HIV-1 exploits CCR5 conformational heterogeneity to escape inhibition by chemokines

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CC chemokine receptor 5 (CCR5) is a receptor for chemokines and the coreceptor for R5 HIV-1 entry into CD4⁺ T lymphocytes. Chemokines exert anti-HIV-1 activity *in vitro*, both by displacing the viral envelope glycoprotein gp120 from binding to CCR5 and by promoting CCR5 endocytosis, suggesting that they play a protective role in HIV infection. However, we showed here that different CCR5 conformations at the cell surface are differentially engaged by chemokines and gp120, making chemokines weaker inhibitors of HIV infection than would be expected from their binding affinity constants for CCR5. These distinct CCR5 conformations rely on CCR5 coupling to nucleotide-free G proteins (^{NF}G proteins). Whereas native CCR5 chemokines bind with subnanomolar affinity to ^{NF}G protein-coupled CCR5, gp120/HIV-1 does not discriminate between ^{NF}G protein-coupled and uncoupled CCR5. Interestingly, the antiviral activity of chemokines is G protein independent, suggesting that “low-chemokine affinity” ^{NF}G protein-uncoupled conformations of CCR5 represent a portal for viral entry. Furthermore, chemokines are weak inducers of CCR5 endocytosis, as is revealed by EC₅₀ values for chemokine-mediated endocytosis reflecting their low-affinity constant value for ^{NF}G protein-uncoupled CCR5. Abolishing CCR5 interaction with ^{NF}G proteins eliminates high-affinity binding of CCR5 chemokines but preserves receptor endocytosis, indicating that chemokines preferentially endocytose low-affinity receptors. Finally, we evidenced that chemokine analogs achieve highly potent HIV-1 inhibition due to high-affinity interactions with internalizing and/or gp120-binding receptors. These data are consistent with HIV-1 evading chemokine inhibition by exploiting CCR5 conformational heterogeneity, shed light into the inhibitory mechanisms of anti-HIV-1 chemokine analogs, and provide insights for the development of unique anti-HIV molecules.

HIV coreceptor | AIDS pathogenesis | β chemokines

C-C chemokine receptor 5 (CCR5) is the principal coreceptor for entry of HIV type 1 (HIV-1), used together with CD4 to enter and infect target cells (1) and a receptor for agonist [C-C chemokine ligand 3 (CCL3)/macrophage inflammatory protein-1 α (MIP-1 α), CCL4/MIP-1 β , CCL5/regulated upon activation, normal T-cell expressed and secreted (RANTES)] and antagonist/weak partial agonist [CCL7/monocyte chemoattractant protein 3 (MCP-3)] chemokines (2, 3). The native agonist chemokine ligands of CCR5 induce conformational changes in the receptor that promote activation of pertussis toxin (PTX)-sensitive, heterotrimeric $\alpha\beta\gamma$ G proteins (Gi/o-type G proteins) by catalyzing an exchange of GTP for GDP on the G α subunit. The GTP-bound G α subunit and the G $\beta\gamma$ dimer then trigger intracellular signaling pathways involved in chemotaxis and activation of leukocytes (4).

Native CCR5 chemokines inhibit infection of R5-tropic HIV-1 *in vitro*. This occurs via two mechanisms: sterically preventing the viral envelope glycoprotein gp120 from binding to the coreceptor and reducing cell surface coreceptor levels by inducing receptor down-regulation (5–7). They are secreted by a number of cell types and in particular immune cells including R5 HIV-1

target cells (6, 8, 9). The potential role of native CCR5 chemokines in blocking HIV-1 transmission and progression has been extensively studied (9–12), but their efficacy as protective factors remains a matter of debate (13, 14). A major paradox relates to the observation that native CCR5 chemokines show lower antiviral potencies than would be expected based on their CCR5 binding affinity constants (15–18), which are in the subnanomolar range (2, 19, 20), much lower than the corresponding value for the HIV-1 envelope glycoprotein gp120, which is $\sim$ 10 nM (19, 21).

