Morphine Induces Gene Expression of CCR5 in Human CEM x174 Lymphocytes*

Received for publication, February 14, 2000, and in revised form, July 6, 2000
Published, JBC Papers in Press, July 7, 2000, DOI 10.1074/jbc.M001269200

Tomoko Miyagishita, Linda F. Chuang†t, Roy H. Doíb, Maria P. Carloss, José V. Torres¶, and Ronald Y. Chuang¶†

From the §Department of Medical Pharmacology and Toxicology, the ¶Section of Molecular and Cellular Biology, and the ¶¶Department of Medical Microbiology and Immunology, University of California, Davis, California 95616

All HIV-1 strains studied to date use CCR5, CXCR4, or both receptors to enter cells. Simian immunodeficiency virus (SIV) infection of non-human primates has served as a useful model for understanding AIDS pathogenesis in humans. Research on several genetically divergent SIV isolates has revealed that SIV uses CCR5, and not CXCR4, for entry. CEM x174, a human lymphoid cell line, has been routinely used to cultivate and maintain various SIV strains. However, questions have arisen about how CEM x174, which reportedly was unable to express detectable amounts of CCR5 transcripts, efficiently supports the growth of SIV. In searching for an answer, we resorted to a sensitive competitive reverse transcriptase-polymerase chain reaction procedure in an attempt to detect as well as quantify the amount of CCR5 expression. Here we present our findings, which indicate that CEM x174 indeed expresses CCR5 and that the amount of CCR5 is increased in cells pretreated with morphine. These results correlate well with our previous observations that morphine treatment causes CEM x174 cells to be more susceptible to SIV infection. Similar morphine effect was not observed on CEM x174 cells infected with simian retroviruses, which do not depend on CCR5 for entry. These findings suggest a plausible mechanism whereby opiate drug users render themselves more susceptible to HIV infection, thereby explaining the vast prevalence of HIV infection among endemic drug use populations.

Using this animal system we have previously found that monkeys dependent upon opioid administration and subsequently infected with SIV will have a faster rate of viral replication in comparison with drug-naive, virus-infected monkeys (1). CEM x174 is a human lymphoid cell line (2) commonly used to co-cultivate SIV isolated from infected monkeys. The syncytium formation of SIV-infected CEM x174 cells was found to be significantly enhanced in the presence of morphine sulfate, with a concomitant increase in the activity of cellular reverse transcriptase and the expression of SIV p27 core antigen (3). Chemokines are small 6–10-kDa polypeptides that are synthesized in response to infection and that function mainly as chemoattracants for phagocytic cells, recruiting monocytes and neutrophils from the vascular system to sites of infection (4). Further studies on the effects of opioids on immune cells revealed that the addition of opioids to the chemokines interleukin-8, RANTES, or MIP-1β reduces the ability of monkey neutrophils and monocytes to migrate toward these chemokines (5). The reaction occurs instantaneously, without the inclusion of a cell opioid preincubation step (6). This suggests that the presence of opioids during SIV/HIV infection immediately disrupts the body’s first line of defense against harmful external pathogens by disrupting the chemotaxis ability of immune cells toward harmful pathogens. Such observations may provide an indirect mechanism to explain why primates or humans dependent upon intravenous drug administration have a higher probability of developing into a full-blown disease than non-drug users when exposed to a viral challenge (1, 7).

Chemokines act on receptors that belong to the G protein-coupled receptor family whose members contain seven transmembrane domains (4). Activation, desensitization, and resensitization of receptor proteins are thought to involve the activity of receptor-specific G protein-coupled receptor kinases and arrestins (8). Chemokines such as RANTES, MIP-1α, and MIP-1β have been implicated in the pathogenesis of HIV disease; they may be selectively secreted from infected individuals and induce inhibition of different strains of HIV-1, HIV-2, and SIV (9). It was later found that chemokine receptors (especially CCR5 and CXCR4) are coreceptors for HIV or SIV entry (10–13). However, regions in CCR5 or CXCR4 required for ligand (chemokine) binding and coreceptor activity are not identical and only partially overlap (10). It was further established that in addition to blocking viral entry through steric hindrance, cognate ligand interaction with chemokine receptors has been shown to result in receptor down-regulation for CCR5 and CXCR4 (14).

