Human (H-) CCR5 is the primary coreceptor for ENV-mediated fusion by R5 strains of human immunodeficiency virus type 1, whereas mouse (M-) CCR5 lacks this function. An array of 23 H/M-CCR5 hybrids containing increasing amounts of H-CCR5 extending from the N terminus generated by random chimeragenesis had a biphasic pattern of coreceptor activity with JRFL and 89.6, revealing active regions in the N-terminal extracellular domain (N-ED) and at the junction of cytoplasmic loop 3. The M-CCR5 mutant in which divergent residues were replaced with the corresponding H-CCR5 N-ED sequence (NyYTsE) gained coreceptor function in fusion but not infection experiments. A M-CCR5 double mutant with substitution of human sequences for divergent residues from the N-ED and cytoplasmic loop 3 had augmented coreceptor activity in fusion assays and gain of function in infection experiments. The SIV-251 ENV utilized H- and M-CCR5 and variants. Flow cytometric analysis of M-CCR5 mutants and bifunctional receptors composed of CD4 domains fused to M-CCR5 mutants excluded the possibility that differences in coreceptor activity resulted from variations in cell surface expression. These results demonstrate that the coreceptor activity of the H-CCR5 N-ED is modulated by intracellular residues, illustrating the complexity of CCR5 requirements for interaction with ENV.

Entry of target cells by HIV-1 is dependent upon the expression of CD4 (1–3) and a fusogenic chemokine receptor on the surface of the target cell (Ref. 4; reviewed in Ref. 5). Although multiple chemokine receptors and related heptahelical proteins have been demonstrated to function as coreceptors (reviewed in Ref. 6), current evidence implicates CCR5 (7) as the principal coreceptor for HIV-1 transmission (8–12). The existence of alleles that confer significant resistance to HIV-1 infection in humans (ccr5Δ32; Refs. 13 and 14) and primates (15, 16) supports the primary role of CCR5. The precedent of protective alleles and the lack of significant consequences of CCR5 deficiency in humans and knockout mice (17) point to its relevance as a target for preventing HIV-1 infection.

The gp120 subunit of the ENV glycoprotein of R5 strains of HIV-1 binds to CCR5 following conformational activation by CD4 (18, 19). Structure-function studies have not provided a clear picture of the domain(s) of CCR5 required for triggering fusion, and various regions have been implicated in this process. Multiple reports have demonstrated that the N-ED of CCR5 is sufficient but not necessary for coreceptor activity with R5 ENV (20–25). Acidic and aromatic residues in this region have been implicated in coreceptor activity in loss of function experiments (24, 26–31), and sulfation of active Tyr residues may also contribute to this function (32). The finding of differences in the binding of anti-CCR5 mAbs between receptors expressed in transfectants and primary cells implies that the conformation of this domain may have an impact on coreceptor function as well (25). Analysis of receptor chimeras and point mutants reveals that the body of the receptor may also play a role in the fusion process (20–25, 28, 30), but it is not clear whether there are multiple sites or one complex structure for interaction with ENV. Interpretation of these findings is complicated by the observations that the domains of CCR5 critical for utilization by different ENV vary (20, 22–25). In contrast, key regions for CXCR4 utilization by R5/X4 ENV have been localized to sequences in or adjacent to the second extracellular loop (ECL) (33, 34), and monoclonal antibodies (mAb) to ECL2 of CXCR4 (35) may block the entry of X4 strains. Similarly, mAbs that bind the corresponding region of CCR5 have been shown to inhibit infection by R5 strains, whereas those that bind the N-ED have lower efficacy (36–38). These findings raise questions regarding the identity of CCR5 residues that are critical for ENV binding and activation.