A number of CCR5 chemokine analogs with improved antiviral potency have been identified, including N-terminally modified RANTES analogs with agonist [aminooxypentane (AOP)-, PSC- or 6P4-RANTES] or antagonist features (5P12- or 2P3-RANTES), which represent promising molecules as topical microbicides (18, 22). Although the enhanced potency of agonist analogs can be explained in terms of their increased capacity to induce CCR5 down-regulation (23), the inhibitory mechanism of antagonist analogs, which neither activate G protein signaling nor induce receptor down-regulation, is more elusive. It was speculated that it might involve increased steric blockade of CCR5, but competition binding assays using labeled CCL4 as a tracer did not show any significant increase in CCR5 binding affinity (22).

In this study, we present evidence that conformationally different CCR5 subpopulations with distinct chemokine binding capacities are present at the surface of HIV-1 target cells. In particular, a fraction of receptors shows strikingly low binding affinity for native CCR5 chemokines, providing an explanation for why native CCR5 chemokines have unexpectedly low anti-HIV-1 potencies. Our results also shed further light on the inhibitory mechanism of chemokine analogs, showing that they overcome the challenge of the chemokine low-affinity CCR5 population through (i) more efficient receptor down-regulation and/or (ii) increased binding affinity for gp120-binding receptors. Overall, these findings explain how R5 HIV-1 could escape from inhibition by native CCR5 chemokines in the course of infection and provide clues for the development of unique chemokine analogs as HIV inhibitors.

Results

Distinct CCR5 Populations Are Differentially Used by HIV-1 gp120 and Chemokines. Chemokines and the CD4-bound form of HIV-1 envelope glycoprotein gp120 competitively bind to overlapping regions of CCR5 (24). Thus, to investigate whether distinct CCR5

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populations interact differently with chemokines and gp120, we first tested unlabeled native chemokines (CCL-3, -4, -5, or -7) or chemokine analogs (AOP-, PSC-, 2P3-, 5P12-, or 6P4-RANTES) for their ability to inhibit binding of either 125 I-labeled CCL3 (125 I-CCL3) or the 35 S-labeled gp120 from the HIV-1 primary strain Bx08 (35 S-gp120_{Bx08}) on membranes from CCR5-expressing HEK 293T cells (HEK-R5 cells) (Fig. 1 and Table 1). Using the data obtained, we calculated the affinity constant values (K_i) of the competing ligands for receptors using the Cheng and Prusoff equation (*SI Materials and Methods*).

Except for CCL7, displacement of 125 I-CCL3 binding revealed high affinities of competitors for CCR5, with K_i values in the nanomolar range or lower (Table 1). The K_i values obtained for the native CCR5 agonists CCL-3, -4, and -5 are comparable to the K_D values determined for these ligands in saturation binding assays (Table 1), consistent with binding of these chemokines to a similar class of high-affinity receptors in both competition and saturation assays. In contrast, CCL-3, -4, and -5, as well as the chemokine analogs AOP- and PSC-RANTES, only partly displaced 35 S-gp120_{Bx08} binding when used at a 100-nM concentration (Fig. 1*B*), suggesting that they have a lower affinity for 35 S-gp120_{Bx08}-binding receptors compared with 125 I-CCL3-binding receptors. In contrast, the observation that 35 S-gp120_{Bx08} binds marginally to CCR5 in the presence of 100 nM 2P3-, 5P12-, or 6P4-RANTES (Fig. 1*B*) suggests that these chemokine analogs preserve high-affinity interactions with 35 S-gp120_{Bx08}-binding CCR5.

