The Amino-terminal Domain of the CCR2 Chemokine Receptor Acts as Coreceptor for HIV-1 Infection

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Abstract

The chemokines are a homologous serum protein family characterized by their ability to induce activation of integrin adhesion molecules and leukocyte migration. Chemokines interact with their receptors, which are composed of a single-chain, seven-helix, membrane-spanning protein coupled to G proteins. Two CC chemokine receptors, CCR3 and CCR5, as well as the CXCR4 chemokine receptor, have been shown necessary for infection by several HIV-1 virus isolates.

We studied the effect of the chemokine monocyte chemoattractant protein 1 (MCP-1) and of a panel of MCP-1 receptor (CCR2)-specific monoclonal antibodies (mAb) on the suppression of HIV-1 replication in peripheral blood mononuclear cells. We have compelling evidence that MCP-1 has potent HIV-1 suppressive activity when HIV-1–infected peripheral blood lymphocytes are used as target cells. Furthermore, mAb specific for the MCP-1R CCR2 which recognize the third extracellular CCR2 domain inhibit all MCP-1 activity and also block MCP-1 suppressive activity. Finally, a set of mAb specific for the CCR2 amino-terminal domain, one of which mimics MCP-1 activity, has a potent suppressive effect on HIV-1 replication in M- and T-tropic HIV-1 viral isolates.

We conjecture a role for CCR2 as a coreceptor for HIV-1 infection and map the HIV-1 binding site to the amino-terminal part of this receptor. This concurs with results showing that the CCR5 amino terminus is relevant in HIV-1 infection, although chimeric fusion of various extracellular domains shows that other domains are also implicated. We discuss the importance of CCR2 structure relative to its coreceptor role and the role of anti-CCR2 receptor antibodies in the prevention of HIV-1 infection. (*J. Clin. Invest.* 1997. 100:497–502.) Key words: chemokine receptor • HIV-1 coreceptor • monoclonal antibodies

Introduction

Chemokines are a family of proinflammatory cytokines which attract and activate specific types of leukocytes. Based on the position of the first two of four canonical cysteine residues and the chromosomal location of the corresponding genes, two main chemokine families, CC or α and CXC or β, have been identified. They act on monocytes, lymphocytes (including natural killer cells), basophils, eosinophils, and neutrophils (1–3). Chemokines mediate their effects via interactions with a seven-transmembrane glycoprotein receptor coupled to a G-protein signaling pathway (4, 5). This type of receptor consists of a single polypeptide chain with an extracellular amino-terminal domain and a cytoplasmic carboxy-terminal domain. The amino-terminal and third extracellular domain have been implicated in receptor–ligand interaction, while the carboxy-terminal and the third intracellular domain cooperate to bind and activate the G proteins (5).

The recent descriptions of the CCR3, CCR5 (6–12), and CXCR4 or fusin (10, 13–15) chemokine receptors as HIV-1 coreceptors, and of the chemokine ligands RANTES, macrophage inflammatory protein-1α (MIP-1α),[1] and MIP-1β as neutralizing agents for HIV-1 infection, assign these molecules a key role in HIV-1 pathogenesis (6–15). Monocyte chemoattractant protein 1 (MCP-1), a CC chemokine family member, is produced by endothelial cells, smooth muscle, and macrophages in response to a variety of stimuli (16). It has been implicated as a mediator of monocyte and macrophage infiltration of tumor cells, where it may contribute to tumor growth suppression (17). MCP-1 interacts with the CCR2 receptor and induces histamine release, calcium influx, regulates integrin expression, and acts as a chemotactic factor for monocytes/macrophages, T cells, natural killer cells, basophils, mast cells, and dendritic cells (18–20). We have assayed the ability of the CCR2 receptor to act as an HIV-1 coreceptor on human PBMC by testing the HIV-1 suppressive activity of MCP-1, as well as the ability of monoclonal anti-CCR2 antibodies to interfere with HIV-1 replication. Our studies show the importance of the CCR2 amino terminus as the domain interacting

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1. *Abbreviations used in this paper:* CCR2, monocyte chemoattractant protein 1 receptor; MCP-1, monocyte chemoattractant protein 1; MIP, macrophage inflammatory protein.

with HIV-1, and complement those analyses performed with chimeras containing human CCR5 and CCR2B domains.