To elucidate the precise molecular structures involved in CCR5 coreceptor function that are lacking in M-CCR5, a random chimeragenesis strategy (39) using H-CCR5 and the inactive mouse homolg was employed to create an array of chimeras with junctions spanning the receptor. Analysis of these hybrids in fusion and infection experiments revealed active sequences in the N-ED and at the fifth transmembrane spanning domain (TM5/C3 junction of H-CCR5. Here, we demonstrate that (i) the motif NyYTsE in the N-ED is sufficient to confer coreceptor activity to M-CCR5 in fusion but not infection experiments; (ii) the substitution of TM5/C3 residues from...
Constructs were linearized and transformed into E. coli DH5α. Ampicillin-resistant colonies, the result of recircularization by homologous recombination between the two cDNAs, were analyzed with restriction endonuclease mapping and DNA sequencing. The molecular model demonstrates the topology of H-CCR5. The arrows represent the junctions of 23 chimeras, and the numeral indicates the residue number in H-CCR5. Divergent amino acids in the N-ED between residues 12 and 25 and at the TM5/C3 interface between residues 214 and 235 are highlighted in red.

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**Experimental Procedures**

**Random Chimeragenesis**—The random chimeragenesis approach (39) was adapted to generate hybrids with H-CCR5 at the N terminus and M-CCR5 at the C terminus (H/M-CCR5; see Fig. 1A). cDNAs encoding H-CCR5 and M-CCR5 were cloned in tandem (5′-H-CCR5-M-CCR5-3′) in pcDNA3, which encodes ampicillin resistance. Two unique sites (NotI and XhoI) were engineered between the cDNAs and used to linearize the final construct and generate incompatible ends. The linear plasmid was transformed into Escherichia coli; and rare ampicillin-resistant colonies were analyzed using a FACScan (Becton Dickinson). Clones containing the programmed mutation(s) were identified by sequencing. The sequence of the open reading frame was confirmed.

**Env-mediated Cell-Cell Fusion Assay**—Coreceptor activity of the CCR5 variants was analyzed as described previously (24, 40). Constructs encoding coreceptor candidates in the pcDNA3 vector, CD4, and luciferase, under the control of a T7 promoter, were cotransfected into Q76 cells using calcium phosphate precipitation. Effector cells were prepared by infection of Q76 cells with recombinant vaccinia viruses that program the expression of ENV on the cell surface and T7 RNA polymerase in the cytoplasm. After 16 h, the target cells were mixed with QT6 effector cells expressing HIV-1 ENV and T7 polymerase. Eight h following mixing of effector and target cells, the medium was aspirated, and detergent lysates were analyzed for luciferase activity using a LucLite Luciferase Reporter Gene Assay Kit (Packard, Downers Grove, IL) in a Top Count luminometer (Packard).

**Infection Assay**—Viral infection assays were performed as described previously (40) using pseudotyped recombinant viruses containing cloned env genes and a luciferase reporter gene (41, 42). Viral stocks were prepared by co-transfecting 293T cells with plasmids encoding the ADA or SIV 251 ENV and the NLA-3 luciferase virus backbone. U87-MG target cells were transfected with plasmids encoding CD4 and coreceptor candidates. After 24 h, the cells were infected with viral stocks in the presence of polybrene. Three days post-infection additional medium was added, and the cells were harvested by resuspension in 0.5% Triton X-100 in phosphate-buffered saline the following day. Aliquots were assayed for luciferase activity.

**Genetic Engineering of Bifunctional Receptors**—Composite receptors composed of the first two Ig-like domains of CD4 fused to CCR5 were prepared using a previously described strategy (43). A segment encoding the signal peptide and Ig domains 1 and 2 of human CD4 was prepared using a downstream primer containing a XbaI site to permit in frame fusion with CCR5. The level of cell surface expression of coreceptor candidates was determined by flow cytometry. QT6 cells transiently expressing CD4/M-CCR5 variants and Chinese hamster ovary cells transiently expressing M-CCR5 mutants were detached from the culture vessel using trypsin or citrate and incubated for 5 h at 37 °C to regenerate receptor proteins. The cells were then incubated with an anti-CD4-mAb phycoerythrin conjugate (22 °C) (MT310, DAKO, Denmark) or 227R (4 °C), respectively, and control myeloma proteins. Cells stained with 227R were subsequently incubated with a phycoerythrin-labeled secondary antibody. Following washing, the cells were analyzed using a FACScan (Becton Dickinson).