In dose-response experiments, 2P3-, 5P12-, or 6P4-RANTES and 35 S-gp120_{Bx08} competed for binding to an apparent single class of receptors, as is revealed by monophasic competitive binding curves (Fig. 1*C* and *D*). The K_i values in the nanomolar range calculated for these chemokine analogs confirm high-affinity interactions with the 35 S-gp120_{Bx08}-binding receptors (Table 1). CCL7 similarly bound to a single class of 35 S-gp120_{Bx08}-binding CCR5 for which the affinity of the chemokine was higher than that for 125 I-CCL3-binding CCR5 ($K_i = 34$ vs. 119 nM). In contrast, displacements of 35 S-gp120_{Bx08} binding by the agonists CCL3 (Fig. 1*C*), CCL4, and PSC-RANTES (Fig. 1*D*) gave biphasic curves, consistent with the presence of two distinct

35 S-gp120_{Bx08} receptor populations, one with high affinity for these chemokines, the other with significantly lower affinity. CCL-3, -4, and PSC-RANTES had K_i values for interaction with the “high-chemokine affinity” receptor population similar to those determined in 125 I-CCL3 displacement assays (Table 1), suggesting that the high-chemokine affinity population of 35 S-gp120_{Bx08}-binding receptors and 125 I-CCL3-binding receptors represent the same receptors. Interestingly, the K_i values obtained for the “low-chemokine affinity” CCR5 population range from a few tens of nM up to more than 10^{-6} M (Table 1), thus exceeding the K_D value for the interaction of 35 S-gp120_{Bx08} with CCR5 determined in saturation assays (~ 10 nM) (19). Hence, this fraction of CCR5 has a higher affinity for gp120 than for native CCR5 chemokines.

Poor Ability of Native CCR5 Chemokines to Displace gp120 Binding to CCR5 Correlates with Low Antiviral Activity. We next investigated whether low anti-HIV potency of native chemokines is related to low-affinity interactions with gp120-binding receptors. For that purpose, we infected activated CD4⁺ T lymphocytes, which represent the major target cells for R5 HIV-1, and HeLa P4C5 cells with infectious NL4-3Ren-based HIV-1 expressing the envelope glycoprotein gp160 of the Bx08 strain and Renilla luciferase as a reporter gene (Bx08Ren viruses; see *SI Materials and Methods* for details) in the presence of native CCR5 chemokines or chemokine analogs (Table 1 and Fig. S1*A* and *B*). Except for the chemokine analog PSC-RANTES, the antiviral potencies of all other chemokines correlated better with their ability to displace the binding of 35 S-gp120_{Bx08} than that of 125 I-CCL3. In particular, the native CCR5 agonists (CCL-3, -4, and -5) that show low-affinity interactions with a proportion of gp120-binding receptors had many more weaker antiviral activities than chemokine analogs, even though both groups of molecules have comparable affinities for 125 I-CCL3-binding CCR5 (Table 1). Similar results were obtained using five other viruses that were generated by inserting the R5 *env* gp120 or *env* gp160 sequences from laboratory-adapted (JRRen) or primary (25Ren, 34Ren, 50Ren, or 58Ren) viruses in place of the corresponding regions of the NL4-3 *env* sequence in the pNL4-3Ren plasmid containing the Renilla luciferase gene in place of the *nef* gene (see *SI Materials and Methods* for details) (Fig. S2).

The potent antiviral activities of the antagonist RANTES analogs 5P12 and 2P3, which do not down-regulate CCR5 (see Fig. S*B* and ref. 22), reflect their increased binding affinities detected using 35 S-gp120_{Bx08} as a tracer (Fig. 1). The enhanced potency of PSC-RANTES, which occurs despite its relatively low capacity to compete with 35 S-gp120_{Bx08} for binding to CCR5, is likely to be due to its enhanced capacity to induce CCR5 down-regulation (23). To test this hypothesis, we performed infection inhibition experiments under conditions where receptor down-regulation is suppressed. HeLa P4C5 cells were preincubated for 2 h with PSC-RANTES (40 nM), 5P12-RANTES (40 nM), or the CCR5 inverse agonist maraviroc (MVC, 20 μ M) at either 37 °C or at 4 °C, a temperature at which receptor endocytosis does not occur. Bx08Ren virus was then added to the cells, which were incubated for a further 2 h at 4 °C, then washed in cold PBS, warmed to 37 °C for 15 min to allow entry of attached viruses, trypsin treated to remove residual viruses, and incubated for 48 h at 37 °C (Fig. 2). Under conditions where CCR5 down-regulation is suppressed, the antiviral activity of PSC-RANTES was almost completely abrogated, but the inhibitory potency of 5P12-RANTES or MVC was unaffected, in accordance with our results in Fig. 1 showing that PSC- and 5P12-RANTES are weak and potent inhibitors of gp120 binding to CCR5. Hence unlike 5P12-RANTES and maraviroc, PSC-RANTES owes a large part of its inhibitory activity to its capacity to induce CCR5 down-regulation. Importantly, these results also validate the notion that the receptors interacting with monomeric gp120/soluble CD4 complexes in the binding assays presented here (Fig. 1*B–D*) and those that are