Methods

Flow cytometry analysis. CCR2B-transfected Jurkat cells and mock-transfected controls were centrifuged, plated in V-bottom 96-well plates (2.5×10^5 cells/well) and incubated with 5 μl/well of biotin-labeled MCP-1R02 or MCP-1R05 mAb (in PBS containing 2% BSA and 2% FCS, staining PBS) for 30 min at 4°C. Cells were washed twice in staining PBS, streptavidin-PE was added, incubated for 30 min at 4°C, and cells again washed twice. Cell staining was determined in a Profile XL cell sorter (Coulter Corp., Miami, FL).

Western blot. CCR2B-transfected Jurkat cells and mock-transfected controls were centrifuged and resuspended in 50 mM Tris, 50 mM NaCl, 5 mM DTT buffer containing a cocktail of protease inhibitors. Cells were subjected to three to five freeze–thaw cycles and centrifuged at 500 *g* for 2 min at 4°C. The supernatant was collected, centrifuged at 15,000 *g* for 15 min at 4°C, and the pellet resuspended in PBS. Lysates were electrophoresed under reducing conditions on 12.5% (wt/vol) SDS-polyacrylamide gels according to Laemmli's method. Gels were transferred to nitrocellulose on a semi-dry blotter (Bio-Rad, Hercules, CA) for 60 min at 250 mA in a 48 nM Tris base, 38 mM glycine, 20% methanol buffer containing 0.037% SDS. The membrane was blocked with 10% nonfat dry milk in PBS, and mAb MCP-1R05 (5 μg/ml in PBS), alone or preincubated with the indicated peptides [CCR2(24–38) and (273–292), 100 μg/ml in PBS, 60 min at room temperature], was added and incubated with agitation for 120 min at room temperature. A peroxidase-labeled goat anti–mouse immunoglobulin antibody (ICN Biochemicals, Inc., Costa Mesa, CA), 1/5,000 dilution, was added and the blot was developed using ECL (Amersham, Little Chalfont, United Kingdom) following the manufacturer's instructions.

Reverse transcriptase-PCR (RT-PCR). Poly(A)$^+$ RNA from transfected Jurkat cells and human PBMC was prepared using the QuickPrep mRNA purification Kit (Pharmacia Biotech AB, Uppsala, Sweden), treated with RNase-free DNase (Boehringer-Mannheim, Mannheim, Germany), and first-strand cDNA was prepared with oligo-dT using the First-Strand cDNA Synthesis Kit (Pharmacia Biotech AB). PCR was performed using *Taq* polymerase in a cycler (9600; Perkin-Elmer Corp., Norwalk, CT).

The following 5′ and 3′ (5′//3′) primer pairs were used to amplify the indicated fragments: CCR1: ATGGAAACTCCAAACACCACAGAGGACTATG // AGGAAAGTGAAGGCTGCAGGTGTGGTGAGTG; CCR2: ATGCTGTCCACATCTCGTTC // TCTATCGATTGTCAGGAGGA; CCR3: GTGTCATCACCAGCATCGTC // CAGATGGCCATAACGAGCAG; CCR4: CATGGTCAGTGGCTGTGTTC // TCACCGCCTTGTTCTTCTTC; CCR5: CCATACAGTCAGTATCAATTC // CCAACCTGTTAGAGCTACTG; and fusin: GTCCACGCCACCAACAGTC // CAGGCAAAGAAAGCTAGGATGAGGAT.

Monoclonal antibodies. Murine mAb were raised against human CCR2B by immunization with KLH-coupled synthetic peptides of CCR2B receptor amino acids 24–38 and 273–292. Spleen and/or lymph node cells were then fused with the P3X63Ag8.653 myeloma line (CRL 1580; American Type Culture Collection, Rockville, MD) using polyethylene glycol 4000 (Merck, Darmstadt, Germany). Antibody specificity was determined by enzyme immunoassay, immunofluorescent cell sorting, immunoprecipitation, and Western blot in cells transfected with the human CCR2 gene. Antibodies were purified and isotypes determined by enzyme immunoassay; the MCP-1R02 and MCP-R105 antibodies are both IgG2a. Since the amino acid sequence selected to produce these antibodies is shared by CCR2A and CCR2B, the mAb should react equally with both receptor forms.

