haggarty, S. Choe, P. Vance, et al. 1996. CD4-independent infection by HIV-2 is mediated by fusin/CXCR4. *Cell.* 87:745–756.
  51. Clapham, P., D. Blanc, and R. Weiss. 1991. Specific cell surface requirements for the infection of CD4-positive cells by human immunodeficiency virus types 1 and by simian immunodeficiency virus. *Virology.* 181:703–715.
  52. Kestler, H., D. Ringler, K. Mori, D. Panicali, P. Sehgal, M. Daniel, and R. Desrosiers. 1991. Importance of the *nef* gene for maintenance of high virus loads and for the development of AIDS. *Cell.* 65:651–662.
  53. Marchese, A., J. Docherty, T. Nguyen, M. Heiber, R. Cheng, H. Heng, L. Tsui, X. Shi, S. George, and B. O'Dowd. 1994. Cloning of human genes encoding novel G protein-coupled receptors. *Genomics.* 23:609–618.
  54. Heiber, M., A. Marchese, T. Nguyen, H. Heng, S. George, and B. O'Dowd. 1996. A novel human gene encoding a G-protein-coupled receptor (gpr15) is located on chromosome 3. *Genomics.* 32:462–465.
  55. O'Dowd, B., M. Heiber, A. Chan, H. Heng, L. Tsui, J. Kennedy, X. Shi, A. Petronis, S. George, and T. Nguyen. 1993. A human gene that shows identity with the gene encoding the angiotensin receptor is located on chromosome 11. *Gene (Amst.).* 136:355–360.
  56. Dobner, T., I. Wolf, T. Emrich, and M. Lipp. 1992. Differentiation-specific expression of a novel G protein-coupled receptor from Burkitt's lymphoma. *Eur. J. Immunol.* 22:2795–2799.
  57. Samson, M., O. Labbe, C. Mollereau, G. Vassart, and M. Parmentier. 1996. Molecular cloning and functional expression of a new human CC-chemokine receptors gene. *Biochemistry.* 35:3362–3367.
  58. Murphy, P.M., and H.L. Tiffany. 1991. Cloning of complementary DNA encoding a functional human interleukin-8 receptor. *Science (Wash. DC).* 253:1280–1283.
  59. Holmes, W.E., J. Lee, W.J. Kuang, G.C. Rice, and W.I. Wood. 1991. Structure and functional expression of a human interleukin-8 receptor. *Science (Wash. DC).* 253:1278–1280.
  60. Loetscher, M., B. Gerber, P. Loetscher, S.A. Jones, L. Piali, I. Clark-Lewis, M. Baggiolini, and B. Moser. 1996. Chemokine receptor specific for IP10 and mig: structure, function and expression in activated T-lymphocytes. *J. Exp. Med.* 184:963–969.
  61. Federspiel, B., I.G. Melhado, A.M. Duncan, A. Delaney, K. Schappert, I. Clarke-Lewis, and F.R. Jirik. 1993. Molecular cloning of the cDNA and chromosomal localization of the gene for a putative seven-transmembrane segment (7-TMS) receptor isolated from human spleen. *Genomics.* 16:706–712.
  62. Methner, A., G. Hermey, B. Schinke, and I. Hermans-Borgmeyer. 1997. A novel G protein-coupled receptor with homology to neuropeptide and chemoattractant receptors expressed during bone development. *Biochem. Biophys. Res. Commun.* 238:836–842.
  63. Birkenbach, M., K. Josefsen, R. Yalamanchili, G. Lenoir, and E. Kieff. 1993. Epstein-Barr virus-induced genes: first lymphocyte-specific G protein-coupled peptide receptors. *J. Virol.* 67:2209–2220.
  64. Liao, F., H.H. Lee, and J.M. Farber. 1997. Cloning of STRL22, a new human gene encoding a G-protein-coupled receptor related to chemokine receptors and located on chromosome 6q27. *Genomics.* 40:175–180.
  65. Heiber, M., J.M. Docherty, G. Shah, T. Nguyen, R. Cheng, H. Heng, A. Marchese, L. Tsui, X. Shi, S.R. George, and B.F. O'Dowd. 1995. Isolation of three novel human genes encoding G protein-coupled receptors. *DNA Cell Biol.* 14:25–35.
  66. Libert, F., M. Parmentier, A. Lefort, J.E. Dumont, and G. Vassart. 1990. Complete nucleotide sequence of a putative G protein coupled receptor: RDC1. *Nucleic Acids Res.* 18:1917.
  67. Napolitano, M., A. Zingoni, G. Bernardini, G. Spinetti, A. Nista, C.T. Storlazzi, M. Rocchi, and A. Santoni. 1996. Molecular cloning of TER1, a chemokine receptor-like gene expressed by lymphoid tissues. *J. Immunol.* 157:2759–2763.
  68. Combadiere, C., S.K. Ahuja, and P.M. Murphy. 1995. Cloning, chromosomal localization, and RNA expression of a human beta chemokine receptor-like gene. *DNA Cell Biol.* 14:673–680.