SIVmac239 replicates most efficiently in the human transformed lymphoid cell line CEM x174 (15). In fact, this cell line is routinely used for preparing high titered stocks of this virus.

Opiate users constitute a large portion of the patient population contracting AIDS. The feasibility and success of human studies have always been hampered by the complexity of an individual’s history of intravenous drug use. Thus, rhesus monkey treated with opioids and infected with simian immunodeficiency virus (SIV) provides an excellent animal model for studying drug abuse and AIDS under a controlled manner (1).

* This work was supported by Grants DA 05901 and DA 10433 from the NIDA, National Institutes of Health and by American Cancer Society Grant RPG-99-338-01-CIM. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Dept. of Medical Pharmacology and Toxicology, School of Medicine, University of California, Davis, CA 95616. Tel.: 530-752-7713; Fax: 530-752-7710; E-mail: rychuang@ucdavis.edu.

‡ The abbreviations used are: SIV, simian immunodeficiency virus; RANTES, regulated upon activation, normal T expressed and secreted; MIP-1α, macrophage inflammatory protein 1α; HIV, human immunodeficiency virus; PCR, polymerase chain reaction; RT, reverse transcriptase; bp, base pair(s); FACS, fluorescence-activated cell sorter; SRV, simian type D retrovirus.
It has been reported that SIVmac239 may use CCR5, BOB (GPR15), or BONZO (STRL33) as a coreceptor for entry into various cell types, including 293T.CD4 (16), 3T3.CD4 (16), and C2Th.CD4 (17). In all of these studies, CCR5 was a preferred coreceptor over BOB or BONZO; cells with CCR5 produced a greater amount of virus than cells with BOB or BONZO. However, CCR5 has not been identified in CEM x174 (11–13). In the course of a study to quantify virus production in CEM x174 cells, we serendipitously found that addition of morphine sulfate to CEM x174 cell cultures significantly increases the replication of SIVmac239 (3). The present study determines which coreceptor is responsible for the observed morphine effect on CEM x174 cells.

**EXPERIMENTAL PROCEDURES**

**Cell Line**—The CEM x174 cell line, a hybrid of the human B cell line 721.174 and human T cell line CEM (2), was maintained in RPMI 1640 medium supplemented with 10% heat-inactivated bovine calf serum, 2 mM t-glutamine, 25 mM HEPES, and penicillin and streptomycin.

**GHOST Parental Cell Line and GHOST Hi-5** (a GHOST cell transfectant with high CCR5 expression) were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% heat-inactivated fetal bovine serum, 500 μg/ml G418, 100 μg/ml hygromycin, and penicillin and streptomycin. For GHOST Hi-5, the medium also contained 1 μg/ml puromycin. All cells were grown at 37 °C in a CO2 incubator.

**Morphine or Naloxone Treatment**—CEM x174 cells in culture were diluted 1:3 with fresh medium every 3–4 days. At the time of dilution, morphine sulfate, naloxone HCl, or H2O (as controls) was added, and incubation was continued for the indicated time. When naloxone was used together with morphine in an experiment, cells were first treated with naloxone HCl for 30 min at 37 °C followed by morphine treatment.

**Construction of Competitor Molecules**—A PCR fragment of CCR5 (1114 bp), BONZO (797 bp), or BOB (563 bp) was cloned into pCRII (Invitrogen), yielding plasmid CCR5/pCRII, BOB/pCRII, or BONZO/pCRII, which was then sequenced to prove identity (Fig. 1A). To construct competitor molecules, small internal deletions were introduced into the inserts. CCR5/pCRII was deleted 96 bp from bases 298 to 393 (inclusive), and BONZO/pCRII was deleted 125 bp from bases 145 to 269 (inclusive) using the Exo mung bean deletion kit (STRATEGENE). BOB/pCRII was deleted 63 bp from bases 404 to 466 (inclusive) after double digestion with HindIII and NdeI (Fig. 1A).