Calcium determinations. Changes in intracellular calcium concentration were monitored using the fluorescent probe Fluo-3 (Calbiochem, San Diego, CA). MonoMac 1 cells (DSM ACC252; German Microorganism and Cell Culture Collection, Braunschweig, Germany) (2.5×10^6 cells/ml) were suspended in RPMI 1640 containing 10 mM Hepes and incubated with 25 μl Fluo-3 (250 mM in DMSO, 15 min at 37°C). Cells were then washed and resuspended in complete medium containing 2 mM $CaCl_2$. To determine the activity of antagonistic mAb, Fluo-3–loaded cells were incubated with various concentrations of purified antibodies in RPMI (30 min at 4°C) before MCP-1 stimulation (PeproTech Inc., London, United Kingdom). Calcium release was then determined in the cell sorter at 525 nm. For agonistic mAb, Fluo-3–loaded cells were stimulated with cytokines or mAb, and Ca^{2+} influx determined as above.

Chemotaxis. MonoMac 1 cells were placed in the upper well of 24-well transmigration chambers (Transwell; Costar Corp., Cambridge, MA) previously coated with mouse brain endothelial cells (5×10^4 cells/well, 48 h at 37°C, 5% CO_2). Chemokines or agonist antibody, diluted in RPMI containing 0.25% BSA, were added to the lower well. Plates were incubated (120 min at 37°C, 5% CO_2), the inserts were removed from the wells, and the cells which had migrated to the lower chamber were counted. To block MCP-1–induced chemotaxis, purified mAb diluted in PBS were added to the upper well simultaneously with the addition of chemokines to the lower well. Chemotaxis determination was as above.

Infectivity of PBL and HIV-1 p24 determination. Human PB mononuclear cells were activated with PHA (10 μg/ml, 48 h at 37°C, 5% CO_2). After washing, cells were incubated with NL4-3 viral stocks (2 ng/10^6 per assay, 37°C overnight). Cells were then washed extensively and cultured in complete RPMI 1640 medium containing rhIL-2 (10 ng/ml) with or without the chemokines MCP-1 or MIP-1β and the antibodies MCP-1R05 or hGH-14. Every 2 d after infection, half of the culture supernatant (500 μl) was removed and replaced with fresh medium containing IL-2, chemokines, and antibodies at their initial concentrations. Cell-free supernatants were tested for HIV-1 p24 antigen content at d 2–8 after infection using a commercial ELISA test (Coulter Corp.).

Results

CCR2B-specific antibodies block MCP-1–triggered cell activation. We have generated a set of mAb to the human CCR2B receptor, specific for either the amino-terminal (amino acids 24–38) or the third extracellular domain (amino acids 273–292), illustrated here by mAb MCP-1R02 and MCP-1R05, respectively. Antibody specificity was analyzed by immunoprecipitation, cytofluorometry, and Western blotting in Jurkat cells, either mock-transfected or transfected with the CCR2B gene. Wild-type Jurkat cells lack CCR2B receptor expression, although they express the CCR4, CCR5, and fusin receptors, as analyzed by RT-PCR (Fig. 1 *A*). Jurkat cells transfected with a CCR2B receptor gene-bearing plasmid also express the CCR2 receptor, as determined by RT-PCR (Fig. 1 *A*), fluorescence-activated cell sorting (Fig. 1 *B*), and Western blot analyses (Fig. 1 *C*). In Western blot, the CCR2B receptor band is abolished when these antibodies are incubated in the presence of the peptides used for immunization (Fig. 1 *C*). In immunofluorescence studies, both antibodies detect the CCR2B receptor expressed in all human monocytes, as defined by anti-CD14 antibodies. They also recognize the CCR2B receptor in a significant fraction (40–70%) of activated peripheral blood– or tonsil-derived human B cells, and in a minor population of resting CD4$^+$ T cells (not shown). These data concur with those of Loetscher et al. (21) showing CCR2 expression in PBMC preparations.