**Results**

**Random Chimeragenesis Produces an Array of H/M-CCR5 Chimeras with Junctions Spanning the Receptor**—The linearized construct containing H-CCR5 and M-CCR5 in tandem was transformed into E. coli, resulting in a low frequency of ampicillin-resistant colonies (Fig. 1A). An insert the length of a single CCR5 cDNA was detected in plasmids from 54 of 114 colonies following digestion with HindIII and ApoI. Mapping
and nucleotide sequencing of the 54 clones revealed 23 chimeras with different junctions between H- and M-CCR5, which spanned the receptor (Fig. 1B).

Multiple Regions of H-CCR5 Are Critical to HIV Coreceptor Function—Analysis of the 23 H/M-CCR5 chimeras in fusion assays revealed a biphasic distribution of activity with JRFL and 89.6 with increasing content of H-CCR5 (Fig. 2A). The chimera containing 12 residues from the N-ED of H-CCR5...
H12/M lacked coreceptor function with both JRFL and 89.6, but that containing the first 25 residues (H25/M) had approximately 50% of the activity of H-CCR5 with both ENV. This activity was consistent in hybrids with junctions between H-CCR5 residues 25–90. Hybrids in which cross-over occurred between residues 118 and 158 of H-CCR5 had coreceptor function similar to that of the human receptor with these ENV. Chimeras with junctions between amino acids 183 and 214 showed decreasing coreceptor activity, with the H214/M chimera having the nadir value (<15% of H-CCR5). The utilization of the remaining chimeras, beginning with H235/M, by JRFL and 89.6 returned to that of H-CCR5. Whereas hybrid receptors with less than 235 residues of H-CCR5 were not effectively utilized as a coreceptor by the RF ENV, H235/M and subsequent chimeras with increasing proportions of the human receptor had coreceptor activity similar to that of H-CCR5.

Analysis of the cell surface expression of the H/M-CCR5 chimeras in transient expression experiments revealed that H25/M through H158/M showed a similar cell surface expression level as wild type H-CCR5, and H183/M through H343/M had identical low level expression using 12D1, a mAb to the N-ED of H-CCR5. This was confirmed for H118/M, H214/M, H235/M, and H248/M using the 227R mAb which also binds to this domain. The H12/M hybrid lacked reactivity with 12D1 and 227R.

**NyYTse Is Responsible for the Coreceptor Activity of the H-CCR5 N Terminus**—The region of the H-CCR5 N-ED between residues 12 and 25 contains four amino acids that are different in M-CCR5 (Fig. 1B); residues 13–18 in H-CCR5 are N13NYTTse18, and the corresponding amino acids in M-CCR53 are D15YGMSE20. Replacement of the corresponding mouse sequences with NyYT or E individually in H12/M did not confer coreceptor activity with JRFL (Fig. 2B). A H12/M variant with substitution by NyYTse, which encodes a primary structure identical to H25/M, was utilized for ENV-mediated fusion with JRFL.

**Intracellular Residues of H-CCR5 Modulate Coreceptor Function of N-ED Motifs**—The H214/M chimera had minimal coreceptor function, and H235/M had activity similar to that of H-CCR5, although they differ only at two residues predicted to be at the TM5/C3 boundary. In H-CCR5 they are Lys-219 and Leu-222, which correspond to His-221 and Phe-224, respectively, in M-CCR5.6 The role of Lys-219 and Leu-222 in coreceptor activity was investigated by site-directed mutagenesis, initially in H214/M and H235/M (Fig. 2C). Conversion of His-221 to Lys in H214/M resulted in an increase in coreceptor activity, which was augmented by the additional replacement of Phe-224 with Leu. Conversely, substitution of the mouse counterparts for these two residues in H235/M resulted in a loss of coreceptor function that was greatest in the double mutant.