**Competitive RT-PCR**—Total RNA was isolated from opioid-treated or control CEM x174 cells using TRIZOL Reagent (Life Technologies, Inc.) In both the reverse transcription and the PCR steps, all the reaction reagents were prepared as master mixes and then aliquoted to each tube to provide uniform reaction conditions and minimize intertube variations. To confirm the detection range of competitive RT-PCR, the relationship between the amount of cDNA generated and the initial concentrations of total RNA used were determined: reverse transcription was performed on 0.5, 1, and 2 μg of total RNA, and the reaction was for 30 min at 42 °C using 200 units of Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc.). The reaction mixture also contained 250 mM Tris-HCl (pH 8.3), 375 mM KCl, 15 mM MgCl2, 1 mM dithiothreitol, 10 μg of RNAse inhibitor (Promega), 1 mM of each dNTP, and 7.5 mM random hexamers in a final volume of 20 μl. For PCR amplification, 20 μl of PCR Master Mix containing 0.5 μM of each primer, 0.5 unit of Taq polymerase (Promega), 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, and 1.5 mM MgCl2 were dispensed into tubes. To the PCR Master Mix 2 μl of the reverse transcription reaction product and various concentrations of competitor molecules were added. The PCR was performed in a MJ Research Thermocycler PTC-200, using the following conditions: after an initial 5-min incubation at 95 °C, PCR amplification was carried out for 20 cycles (BOB), 27 cycles (BONZO), or 31 cycles (CCR5) at 95 °C for 40 s, 60 °C for 40 s, and 72 °C for 1 min. The last primer extension step was conducted for 10 min. The primers for BOB were 5'-CATCTGCTTCTTGGTGATG-3' and 5'-ATGTGCACAACTCTGACTG-3', corresponding to bases 44–63 and 1041–1065 of the published STRL33 (or BONZO) sequence (16). The primers for CCR5 were 5'-GGTGGCAACAAGTGAGTTTATG-3' and 5'-ATGTGCACAACTCTGACTG-3', corresponding to bases 269–289 and 1041–1065 of the published STRL33 (or BONZO) sequence (16). The primers for CCR5 were 5'-GGTGGCAACAAGTGAGTTTATG-3' and 5'-ATGTGCACAACTCTGACTG-3', corresponding to bases 44–63 and 1139–1157 of the published CCR5 sequence (15). PCR products separated on 2% agarose gel were visualized by ethidium bromide staining and photographed under UV illumination.

**Figure 1. Diagram illustration of competitive RT-PCR.** a, construction of competitor molecules. A BamHI-EcoRI fragment of CCR5 (1114 bp), EcoRI-EcoRI fragment of BONZO (797 bp), or EcoRI-EcoRI fragment of BOB (563 bp) was deleted 63 bp from bases 298 to 393 (inclusive), and BONZO/pCRII was deleted 125 bp from bases 145 to 269 (inclusive) using the Exo mung bean deletion kit (STRATEGENE). BOB/pCRII was deleted 63 bp from bases 404 to 466 (inclusive) after double digestion with HindIII and NdeI (Fig. 1A).