MCP-1 induces Ca^{2+} influx in the monocyte cell line MonoMac 1 and in human peripheral T cells (not shown). This Ca^{2+}

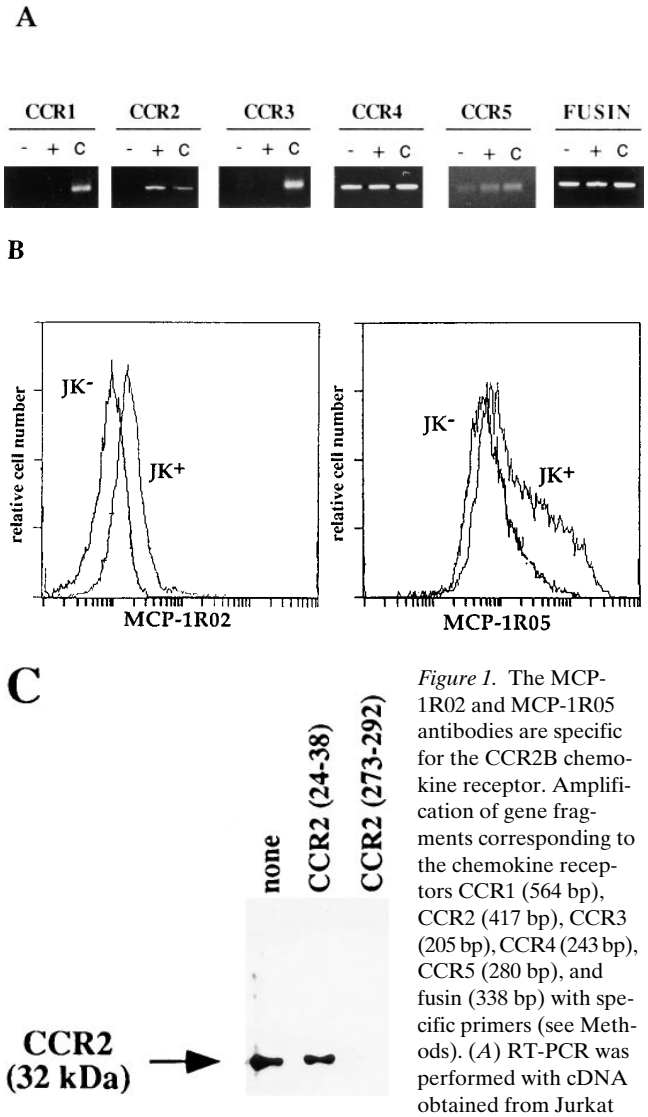

Figure 1. The MCP-1R02 and MCP-1R05 antibodies are specific for the CCR2B chemokine receptor. Amplification of gene fragments corresponding to the chemokine receptors CCR1 (564 bp), CCR2 (417 bp), CCR3 (205 bp), CCR4 (243 bp), CCR5 (280 bp), and fusin (338 bp) with specific primers (see Methods). (*A*) RT-PCR was performed with cDNA obtained from Jurkat cells transfected with pCDNA-3 (−), with pCDNA-3 encoding the CCR2B gene (+) and with human PBMC (*C*). CCR2B-transfected Jurkat cells (JK^+) or mock-transfected controls (JK^-) were incubated with the biotin-labeled antibodies MCP-1R02 and MCP-1R05, as indicated, followed by streptavidin-PE (*B*). The figure shows mAb binding to JK^+ cells compared with JK^-. Western blot (*C*) analysis of JK^+ cell lysates was performed, using mAb MCP-1R05 alone or preincubated with the immunizing peptide, CCR2B (273–292), or with the amino-terminal receptor region peptide CCR2B (24–38), as indicated.

influx is blocked by the mAb specific for the third extracellular domain of the CCR2B (MCP-1R05) (Fig. 2 *B*), as well as by the two CCR2B receptor amino-terminal domain-specific antibodies MCP-1R01 and MCP-1R02 (not shown), but not by an irrelevant, isotype-matched mAb (Fig. 2 *A*). MCP-1R05 and MCP-1R02 also block the MCP-1–triggered chemotactic response in human monocytes in a concentration-dependent fashion (not shown), as they do in MonoMac 1 cells (Fig. 2 *C*).

CCR2 acts a coreceptor for HIV-1 infection. Three other CC chemokine family members, RANTES, MIP-1α, and MIP-1β, all of which interact with CCR5, display the HIV-1 suppressive activity which has been implicated in infection control of this virus (6–12). We demonstrate here that MCP-1 has suppressive activity against the NL4-3 HIV-1 strain, which was derived from NY5 (5′) and LAV (3′) isolated from PBMC (22, 23); it replicates in peripheral blood lymphocytes and was cultured in the Jurkat-derived $CD4^+$ T cell line J-Jhan. Dose-dependent inhibition of extracellular HIV-1 p24 antigen release was observed for MIP-1β and RANTES as described (6), but also for MCP-1, with an end dilution (ED_{95}) of 1–20 nM (Fig. 3). This concurs with earlier results for MIP-1β, correlating the concentration range at which MCP-1 displays HIV-1 suppressive activity with that required for biological responses (0.01–10 nM), as analyzed by Ca^{2+} influx, chemotactic responses, and T cell signaling. We have excluded the possibility that this antiviral activity is due to a negative effect on cellular proliferation, as MCP-1 does not affect human peripheral blood T cell proliferative responses to PHA stimulation (not shown).