**Residues in the N-ED and TM5/C3 Region of H-CCR5 Cooperate to Confer Coreceptor Activity to M-CCR5 in the Cell-Cell Fusion Model**—The structure-function analysis on the H/M-CCR5 chimeras identified the involvement of residues in two regions of H-CCR5 in coreceptor function. The critical residues in the N-ED and TM5/C3 region of H-CCR5 were introduced into M-CCR5 to determine whether they were sufficient to confer coreceptor activity to the mouse receptor in ENV-mediated fusion assays. The M-CCR5 mutants that were analyzed are listed in Table I. Although the coreceptor activity of M(NyYT) did not differ significantly from that of M-CCR5 (Fig. 2D), M(NyYTse) demonstrated low but consistent levels of utilization by JRFL. The conversion of the TM5/C3 residues in M-CCR5 to the human counterparts, either singly, M(K), or in tandem, M(KL), resulted in a minimal increase over the values with M-CCR5. Double mutants of M-CCR5 containing the human N-ED and TM5/C3 residues showed a significant increase in activity that was greater than the sum of their individual effects.

**Introduction of H-CCR5 N-ED and Intracellular Residues into M-CCR5 Is Necessary to Confer Sensitivity to Infection**—These M-CCR5 variants were tested in infection assays using pseudotyped viruses containing a reporter gene and the R5 ADA ENV (Fig. 3A). The sensitivity of cells transiently expressing M(NyYT) to infection was similar to that of those expressing M-CCR5. Similarly, the sensitivity of target cells expressing M(NyYTse) did not appear to be significantly different from those expressing M-CCR5. M-CCR5 mutants in which TM5/C3 residues were replaced with the corresponding amino acids from H-CCR5 had no effect on utilization in the infection experiments. Target cells transiently expressing M(NyYTse+E) had a significant increase in viral entry over wild type M-CCR5 and the mutants containing H-CCR5 sequences from either the N-ED or TM5/C3 regions. There appeared to be some enhancement of activity by the additional substitution of the second H-CCR5 TM5/C3 residue at position 224 (M(NyYTse+E+KL)).

**M-CCR5 Is a Coreceptor for SIV**—Representative H/M-CCR5 chimeras were tested for utilization by the SIV 251 ENV in infection assays. H-CCR5, M-CCR5, and representative chimeras demonstrated consistent levels of coreceptor activity (data not shown). Analysis of wild type M-CCR5 and the mutants revealed that all were utilized as coreceptors by SIV 251 ENV at similar levels (Fig. 3B).

**Differences in Coreceptor Activities of M-CCR5 Mutants Do Not Result from Variation in Cell Surface Expression**—None of the available anti-H-CCR5 mAbs bound to M-CCR5, but mapping studies revealed that the epitope recognized by 227R, included residues between Asp-11 and Thr-16 of H-CCR5. Because Asp-11 and Ile-12 are conserved in the mouse homolog, M-CCR5 variants containing the NyYT mutation can be stained using this mAb. Therefore, a representative panel of M-CCR5 mutants containing this epitope were evaluated for levels of cell surface expression with 227R. Staining of cells transiently expressing M-CCR5 variants revealed no differences in the level of expression between the following mutations, NyYT, NyYTse, NyYTse+E+K, and NyYTse+E+KL (Fig. 4), which demonstrate a spectrum of coreceptor activities.