**Competitive RT-PCR**—Total RNA was isolated from opioid-treated or control CEM x174 cells using TRIZOL Reagent (Life Technologies, Inc.). In both the reverse transcription and the PCR steps, all the reaction reagents were prepared as master mixes and then aliquoted to each tube to provide uniform reaction conditions and minimize intertube variations. To confirm the detection range of competitive RT-PCR, the relationship between the amount of cDNA generated and the initial concentrations of total RNA used were determined: reverse transcription was performed on 0.5, 1, and 2 μg of total RNA, and the reaction was for 30 min at 42 °C using 200 units of Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc.). The reaction mixture also contained 250 mM Tris-HCl (pH 8.3), 375 mM KCl, 15 mM MgCl2, 1 mM dithiothreitol, 10 μg of RNAse inhibitor (Promega), 1 mM of each dNTP, and 7.5 mM random hexamers in a final volume of 20 μl. For PCR amplification, 20 μl of PCR Master Mix containing 0.5 μM of each primer, 0.5 unit of Taq polymerase (Promega), 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, and 1.5 mM MgCl2 were dispensed into tubes. To the PCR Master Mix 2 μl of the reverse transcription reaction product and various concentrations of competitor molecules were added. The PCR was performed in a MJ Research Thermocycler PTC-200, using the following conditions: after an initial 5-min incubation at 95 °C, PCR amplification was carried out for 20 cycles (BOB), 27 cycles (BONZO), or 31 cycles (CCR5) at 95 °C for 40 s, 60 °C for 40 s, and 72 °C for 1 min. The last primer extension step was conducted for 10 min. The primers for BOB were 5'-CATCTGCTTCTTGGTGATG-3' and 5'-ATGTGCACAACTCTGACTG-3', corresponding to bases 66–85 and 667–682 of the published BOB sequence (16). The primers for BONZO were 5'-AGCGGCTGAGTGTT-3' and 5'-CAAAGCCCTAATGGAAGGACGTT-3', corresponding to bases 269–289 and 1041–1065 of the published STRL33 (or BONZO) sequence (16). The primers for CCR5 were 5'-GGTGGCAACAAGTGAGTTTATG-3' and 5'-ATGTGCACAACTCTGACTG-3', corresponding to bases 44–63 and 1139–1157 of the published CCR5 sequence (15). PCR products separated on 2% agarose gel were visualized by ethidium bromide staining and photographed under UV illumination.

**Flow Cytometry and Western Blot Analysis**—The expression of CCR5 on CEM x174 cells treated with or without morphine was evaluated with the public domain NIH Image program (developed at the U.S. National Institutes of Health). For analyzing the results, the log of the ratio of amplified target to competitor products was graphed as a function of the known amount of competitor added to the PCR reaction (Fig. 1c). CA-Cricket Graph III (Computer Associates) was used for the regression analysis and calculation of x intercepts at which the log ratios equaled zero. c is a representative figure of the experiment, drawn from data obtained from a RT-PCR analysis of BONZO cDNA synthesized from 0.5 μg ([]), 1.0 μg (▲), or 2.0 μg (●) RNA.
Infection of CEM x174 cells with SIVmac239 or SRV—Methods for the experimental infection of CEM x174 cells with SIVmac239 or SRV and the subsequent assay of the reverse transcriptase activity of the infected cultures were detailed elsewhere (3, 19). SIVmac239 was propagated and titered in CEM x174 cells (3), and titers for SRV serotypes 1–3 were determined by the Raji cell infectivity assay as described previously (19). The Raji cell line was obtained from American Type Culture Collection.

RESULTS

Fig. 2. CCR5 on CEM x174 as analyzed by RT-PCR (a) or flow cytometry (b). a, CCR5 mRNA from CEM x174 (lanes 2 and 3), GHOST Hi-5 (lanes 4 and 5), or GHOST Parental cells (lanes 6 and 7) was determined by RT-PCR. Lanes 3, 5, and 7, RT was omitted in the reactions. Lane 1, 100-bp DNA ladder. b, cells were stained with either fluorescein isothiocyanate-conjugated mouse anti-human CCR5 monoclonal antibody (upper panel) or carboxyfluorescein-conjugated mouse IgG1 isotype control (lower panel) and subjected to FACS analysis. FL1-H, green fluorescence; FL2-H, red fluorescence (auto fluorescence measured in “red” channel). The data were reproducible in three independent experiments.

Fig. 3. Effect of morphine treatment on SIV coreceptor expression. The amount of CCR5 (a), BOB (b), or BONZO (c) expressed in CEM x174 cells was determined by the competitive RT-PCR after treatment of the cells with 10 μM (solid column) or 10 nM (column with wavy lines) morphine sulfate for the indicated time. Control (open column), H2O-treated cells. The data were reproducible in at least four independent experiments.