As shown, MCP-1R05 eliminates the MCP-1 suppressive activity in a dose-dependent manner (Fig. 3), while it has little or no effect on the HIV-1 suppressive activity of MIP-1β and RANTES (not shown). Isotype-matched antibodies of irrelevant specificity have no effect on extracellular p24 release after HIV-1 infection of PBL (Fig. 3). The MCP-1R05 antibody blocks virtually all HIV-1 suppressive activity promoted by MCP-1. The blocking effect of MCP-1R05 is dose dependent and, at the highest concentration used, no suppressive MCP-1 activity remains (Fig. 3). In fact, at 15 μg/ml, the p24 concentration observed is at the same level as in the absence of MCP-1. The specificity of MCP-1 inhibition by MCP-1R05 is in accordance with results obtained in Ca^{2+} influx and chemotactic response inhibition studies.

In a second approach to the unraveling of CCR2 function, we tested two mAb (MCP-1R01 and MCP-1R02) specific for the amino-terminal part of this human chemokine receptor (amino acids 24–38). This sequence was chosen for its possible implication in ligand binding, based on data for other chemokine receptors such as the IL-8R (24). In contrast to MCP-1R05, the MCP-1R01 and MCP-1R02 antibodies display dose-dependent HIV-1 suppressive activity, with an ED_{95} of 1–10 nM, mimicking the activity of MCP-1 itself (Fig. 4 *A*). These results clearly dissociate the chemokine ligand-binding domains of the CCR2 receptor from those used by HIV-1 as a coreceptor. MCP-1 suppressive activity was tested in other HIV-1 viral strains; MCP-1, as well as MCP-1R02 and MCP-1R01, clearly suppresses other M-tropic HIV-1 viral isolates such as BaL (Fig. 4 *B*), but was ineffective with the T-tropic JC-CSF isolate (not shown).

At least five different β-chemokine receptors have been involved in chemokine binding. Three of them, CCR3, CCR5, and fusin, act as coreceptors for HIV-1 (6–12, 25), and the presence of the appropriate chemokine prevents viral infection. Here we show compelling evidence that CCR2 also acts as an HIV-1 coreceptor. Our data concur with the very recent results obtained using the HIV-1 89.6 isolate, in which the CCR2 receptor is implicated in syncytium formation (10). MIP-1β also suppresses NL4-3 replication, implying that this HIV-1 isolate might also use CCR5 as coreceptor. If this is indeed the case, the suppression of NL4-3 replication by both MCP-1 and MIP-1β may be explained by cross-desensitization of the two receptors used by the chemokines upon specific receptor binding (not shown).