A novel strategy was developed to provide further evidence

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**Table I**

| Designation of M-CCR5 mutant | Mutated residues (M-CCR5→H-CCR5) | Cell surface expressiona | Coreceptor activityb |
|-------------------------------|----------------------------------|-------------------------|----------------------|
| M(NyYT)                      | D15YGM18                                 | ++                     | −                    |
| M(NyYTse)                    | D15YGM18                                 | ++                     | +                    |
| M(K)                         | H221→K                                   | NA3                    | +                    |
| M(KL)                        | H221→P224                                 | NA3                    | −                    |
| M(NyYT+K)                    | D15YGM18H221                                 | +                      | +++                  |
| M(NyYTse+K)                  | D15YGM18H221                                 | +                      | +++                  |
| M(NyYTse+KL)                 | D15YGM18H221                                 | ++                    | +++                  |

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3 A two codon insertion in the N-ED of M-CCR5 is responsible for the difference in residue number between the human and mouse receptors.

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a Hyphens used in sequences represent gaps.

b Transient expression in Chinese hamster ovary cells (Fig. 4).

c Cell-cell fusion assay (Fig. 2D).

d NA, not analyzed.
that differences in coreceptor activity of CCR5 variants did not result from variation in the level of cell surface expression. Bifunctional receptors were engineered in which the first two Ig loops of CD4 were fused to the N terminus of the M-CCR5 variants (Fig. 5A). Analysis of the CCR5 bifunctional receptors with a mAb to CD4 demonstrated that they were all expressed at similar levels on the cell surface (Fig. 5B). Analysis of the CD4/M-CCR5 hybrids in fusion assays using the JRFL ENV (Fig. 5C) revealed that the M(NyYTsE + KL) and M(NyYTsE + KL) bifunctional receptors had greater activity than those containing only the N-ED or C3 sequences. The relative values of wild type H- and M-CCR5 and the mutant forms of M-CCR5 were similar to those obtained in standard cell-cell fusion assays, in which CD4 and the coreceptor candidates were transfected as individual proteins encoded by separate constructs.

**DISCUSSION**

In the present study, analysis of an array of H/M-CCR5 chimeras with increasing amounts of the human receptor, which was generated by random chimeragenesis, implicated the involvement of sequences in two regions of the protein, at the N-ED and at the TM5/C3 junction. Conversion of six divergent residues identified by this analysis, four in the N terminus and two in the TM5/C3 region, to the corresponding amino acids in H-CCR5 was sufficient to confer coreceptor function to the mouse homolog in the fusion model and infection assays. The NyYTsE motif in the N-ED of H-CCR5 was sufficient to impart limited coreceptor function to M-CCR5 in fusion but not in infection experiments. Introduction of the two H-CCR5 residues in the TM5/C3 region had a modest effect on coreceptor activity of M-CCR5 alone, but their addition significantly augmented this function of M(NyYTsE).

Because substitution of the human TM5/C3 residues into M-CCR5 exerted a limited direct effect in either fusion or infection assays, it is proposed that they enhance coreceptor activity indirectly by influencing the conformation of the protein. The current finding of a biphasic pattern of coreceptor activity with JRFL and 89.6 ENV also favors the interpretation that cooperativity between the N-ED and other regions of
CCR5 is important for this function. The significance of the nadir in coreceptor activity that was observed in the H214/M chimera is supported by the inability of RF, an R5/X4 ENV that is sensitive to changes in coreceptor structure (34), to utilize any of the chimeras containing fewer than 235 residues from H-CCR5. Molecular modeling predicts that residues His-221 and Phe-224 are in TM5 of M-CCR5. When His-221 is converted to Lys in H-CCR5. Molecular modeling predicts that residues His-221 and Phe-224 are in TM5 of M-CCR5. When His-221 is converted to Lys in H-CCR5, the location of this residue is predicted to be at the boundary of TM5 and C3. Therefore, it is possible that conversion of His-221 to Lys and Cys-20 (31), Dragic et al. (26) and Farzan et al. (29) have shown that removal of acidic residues and aromatic residues, respectively, from the CCR5 N-ED results in loss of ENV binding. The conformation of this domain may also be dynamic, because Hill et al. (25) have noted differences in the binding of mAbs to the N-ED of CCR5 expressed in transfected and primary cells. Fusion of the intact ectodomain of CD4 to CCR5 has recently been reported to yield an active bifunctional coreceptor but not to compensate for loss of function resulting from truncation of the majority of the N-ED of CCR5 (25).