Fig. 1. Chemokine- and gp120-binding CCR5 represent different receptor populations. Binding of 0.1 nM 125 I-CCL3 (*A*) or 10 nM 35 S-gp120_{Bx08} (in the presence of 30 nM soluble CD4) (*B–D*) was displaced by increasing amounts (*A*, *C*, and *D*) or a 100-nM concentration (*B*) of unlabeled chemokines. Results were normalized for nonspecific binding (0%) and specific binding in the absence of competitors (B_0 , 100%) and fitted according to a one-site (*A*, 6P4 and CCL7 in *C*, and 2P3 and 5P12 in *D*) or a two-site ($16.2 < F$ value < 93.3 with $P < 0.0001$ for CCL3 in *C* and CCL4 and PSC in *D*) competitive binding model. In *B*, data points are means $\pm$ SEM of five independent determinations. *A*, *C*, and *D* show representative experiments of three to five performed independently.

Table 1. CCR5 binding affinity constants of native chemokines and RANTES analogs and their half-maximal inhibitory concentrations (IC₅₀) of HIV-1 infection in CD4⁺ T-cells and HeLa P4C5 cells

Chemokines	K _D (nM)	K _i ^{CCL3} (nM)	K _i ^{9p120} (nM) - Gpp(NH)p	K _i ^{9p120} (nM) + Gpp(NH)p	IC ₅₀ (nM) T-CD4 ⁺ -PTX	IC ₅₀ (nM) T-CD4 ⁺ +PTX	IC ₅₀ (nM) P4C5 -PTX	IC ₅₀ (nM) P4C5 +PTX
CCL3	0.25 ± 0.05	0.06 ± 0.002	High 0.6 ± 0.1 (43.6 ± 9.7%); low > 1,000	High 0.58 ± 0.4 (16.4 ± 6.6 %); low 597 ± 185.4	106.9 ± 42.7	143	> 1,000	> 1,000
CCL4	0.37 (a)	0.21 ± 0.1	High 0.43 ± 0.1 (25 ± 3 %); low 44.7 ± 6.5	High und (b) low 86 ± 11 (b)	4.5 ± 1.8	6.7 ± 2.8	444 ± 59	366 ± 62
CCL5	0.75 ± 0.15	3 ± 1.1	> 1,000	-	-	-	> 1,000	> 1,000
CCL7	-	119.4 ± 31.7	34.1 ± 8.2	-	> 1,000	-	> 1,000	> 1,000
AOP	-	1.13 ± 0.18	1.47	-	-	-	-	-
PSC	-	1.89 ± 0.95	High 1.47 ± 0.03 (31.7 ± 0.5 %); low 215 ± 35.7	-	0.14 ± 0.03	0.12 ± 0.03	0.44 ± 0.2	0.48 ± 0.02
2P3	-	0.31 ± 0.11	2.72 ± 0.21	-	0.96 ± 0.31	-	-	-
5P12	-	0.26 ± 0.13	3.51 ± 1.8	-	0.036 ± 0.015	0.038 ± 0.013	0.87 ± 0.45	0.96 ± 0.45
6P4	-	0.055 ± 0.02	2.93 ± 0.23	-	0.046 ± 0.01	0.047	0.82 ± 0.12	0.64 ± 0.12