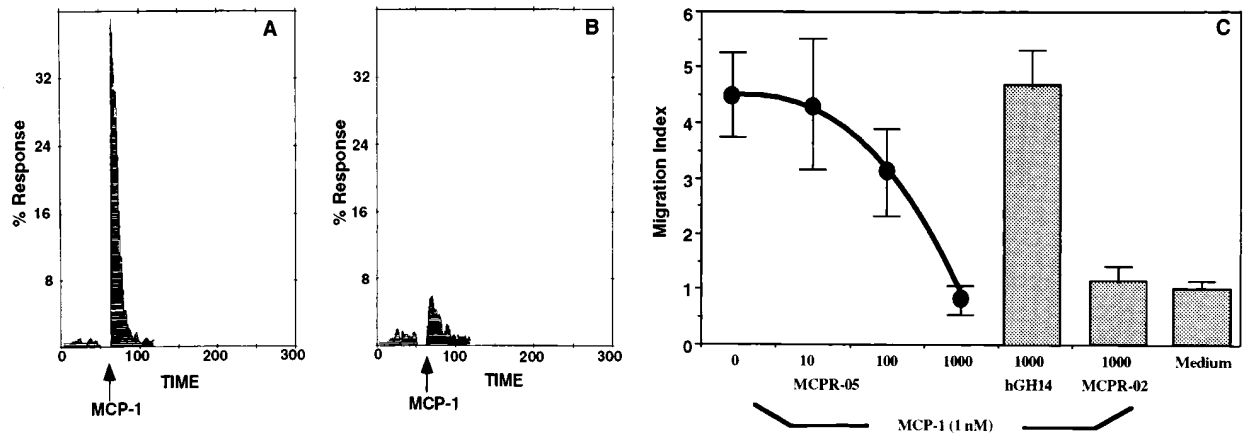

Figure 2. Antibodies specific for the human CCR2B block MCP-1–triggered Ca^{2+} influx and transmigration. Induction of Ca^{2+} influx was promoted by human MCP-1 in MonoMac 1 cells preloaded with Fluo-3 and incubated with purified hGH-14 (*A*), MCP-1R05, or MCP-1R02 (*B*) antibodies before stimulation with MCP-1 (5 nM). Calcium influx was determined in cell sorting at 525 nm. The negative control mAb hGH-14, isotype-matched to MCP-1R05, is specific for human growth hormone (*hGH*). The figure depicts one of five experiments performed. Chemotaxis (*C*) was assessed in endothelial cell–coated porous cell culture inserts. MonoMac 1 cells were incubated with increasing concentrations of MCP-1R05, MCP-1R02, or control hGH-14 antibody in the upper chamber, and MCP-1 (1 nM in culture medium) was added to the lower chamber. Cells that migrated to the lower well were counted and expressed as a migration index, calculated as the *x*-fold increase in migration observed over the negative control (*Medium*). Data represent the mean of triplicate determinations, with the SD indicated. One experiment of four is represented.

Discussion

Chemokines interact with their receptors in a complex, multisite manner (26, 27). The amino terminus and the third extracellular domain of the receptor are thought to fold into proximity on the intact receptor and join by a disulfide bridge (28). Antibodies specific for the chemokine receptor fusin have been shown to prevent cell fusion and infection of $CD4^+$ T cells with HIV-1 (13), but no further characterization of the specificity of these antibodies was demonstrated. We now present cogent data on the role of CCR2 as a coreceptor for HIV-1. Using CCR2B-specific mAb, we have identified the HIV-1 binding site on this coreceptor by showing that the receptor ligand-binding domain overlaps the HIV-1– and mAb-binding domains, which do not themselves overlap. First, MCP-1 which binds to CCR2B (19) shows HIV-1 suppressive activity. Second, the MCP-1R05 antibody to the CCR2 receptor third extracellular domain, and MCP-1R01 and MCP-R02 mAb to the amino terminus block MCP-1–triggered effects such as Ca^{2+} influx and chemotaxis, as well as the suppressive effect of MCP-1 on HIV-1 replication. Third, the two anti–amino-terminal antibodies, MCP-1R01 and MCP-1R02, but not MCP-1R05, behave like MCP-1 and block HIV-1 virus infection. The amino-terminal residues of the CCR2B receptor appear to be necessary for HIV-1 binding; this contrasts with the situation of the chemokines, which in addition require the third extracellular domain. This difference might reflect the diverse nature of the ligands and may be used to advantage to design therapeutic tools which prevent HIV-1 infection but preserve chemokine responses. Similar results in studies using cells transfected with chimeric constructs of the CCR5 receptor have recently permitted mapping of the amino-terminal domain of this receptor as implicated in the HIV-1 virus interaction (29, 30). Our data support earlier studies in indicating the importance of the chemokine receptor amino terminus for chemokine activity (29, 30). The use of CCR5 receptor chimeras shows that simple analysis of deletion mutants cannot elucidate which receptor elements are significant in its role as an HIV-1 coreceptor (29, 31). M-tropic HIV-1 envelope protein fusion thus requires the amino-terminal domain or the first intracellular domain of the CCR5 receptor or, alternatively, a chimeric gene containing the CCR5 amino-terminal domain