Multiple regions of H-CCR5 contribute to coreceptor function, either directly or indirectly. We used random chimeragenesis to pinpoint important regions and gain of function experiments to identify sequences in the N-ED and two residues in the TM5/C3 region that are sufficient to confer coreceptor activity to M-CCR5. The N-ED motif appears to contribute directly to this function, whereas the TM5/C3 residues enhance the activity while exerting a limited direct effect. The involvement of residues predicted to be in segments of the receptor that are not exposed on the extracellular surface in this function suggests that conformation is critical to coreceptor activity. These findings invite the premise that the presence of active residues is not sufficient to impart coreceptor activity unless they are in the proper context.

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REFERENCES

1. Dalgleish, A. G., Beverley, P. C. L., Clapham, P. R., Crawford, D. H., Greaves, M. F., and Weiss, R. A. (1984) Nature 312, 763–767.
2. Maddon, P. J., Dalgleish, A. G., McDoigal, J. S., Clapham, P. R., Weiss. R. A., and Axel, R. (1986) Cell 47, 333–348.
3. McDoigal, J. S., Kennedy, M. S., Sligh, J. M., Cort, S. P., Mawle, A., and Nicholson, J. K. A. (1986) Science 231, 382–385.
4. Feng, Y., Broder, C. C., Kennedy, P. E., and Berger, E. A. (1996) Science 272, 872–877.
5. Doms, R. W., and Peiper, S. C. (1997) Virology 235, 179–190.
6. Littman, D. R. (1998) Cell 93, 677–680.
7. Samson, M., Labbe, O., Mollereau, C., Vassart, G., and Parmentier, M. (1996) Biochemistry 35, 3362–3368.
8. Alkhatib, G., Combaicier, B., Broder, C. C., Feng, Y., Kennedy, P. E., Murphy, P. M., and Berger, E. A. (1998) Science 272, 1955–1958.
9. Deng, H., Liu, R., Eillen, M., Wei, S., Unutmaz, D., Burkhardt, M., Marzio, P. D., Mormon, S., Sutton, R. E., Hill, C. M., Davis, C. B., Peiper, S. C., Schall, T. J., Littman, D. R., and Landau, N. R. (1996) Nature 381, 661–666.
10. Dragic, T., Litvina, V., Allaway, G. P., Martin, S. R., Huang, Y., Nagashima, K. A., Cuiyanan, C., Maddon, P. J., Koup, R. A., Moore, J. P., and Paxton, W. A. (1996) Nature 381, 667–673.
11. Choe, H., Farzan, M., Sun, Y., Sullivan, N., Rollins, B., Panath, P. D., Wu, L., Mackay, C. R., LaRossa, G., Newman, W., Gerard, N., Gerard, C., and Sodroski, J. (1996) Cell 85, 1135–1148.
12. Doranz, B. J., Rucker, J. Y., Smyth, R. J., Samson, M., Peiper, S. C., Parmentier, M., Collman, R. G., and Doms, R. W. (1998) Cell 85, 1149–1158.
13. Samson, M., Libert, F., Doranz, B. J., Rucker, J., Liemard, C., Faber, C. M., Saragosti, S., Lapoumeroulie, C., Cogniaux, J., Forceille, C., Muyldermans, G., Verhofstede, C., Burtonboy, G., Georges, M., Imai, T., Rana, S., Yi, Y., Smyth, R. J., Collman, R. G., Doms, R. W., Vassart, G., and Parmentier, M. (1996) Nature 382, 722–725.