K_i^{CCL3} and K_i^{gp120} represent the equilibrium dissociation constants for interaction of chemokines with CCR5 determined in competition assays using either ^{125}I -CCL3 or ^{35}S -gp120 as a tracer, respectively, in the presence or in the absence of Gpp(NH)p. K_D values are the equilibrium dissociation constants of radiolabeled chemokine-CCR5 complexes deduced from saturation binding experiments. The K_D values for ^{125}I -CCL3 and ^{125}I -CCL5 are deduced from the experiments shown in Figs. 3B and 3C, respectively. For details on the B_{max} values, see the text covering these figures. (a) The reported K_D value is from ref. 20. (b) The reported K_i values for interaction of CCL4 with ^{35}S -gp120-binding CCR5 in the presence of Gpp(NH)p is from ref. 19. Except for inhibition of infection of T-cells by CCL3 or 6P4 in the presence of PTX, which was performed once, values represent means $\pm$ SD of at least three independent determinations. The independent experiments in CD4⁺ T-cells represent experiments run in cells obtained from different donors. Numbers in parentheses represent the percentages of receptors, which are in a high-affinity state for the considered agonist chemokines. Und., undetectable.

used by infectious virus particles at the surface of intact cells in infection assays extensively overlap and would represent similar receptor populations.

CCR5 Coupling to Nucleotide-Free G Proteins Differentially Regulates Native Agonist Chemokine and gp120 Binding. It has been established that conformations of G protein-coupled receptors with high-affinity for agonists are stabilized by coupling to guanine nucleotide-free G proteins (^{NF}G proteins), and that the receptors are induced to shift toward low-affinity conformations as soon as G proteins are occupied by nucleotides (25). Similarly to what we and others showed in mammalian cell lines (19, 26), we observed that CCR5 coupling to ^{NF}G proteins also stabilizes the receptor in a high-affinity conformation for agonists in HIV-1 target cells. Indeed, the non hydrolysable GTP analogs guanosine 5'-O-(γ-thio)-triphosphate (GTPγS) and guanosine 5'-(β,γ-imido)triphosphate [Gpp(NH)p] or PTX, which inactivates G_{i/o}-proteins, decreased ¹²⁵I-CCl3 binding to CCR5 expressed in human lymphoblastoid CD4⁺ T-cell lines (A3.01-R5 cells) or primary T lymphocytes to levels approaching that of nonspecific

binding (Fig. 3A). Saturation binding of ^{125}I -CCL3 to membranes from HEK-R5 cells further revealed that Gpp(NH)p decreases the maximum number of binding sites for the chemokine (B_{max}) from 10.7 ± 1.3 – 2.9 ± 0.7 pmol/mg of protein (Fig. 3B), while only slightly affecting the K_D value from 0.25 ± 0.05 – 0.46 ± 0.08 nM, indicating that Gpp(NH)p reduces the number of receptors that are of high affinity for ^{125}I -CCL3. In line with this, 0.1 nM ^{125}I -CCL3 showed only background levels of binding to membranes from HEK cells expressing the R126N-CCR5 mutant, which does not activate G proteins (27) (Fig. 3D). Similarly to CCL3 and CCL4 (26), high-affinity binding of CCL5 also required CCR5 coupling to NF_G proteins (Fig. 3C).