Infection of CEM x174 cells with SIVmac239 or SRV—Methods for the experimental infection of CEM x174 cells with SIVmac239 or SRV and the subsequent assay of the reverse transcriptase activity of the infected cultures were detailed elsewhere (3, 19). SIVmac239 was propagated and titered in CEM x174 cells (3), and titers for SRV serotypes 1–3 were determined by the Raji cell infectivity assay as described previously (19). The Raji cell line was obtained from American Type Culture Collection.

RESULTS

Fig. 2a is a representative figure of the results from an RT-PCR analysis of CEM x174 CCR5. Using primer sequences corresponding to bases 44–63 and 1139–1157 of the published human CCR5 sequence (13), CEM x174 cells synthesized a 1114-bp segment (lane 2) that was identical in size to the segment synthesized by GHOST Hi-5 (a GHOST cell transfec-tant encoding CCR5, lane 4) and that was not found in GHOST Parental cells (lane 6). Using primer pairs corresponding to bases 66–85 and 607–628 of the published BOB sequence (16) and bases 269–289 and 1041–1065 of the published BONZO sequence (16), we also found that CEM x174 cells synthesized cDNA segments of 563 and 797 bp, respectively, in length (data not shown). The cDNA synthesized in each case was sequenced to prove identity. It was found that CEM x174 cells express receptors with gene sequences identical to the published human CCR5, BOB, and BONZO sequences (13, 16) (data not shown).

To further establish that the CCR5 transcripts detected in CEM x174 cells are translated into receptor proteins, we performed both flow cytometry (Fig. 2b) and Western blot analysis (see Fig. 4b, inset) using fluorescein-conjugated mouse monoclonal anti-human CCR5 (for flow cytometry) and rabbit polyclonal anti-human CCR5 (for Western blot). Both procedures confirmed the presence of CCR5 molecules on CEM x174 cells (Figs. 2b and 4b). Therefore, we demonstrated that in addition to BOB and BONZO, CEM x174 cells indeed express significant amounts of CCR5, the major coreceptor for SIVmac entry.

To determine coreceptor densities on CEM x174 cells, plasmids containing segments of CCR5, BOB, and BONZO, and plasmids containing CCR5, BOB, and BONZO segments with 96-bp (CCR5), 63-bp (BOB), and 125-bp (BONZO) deletions were constructed (see “Experimental Procedures” and Fig. 1a). Plasmids with deleted segments were used in quantitative RT-PCR as external controls for quantifying the expression of
chemokine receptor genes in CEM x174. To select a cycle number to achieve an exponential amplification phase, the densities of amplified fragments from different cyclical amplifications were measured after gel electrophoresis and ethidium bromide staining. Cycle numbers 31, 20, and 27 were selected for experiments on CCR5, BOB, and BONZO, respectively (data not shown).

To confirm the detection range of competitive RT-PCR, the relationship between the amount of cDNA generated and the initial concentrations of total RNA used were determined. For each RT reaction of 0.5, 1.0, or 2.0 μg of total RNA, five different concentrations of competitors were used for PCR amplification (Fig. 1b). The results showed that the amount of cDNA generated was in proportion to the amount of initial RNA used (Fig. 1c).

To investigate the effect of morphine treatment on the gene expression of CCR5, BOB, and BONZO, the amount of cDNA amplified by competitive RT-PCR from cells treated with morphine sulfate was compared with that of untreated cells. Morphine treatment, if used, was either 10 μM or 10 nM; these are physiological morphine concentrations in morphine-dependent animals (20). Samples were taken 0, 12, 24, and 36 h post-morphine treatment for analysis. It was found that after the dilution of the cultures at time 0 (see “Experimental Procedures”), the cells synthesized an increasing amount of CCR5 (Fig. 3a) or BOB (Fig. 3b), with the amount reaching a plateau 12–24 h post-dilution. However, it was also found that in comparison with the control cells at each time point, 10 μM morphine treatment increased CCR5 expression by 207% by 12 h post-treatment, whereas 10 nM morphine treatment induced a 240% increase of CCR5 by 24 h post-treatment (Fig. 3a). On the contrary, morphine treatment did not affect the expression of BOB (Fig. 3b) or BONZO (Fig. 3c) in CEM x174 cells. Further experiments showed that the morphine-induced increase in CCR5 expression correlated with the amount of CCR5 proteins on the cell surface, as determined by
flow cytometry (Fig. 4a) as well as Western blot analysis (Fig. 4b) and that the effect was opioid receptor-mediated, because it could be completely ablated when cells were pretreated with naloxone, a μ opioid receptor antagonist (Fig. 5).