14. Liu, R., Paxton, W. A., Choe, S., Ceradini, D., Martin, S. R., Horuk, R., MacDonald, M. E., Stuhlmann, H., Koup, R. A., and Landau, N. R. (1996) Nature 382, 367–377.
15. Palacios, E., Digilio, M., McClure, H. M., Chen, Z., Marx, P. A., Goldsmith, M. A., and Grant, R. M. (1998) Current Biol. 8, 943–946.
16. Chen, Z., Kwon, D., Jin, Z., Monard, S., Telfer, P., Jones, M. S., Lu, C. Y., Aguilar, R. F., Ho, D. D., and Marx, P. A. (1998) J. Exp. Med. 188, 2057–2065.
17. Zhang, Y., Kurihara, T., Byrcek, R. P., Yang, Y., Ryan, C., Loy, J., Warr, G., and Bravo, R. (1998) J. Immunol. 160, 4918–4925.
18. Wu, L., Gerard, N. P., Wyatt, R., Choe, H., Parolin, C., Ruffing, N., Bersett, A., Cardoso, A. A., Desjardin, E., Newman, W., Gerard, C., and Sodroski, J. (1996) Nature 384, 179–183.
19. Trkola, A., Draic, T., Arthos, J., Pinley, J. M., Olson, W. C., Allaway, G. P., Cheng-Mayer, C., Robinson, J., Maddon, P. J., and Moore, J. P. (1996) Nature 384, 184–187.
20. Rucker, J., Samson, M., Doranz, B. J., Libert, F., Berson, J., Yi, Y., Collman, R. G., Vassart, G., Broder, C. C., Doms, R. W., and Parmentier, M. (1996) *Cell* **87,** 437–446
21. Atchison, R. E., Gosling, J., Montecelaro, F. S., Digilio, L., Charo, I. F., and Goldsmith, M. A. (1996) *Science* **274,** 1924–1926
22. Bieniasz, P. D., Fridei, R. A., Aramori, I., Ferguson, S. S., Caron, M. G., and Cullen, B. R. (1997) *EMBO J.* **16,** 2599–2609
23. Picard, L., Simmons, G., Power, C. A., Meyer, A., Weiss, R. A., and Clapham, P. R. (1997) *J. Virol.* **71,** 5003–5011
24. Doranz, B. J., Lu, Z.-H., Rucker, J., Zhang, T.-Y., Sharron, M., Cen, Y.-H., Wang, Z.-X., Guo, H.-H., Du, J.-G., Accavitti, M. A., Doms, R. W., and Peiper, S. C. (1997) *J. Virol.* **71,** 6305–6314
25. Hill, C. M., Kwon, D., Jones, M., Davis, C. B., Marmon, S., Daugherty, B. L., DeMartino, J. A., Springer, M. S., Unutmaz, D., and Littman, D. R. (1998) *Virology* **248,** 357–371
26. Dragic, T., Trkola, A., Lin, S. W., Nagashima, K. A., Kajumo, F., Zhao, L., Olson, W. C., Wu, L., Mackay, C. R., Allen, W. C., Wu, L., Mandon, J. P., and Dragic, T. (1998) *J. Virol.* **72,** 279–285
27. Martin, K. A., Wyatt, R., Farzan, M., Choe, H., Marcon, L., Desjardins, E., Robinson, J., Sodroski, J., Gerard, C., and Gerard, N. P. (1997) *Science* **278,** 1470–1473
28. Kuhmann, S. E., Platt, E. J., Kozak, S. L., and Kabat, D. (1997) *J. Virol.* **71,** 8642–8656
29. Farzan, M., Choe, H., Vaca, L., Martin, K., Sun, Y., Desjardins, E., Ruffing, N., Wu, L., Wyatt, R., Gerard, N., Gerarbl, C., and Sodroski, J. (1998) *J. Virol.* **72,** 1160–1164