In contrast to native CCR5 agonist chemokines, R5 HIV-1 gp120 acts as an antagonist/weak partial agonist for CCR5, as it does not discriminate between ^{NF}G protein-coupled or uncoupled CCR5 and binds equally well to R126N-CCR5 and wild-type CCR5, both in the presence and absence of Gpp(NH)p (Fig. 3E). This led us to hypothesize that the biphasic competitive binding curves obtained with native CCR5 agonist chemokines using ³⁵S-gp120_{Bx08} as a tracer is a reflection of (i) the existence of populations of both ^{NF}G protein-coupled and ^{NF}G protein-uncoupled receptors with respectively high and low affinity for these chemokines and (ii) the capacity of gp120 to bind indiscriminately to either population. We tested this hypothesis by repeating the competition experiments of ³⁵S-gp120_{Bx08} binding by CCL3, in the presence and absence of Gpp(NH)p (Fig. 3F). Treatment with Gpp(NH)p decreased the proportion of high-chemokine affinity receptors versus low-chemokine affinity receptors from 43% to 16% ($P = 0.016$ in unpaired, two-tailed Student t test), without affecting the K_i value of the low-chemokine affinity receptor population (Table 1 and Fig. 3F). This result is consistent with our previous observation that Gpp(NH)p eliminates the fraction of ³⁵S-gp120_{Bx08}-binding CCR5 that binds CCL4 with high affinity (19).

Chemokine-Mediated Inhibition of HIV Infection and CCR5 Endocytosis Are G Protein-Independent Processes. Based on the observation that HIV envelope binds indiscriminately to high-chemokine affinity ^{NF}G protein-coupled CCR5 and low-chemokine affinity

Fig. 2. Steric inhibition of gp120 binding to CCR5 does not contribute to the anti-HIV-1 activity of PSC-RANTES. HeLa P4C5 cells were incubated with MVC, 5P12-, or PSC-RANTES at 37 °C or 4 °C before being infected by Bx08Ren viruses and treated as indicated in the text. Results represent the luciferase activity in the cell lysates, expressed as relative light units (RLUs). One representative experiment of five independent determinations is shown. Uninfected cells (NI) represent negative controls.

a β -arrestin-coupled conformation of CCR5 for which it maintains a high affinity, similarly to other receptors, which are in a high-affinity state for agonists when complexed with arrestins (35). Finally, the robust CCR5 down-regulation induced by PSC-RANTES explains why the molecule preserves a strong antiviral activity despite having a low affinity for gp120-binding receptors. Indeed, preventing CCR5 endocytosis virtually abrogates PSC-RANTES-mediated inhibition of HIV infection (Fig. 2), indicating that steric inhibition of gp120 binding to CCR5 plays a marginal role in the antiviral activity of PSC-RANTES.

The antagonists 5P12- and 2P3-RANTES appeared instead to act solely by potentially blocking the interaction between gp120 and CCR5 (Fig. 1). Using these antagonists together with CCR5-internalizing molecules such as PSC-RANTES could in principle represent an interesting therapeutic perspective, albeit no studies have shown yet whether these different analogs have additive inhibitory effects in HIV infection. Interestingly however, we showed here that 6P4-RANTES resembles both 5P12- and PSC-RANTES in that it preserves high affinity for gp120 binding receptors (Fig. 1C) and down-regulates CCR5 at nanomolar concentrations. Considered altogether, these results are consistent with 5P12-, 6P4-, and PSC-RANTES stabilizing different CCR5 conformations. In line with this, mutations in the transmembrane domains of CCR5 were found to modulate in different ways their ability to inhibit HIV infection, indicating that they have different structural constraints for HIV-1 inhibition (36). Notably, these mutations did not change the ability of the RANTES analogs to inhibit 125 I-CCL3 binding to CCR5 (36), again strengthening the notion that chemokines have different structural requirements for

interacting with NF G protein coupled, CCL3-binding receptors, and inhibiting gp120 binding and HIV infection.

Overall, our findings document that both mechanisms whereby native CCR5 chemokines exert their anti-HIV activity, inhibition of gp120/CCR5 interactions, and CCR5 down-regulation, are strongly limited by virtue of their low-affinity interactions with a proportion of CCR5 conformations. Overcoming these limitations explains why RANTES analogs show improved antiviral potencies compared with their natural counterparts and should help guide the development of new anti-HIV agents. Finally, these limitations could make it difficult to accomplish the blockade of R5 HIV-1 isolates by chemokines in vivo and contribute to their preferential transmission and propagation in the early stages of infection.