To correlate the observed morphine effect on CCR5 up-regulation with viral infectivity, CEM x174 cells with or without morphine treatment were assayed for viral susceptibility after infection with SIVmac239 or with SRV in parallel experiments. SRV infect B cells, T cells (CD4+ and CD8+), macrophages, and epithelial cells. Like SIVmac239, SRV cause an acquired immunodeficiency syndrome in monkeys (19). The receptor for SRV is not CCR5 but a neutral amino acid transporter that has been identified and cloned (19). The downstream molecular mechanisms induced by receptor activation through which morphine affects CCR5 expression awaits further investigation. Morphine, which inhibits chemokine-induced chemotaxis, nevertheless does not perturb chemokine binding to CCR5 (26). It is therefore attractive to propose that morphine, by binding to its own cell surface receptor, initiates a series of G protein-coupled signal transduction pathways (18) that thereby hetero-sensitize (or up-regulate) CCR5.

The current study shows that activation of opioid receptors of human lymphocytes, probably of the μ subtype, by morphine up-regulates the expression of the chemokine receptor CCR5. The downstream molecular mechanisms induced by receptor activation through which morphine affects CCR5 expression awaits further investigation. Morphine, which inhibits chemokine-induced chemotaxis, nevertheless does not perturb chemokine binding to CCR5 (26). It is therefore attractive to propose that morphine, by binding to its own cell surface receptor, initiates a series of G protein-coupled signal transduction pathways (18) that thereby hetero-sensitize (or up-regulate) CCR5.

DISCUSSION

Immune cells have been shown to express brain-like opioid receptors (22–24). Similar to chemokine receptors, opioid receptors are also G protein coupled, seven-transmembrane domain receptors (25). Human CEM x174 lymphocytes possess all three subtypes of opioid receptors, μ, κ, and δ (22–24). The current study shows that activation of opioid receptors of human lymphocytes, probably of the μ subtype, by morphine up-regulates the expression of the chemokine receptor CCR5.

The downstream molecular mechanisms induced by receptor activation through which morphine affects CCR5 expression awaits further investigation. Morphine, which inhibits chemokine-induced chemotaxis, nevertheless does not perturb chemokine binding to CCR5 (26). It is therefore attractive to propose that morphine, by binding to its own cell surface receptor, initiates a series of G protein-coupled signal transduction pathways (18) that thereby hetero-sensitize (or up-regulate) CCR5. Many facts support this proposal. For instance, morphine has been shown to modulate the expression of other cellular proteins that may induce CCR5 expression. Specifically, morphine reportedly modulates the cellular activation of NFκB and TNF-α in macrophages (27) and interleukin-2 in lymphocytes (28); activation of NFκB, TNF-α, and interleukin-2 has been found to up-regulate CCR5 expression (10, 29). Alternatively, morphine may up-regulate CCR5 by inhibiting chemokine synthesis and thus chemotaxis. Under certain conditions, opiates such as morphine as well as opioid peptides may down-regulate
cytokine synthesis and release (30, 31). Chemokines (chemo-
tactic cytokines) such as RANTES, MIP-1α, and MIP-1β may
induce receptor internalization and decrease cell surface ex-
pression of CCR5 (14, 32), which ultimately contributes to
anti-HIV-1 activity (9, 14). Thus, one yet-to-be-proved mecha-
ism of morphine-mediated up-regulation of CCR5 is through
the down-regulation of chemokine synthesis.