30. Ross, T. M., Bieniasz, P. D., and Cullen, B. R. (1998) *J. Virol.* **72,** 1989–1992
31. Rabut, G. E., Konner, J. A., Kajumo, F., Moore, J. P., and Dragic, T. (1998) *J. Virol.* **72,** 3464–3468
32. Farzan, M., Mirzabekov, T., Kolchinsky, P., Wyatt, R., Cayabyab, M., Gerard, N. P., Gerard, C., Sodroski, J., and Choe, H. (1999) *Cell* **96,** 667–676
33. Brelot, A., Heveker, N., Pleskoff, O., Sol, N., and Alizon, M. (1999) *J. Virol.* **71,** 4744–4751
34. Lu, Z.-H., Berson, J., Cen, Y.-H., Turner, J. D., Zhang, T.-Y., Sharron, M., Jenks, M. H., Wang, Z.-X., Kin, J., Rucker, J., Hoxie, J. A., Peiper, S. C., and Doms, R. W. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 6428–6431
35. Endres, M. J., Clapham, P. R., March, M., Abuja, M., Turner, J. D., McKnight, A., Thomas, J. F., Stoebenau-Haggarty, B., Choe, S., Vance, P. J., Wells, T. N., Power, C. A., Sutterwala, S. S., Doms, R. W., Landau, N. R., and Hoxie, J. A. (1996) *Cell* **87,** 745–756
36. Wu, L., LaRosa, B., Kassam, N., Gordon, C. J., Heath, H., Ruffing, N., Chen, H., Humblies, J., Samson, M., Parmentier, M., Moore, J. P., and Mackay, C. R. (1997) *J. Exp. Med.* **186,** 1373–1381
37. Osen, W. C., Rabut, G. E. E., Nagashima, K. A., Tran, D. N. H., Anselma, D. J., Monard, S. P., Segal, J. P., Thompson, D. A. D., Kajumo, F., Gao, Y., Moore, J. P., Mandon, J. P., and Dragic, T. (1999) *J. Virol.* **73,** 4145–4155
38. Lee, B., Sharron, M., Blanpain, C., Doranz, B. J., Vakili, J., Setoh, P., Berg, E., Liu, G., Guy, H. R., Durell, S. R., Parmentier, M., Chang, C. N., Price, K., Tsang, M., and Doms, R. W. (1999) *J. Biol. Chem.* **274,** 9617–9626
39. Kim, J. Y., and Devreotes, P. N. (1994) *J. Biol. Chem.* **269,** 13724–13727
40. Wang, Z., Berson, J. F., Zhang, T., Cen, Y. H., Sun, Y., Sharron, M., Lu, Z., and Peiper, S. C. (1998) *J. Biol. Chem.* **273,** 15007–15015
41. Chen, B. K., Saksela, K., Andino, R., and Baltimore, D. (1994) *J. Virol.* **68,** 654–660
42. Connor, R. I., Chen, B. K., Choe, S., and Landau, N. R. (1995) *Virology* **206,** 935–944
43. Peiper, S. C., Lu, Z. H., Zhang, T. Y., and Wang, Z. X. (1997) *Methods Enzymol.* **288,** 56–70
44. Alkhatib, G., Berger, E. A., Murphy, P. M., and Pease, J. E. (1997) *J. Biol. Chem.* **272,** 20342–20346
45. Siciliano, S. J., Kuhmann, S. E., Weng, Y., Madani, N., Springer, M. S., Lineberger, J. E., Danzeisen, R., Miller, M. D., Kavanaugh, M. P., DeMartino, J. A., and Kabat, D. (1999) *J. Biol. Chem.* **274,** 19055–19013
46. Cocchi, F., DeVico, A. L., Garzino-Demo, A., Arya, S. K., Gallo, R. C., and Lusso, P. (1995) *Science* **270,** 1811–1815
47. Samson, M., LaRosa, G., Libert, F., Paindavoine, P., Dethieux, M., Vassart, G., and Parmentier, M. (1997) *J. Biol. Chem.* **272,** 24814–24811