Materials and Methods

Radioactive chemokines were from PerkinElmer Life Sciences. CCL3 and CCL7 were purchased from R&D Systems. Other information regarding materials (chemokines, HIV-1 glycoproteins, viruses, and cells) and experimental procedures (radioligand binding, chemotaxis, receptor down-regulation, and infection inhibition assays) is provided in *SI Materials and Methods*.

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- Alkhatib G, et al. (1996) CC CKR5: A RANTES, MIP-1 α , MIP-1 β receptor as a fusion cofactor for macrophage-tropic HIV-1. *Science* 272(5270):1955–1958.
- Blanpain C, et al. (1999) CCR5 binds multiple CC-chemokines: MCP-3 acts as a natural antagonist. *Blood* 94(6):1899–1905.
- Mueller A, Mahmoud NG, Strange PG (2006) Diverse signalling by different chemokines through the chemokine receptor CCR5. *Biochem Pharmacol* 72(6):739–748.
- Sorce S, Myburgh R, Krause KH (2011) The chemokine receptor CCR5 in the central nervous system. *Prog Neurobiol* 93(2):297–311.
- Alkhatib G, Locati M, Kennedy PE, Murphy PM, Berger EA (1997) HIV-1 coreceptor activity of CCR5 and its inhibition by chemokines: Independence from G protein signaling and importance of coreceptor downmodulation. *Virology* 234(2):340–348.
- Cocchi F, et al. (1995) Identification of RANTES, MIP-1 α , and MIP-1 β as the major HIV-suppressive factors produced by CD8+ T cells. *Science* 270(5243):1811–1815.
- Oberlin E, et al. (1996) The CXCR chemokine SDF-1 is the ligand for LESTR/fusin and prevents infection by T-cell-line-adapted HIV-1. *Nature* 382(6594):833–835.
- Fantuzzi L, Belardelli F, Gessani S (2003) Monocyte/macrophage-derived CC chemokines and their modulation by HIV-1 and cytokines: A complex network of interactions influencing viral replication and AIDS pathogenesis. *J Leukoc Biol* 74(5):719–725.
- Rosenberg ES, et al. (1997) Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. *Science* 278(5342):1447–1450.
- Gonzalez E, et al. (2005) The influence of CCL3L1 gene-containing segmental duplications on HIV-1/AIDS susceptibility. *Science* 307(5714):1434–1440.
- Paxton WA, et al. (1998) Reduced HIV-1 infectability of CD4+ lymphocytes from exposed-uninfected individuals: Association with low expression of CCR5 and high production of beta-chemokines. *Virology* 244(1):66–73.
- Zagury D, et al. (1998) C-C chemokines, pivotal in protection against HIV type 1 infection. *Proc Natl Acad Sci USA* 95(7):3857–3861.
- Barretina J, et al. (2000) Evaluation of the putative role of C-C chemokines as protective factors of HIV-1 infection in seronegative hemophiliacs exposed to contaminated hemoderivatives. *Transfusion* 40(4):461–467.
- Nakajima T, Kaur G, Mehra N, Kimura A (2008) HIV-1/AIDS susceptibility and copy number variation in CCL3L1, a gene encoding a natural ligand for HIV-1 co-receptor CCR5. *Cytogenet Genome Res* 123(1–4):156–160.
- Gaertner H, et al. (2008) Highly potent HIV inhibition: Engineering a key anti-HIV structure from PSC-RANTES into MIP-1 β /CCL4. *Protein Eng Des Sel* 21(2):65–72.
- Oravecz T, et al. (1997) Regulation of anti-HIV-1 activity of RANTES by heparan sulfate proteoglycans. *J Immunol* 159(9):4587–4592.
- Schols D, et al. (1998) CD26-processed RANTES(3–68), but not intact RANTES, has potent anti-HIV-1 activity. *Antiviral Res* 39(3):175–187.