SRV are endemic in wild macaques of India and Indonesia
and in captive macaques important for medical research (33).
Five distinct neutralization serotypes (SRV1–SRV5) have
been described, of which three have been molecularly cloned (SRV1–SRV3). The present study shows that unlike SIVmac239 infec-
tion, morphine treatment will not increase the infectivity of
SRV 1–3 (Fig. 6). Instead, morphine may induce a “protective”
effect against SRV infection (Tables II and III). Similar phe-
nomenon has been reported for murine Friend retrovirus infec-
tion (34). Like SRV, the Friend retrovirus infection model has
been described as relevant to several aspects of AIDS; in par-
cular, there are significant changes in immune function sim-
ilar to those observed in HIV infection. The receptor for such
virus is again not CCR5 but murine cationic amino acid acid
transporter 1 (35), and morphine was found to attenuate the patho-
logical manifestations of the virus in infected animals (34).
Therefore, it appears that morphine-induced CCR5 in human
lymphocytes facilitates only SIVmac239 infection, for which
CCR5 is a co-receptor for viral entry.

This study shows for the first time that lymphocytes ex-
press CCR5 at higher levels when treated with morphine
sulfate. Previous studies that were unable to detect CCR5 in
CEM x174 may have been due to the low levels of CCR5 expression inherent in cells; this level is below the threshold of many conventional detection methods. These low levels of CCR5 expression are significant nevertheless, especially when these low levels of CCR5 expression are augmented by
morphine treatment. In addition, recent studies have shown
that in cells with low amounts of CD4, a low trace of CCR5 was sufficient for susceptibility to virus infection (36).
Therefore, in cells with low levels of CCR5 expression, morphine treatment may bring CCR5 concentrations above threshold levels for maximal infection. Morphine does not affect the
gene expression of BOB or BONZO (Fig. 3). In this regard,
the induction of the chemokine receptor CCR5 gene expres-
sion by morphine may provide a mechanism by which mor-
phine sulfate enhances HIV/SIV infection and hence exacer-
bates the Simian AIDS or AIDS pathogenesis.

Acknowledgments—The following reagents were obtained through
the AIDS Research and Reference Reagent Program, Division of AIDS,
NIADD, National Institutes of Health: GHOST Parental Cell Line and
GHOST Hi-5 (with high CCR5 expression), from Dr. Vineet N. Kewal-
Ramani and Dr. Dan R. Littman. We also thank Dr. Vineet N. Kewal-
Ramani for useful advice.