- Simmons G, et al. (1997) Potent inhibition of HIV-1 infectivity in macrophages and lymphocytes by a novel CCR5 antagonist. *Science* 276(5310):276–279.
- García-Pérez J, et al. (2011) New insights into the mechanisms whereby low molecular weight CCR5 ligands inhibit HIV-1 infection. *J Biol Chem* 286(7):4978–4990.
- Shin N, et al. (2011) Identification and characterization of INCB9471, an allosteric noncompetitive small-molecule antagonist of C-C chemokine receptor 5 with potent inhibitory activity against monocyte migration and HIV-1 infection. *J Pharmacol Exp Ther* 338(1):228–239.
- Doranz BJ, Baik SS, Doms RW (1999) Use of a gp120 binding assay to dissect the requirements and kinetics of human immunodeficiency virus fusion events. *J Virol* 73(12):10346–10358.
- Gaertner H, et al. (2008) Highly potent, fully recombinant anti-HIV chemokines: Re-engineering a low-cost microbicide. *Proc Natl Acad Sci USA* 105(46):17706–17711.
- Escola JM, Kuenzi G, Gaertner H, Foti M, Hartley O (2010) CC chemokine receptor 5 (CCR5) desensitization: Cycling receptors accumulate in the trans-Golgi network. *J Biol Chem* 285(53):41772–41780.
- García-Pérez J, et al. (2011) Allosteric model of maraviroc binding to CC chemokine receptor 5 (CCR5). *J Biol Chem* 286(38):33409–33421.
- Chabre M, Deterre P, Antonny B (2009) The apparent cooperativity of some GPCRs does not necessarily imply dimerization. *Trends Pharmacol Sci* 30(4):182–187.
- Springael JY, et al. (2006) Allosteric modulation of binding properties between units of chemokine receptor homo- and hetero-oligomers. *Mol Pharmacol* 69(5):1652–1661.
- Lagane B, et al. (2005) Mutation of the DRY motif reveals different structural requirements for the CC chemokine receptor 5-mediated signaling and receptor endocytosis. *Mol Pharmacol* 67(6):1966–1976.
- Delhay M, et al. (2007) Identification of a postendocytic sorting sequence in CCR5. *Mol Pharmacol* 72(6):1497–1507.
- Berro R, et al. (2011) Multiple CCR5 conformations on the cell surface are used differentially by human immunodeficiency viruses resistant or sensitive to CCR5 inhibitors. *J Virol* 85(16):8227–8240.
- Amara A, et al. (2003) G protein-dependent CCR5 signaling is not required for efficient infection of primary T lymphocytes and macrophages by R5 human immunodeficiency virus type 1 isolates. *J Virol* 77(4):2550–2558.
- Galés C, et al. (2006) Probing the activation-promoted structural rearrangements in preassembled receptor-G protein complexes. *Nat Struct Mol Biol* 13(9):778–786.
- Kinter A, Arthos J, Cicala C, Fauci AS (2000) Chemokines, cytokines and HIV: a complex network of interactions that influence HIV pathogenesis. *Immunol Rev* 177:88–98.
- Kobilka BK, Deupi X (2007) Conformational complexity of G-protein-coupled receptors. *Trends Pharmacol Sci* 28(8):397–406.
- Zidar DA, Violin JD, Whalen EJ, Lefkowitz RJ (2009) Selective engagement of G protein coupled receptor kinases (GRKs) encodes distinct functions of biased ligands. *Proc Natl Acad Sci USA* 106(24):9649–9654.
- Gurevich VV, Pals-Rylaarsdam R, Benovic JL, Hosey MM, Onorato JJ (1997) Agonist-receptor-arrestin, an alternative ternary complex with high agonist affinity. *J Biol Chem* 272(46):28849–28852.
- Choi WT, et al. (2012) CCR5 mutations distinguish N-terminal modifications of RANTES (CCL5) with agonist versus antagonist activity. *J Virol* 86(18):10218–10220.