REFERENCES
1. Chuang, L. F., Killam, K. F., Jr., and Chuang, R. Y. (1997) Addiction Biol. 2, 421–430
2. Salter, R. D., Howell, D. N., and Cresswell, P. (1985) Immunogenetics 21, 235–246
3. Chuang, L. F., Killam, K. F., Jr., and Chuang, R. Y. (1993) Biochim. Biophys. Res. Commun. 195, 1165–1173
4. Kelvin, D. J., Michiel, D. F., Johnston, J. A., Lloyd, A. R., Sprenger, H., Oppenheim, J. J., and Wang, J. M. (1993) J. Leukocyte Biol. 54, 604–612
5. Choi, Y., Chuang, L. F., Lam, K. M., Kung, H.-F., Wang, J. M., Osburn, B. I., and Chuang, R. Y. (1999) In Vivo 13, 389–396
6. Miyagi, T., Chuang, L. F., Lam, K. M., Kung, H.-F., Wang, J. M., Osburn, B. I., and Chuang, R. Y. (2000) Immunopharmacology 47, 53–62
7. Mazzone, A., Mazzucchelli, I., Possati, G., Gritti, D., Pea, M., and Ricevuti, G. (1994) Int. J. Immunopharmacol. 16, 959–967
8. Ferguson, S. S., Barak, L. S., Zhang, J., and Caron, M. G. (1996) Can. J. Physiol. Pharmacol. 74, 1085–1110
9. Cocchi, P., DeVico, A. L., Garzino-Demo, A., Arya, S. K., Gallo, R. C., and Lusso, P. (1995) Science 270, 1811–1815
10. Lee, B., and Montanier, L. J. (1999) J. Leukocyte Biol. 65, 552–565
11. Chen, L., Zhou, P., Ho, D. D., Littman, D. R., and Moore, P. A. (1997) J. Biol. Chem. 272, 2705–2714
12. Vodicka, M. A., Goh, W. C., Wu, L. I., Rogel, M. E., Bartz, S. R., Schweickart, V. L., Raport, C. J., and Emerman, M. (1997) Virology 233, 193–198
13. Kirchhoff, F., Pohlmann, S., Hamacher, M., Means, R. E., Kraus, T., Uberla, K., and Di Mario, P. (1997) J. Virol. 71, 1165–1173
14. Trkola, A., Paxton, W. A., Monard, S. P., Sun, Y., Marcon, L., Cayabyab, M., Berman, M., Dorf, M. E., Gerard, N., Gerard, C., and Sodroski, J. (1998) J. Virol. 72, 6113–6118
15. Chuang, L. F., Killam, K. F., Jr., and Chuang, R. Y. (1997) J. Biol. Chem. 272, 26815–26817
16. Torres, J. V., Werner, L. L., Malley, A., and Benjamini, E. (1991) J. Med. Primatol. 20, 218–221
17. Liu, Y., Blackbourn, D. J., Chuang, L. F., Killam, K. F., Jr., and Chuang, R. Y. (1992) J. Pharmacol. Exp. Ther. 263, 533–539
18. Anderson, D., and Torres, J. V. (1999) Viral Immunology 12, 47–56
19. Chuang, L. F., Chuang, T. K., Killam, K. F., Jr., Chuang, A. J., Kung, H.-F., Yu, L., and Chuang, R. Y. (1994) Biochim. Biophys. Res. Commun. 202, 1291–1299
20. Choo, H., Parzan, M., Konkel, M., Martin, K., Sun, Y., Marcel, L., Carayabah, M., Berman, M., Dorf, M. E., Gerard, N., Gerard, C., and Sodroski, J. (1998) J. Virol. 72, 6113–6118
21. Chuang, L. F., Killam, K. F., Jr., and Chuang, R. Y. (1997) J. Med. Chem. 272, 26815–26817
22. Torres, J. V., Werner, L. L., Malley, A., and Benjamini, E. (1991) J. Med. Primatol. 20, 218–221
23. Liu, Y., Blackbourn, D. J., Chuang, L. F., Killam, K. F., Jr., and Chuang, R. Y. (1992) J. Pharmacol. Exp. Ther. 263, 533–539
24. Anderson, D., and Torres, J. V. (1999) Viral Immunology 12, 47–56
25. Choo, H., Parzan, M., Konkel, M., Martin, K., Sun, Y., Marcel, L., Carayabah, M., Berman, M., Dorf, M. E., Gerard, N., Gerard, C., and Sodroski, J. (1998) J. Virol. 72, 6113–6118
26. Chao, C. C., Molitor, T. W., Close, K., Hu, S., and Peterson, P. K. (1993) Int. J. Immunopharmacol. 15, 447–543
27. Morgan, E. L. (1996) J. Neuroimmunol. 65, 21–30
28. Franchin, G., Zymbart, G., Dai, W. W., Hu, S., and Oppenheim, J. J. (1993) J. Immunol. 149, 889-889
29. Platt, E. J., Wehrly, K., Kuhmann, S. E., Chesebro, B., and Kabat, D. (1998) J. Virol. 72, 2855–2864
30. Chao, C. C., Molitor, T. W., Close, K., Hu, S., and Peterson, P. K. (1993) Int. J. Immunopharmacol. 15, 447–543
31. Morgan, E. L. (1996) J. Neuroimmunol. 65, 21–30
32. Franchin, G., Zymbart, G., Dai, W. W., Hu, S., and Oppenheim, J. J. (1993) J. Immunol. 149, 889-889
33. Platt, E. J., Wehrly, K., Kuhmann, S. E., Chesebro, B., and Kabat, D. (1998) J. Virol. 72, 2855–2864