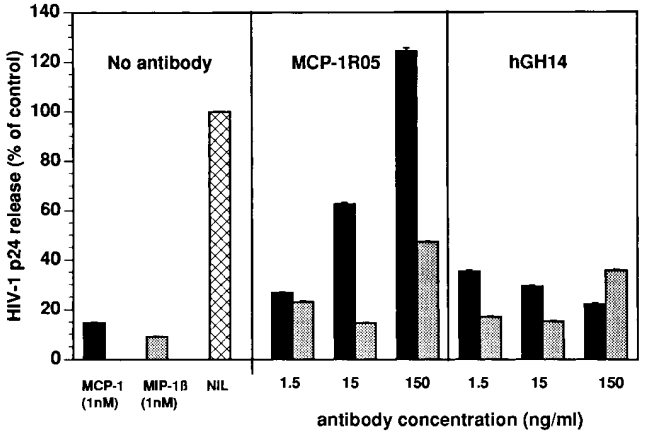

Figure 3. MCP-1 displays suppressive HIV-1 activity that is blocked by MCP-1 antagonist antibodies specific for the CCR2B receptor. Several concentrations of MCP-1 and MIP-1β were tested for HIV-1 suppressive activity (0.01–10 nM). The concentration of MCP-1 (*filled bars*) and MIP-1β (*dotted bars*) yielding maximal HIV-1 inhibitory activity on activated PB mononuclear cell cultures (1 nM) was selected, using untreated culture supernatants (*NIL*) as control. The suppressive activity of MCP-1 (1 nM) and of MIP-1β (1 nM) was blocked in the presence of the indicated concentrations of the MCP-1R05 or the MCP-1R01 antibody, using the hGH-14 antibody as control. The figure shows the results of one experiment of three performed.

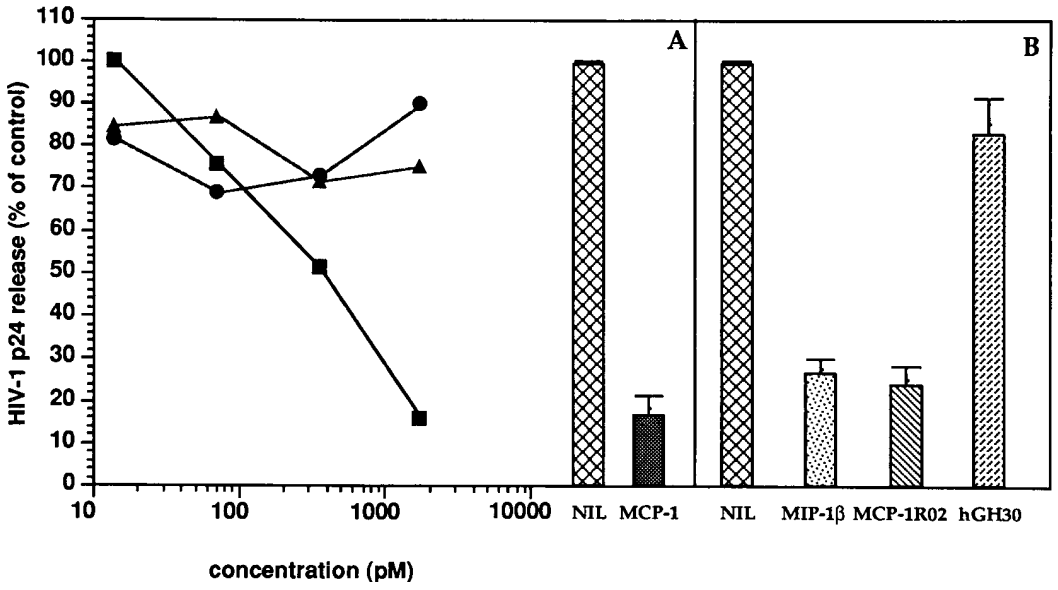

Figure 4. MCP-1 agonist antibody specific for the human CCR2B shows HIV-1 suppressive activity. (*A*) The HIV-1 suppressive activity of the MCP-1R02 mAb (■) was tested as described in the legend to Fig. 3, and compared with that of the control hGH-30 mAb (●) and MCP-1R05 antagonist mAb (▲) using the NL4-3 viral strain and PBL as target cells. (*B*) The suppressive activity of the MCP-1R02 mAb (200 pM) was also tested using the BaL viral strain. As positive controls, the suppressive activities of MCP-1 (1 nM) and MIP-1β (1 nM) are shown. Data represent the means of triplicate determinations±SD. The figure shows one representative experiment of three performed.

linked to the CCR2B receptor. Complementation studies such as the one performed here might thus improve understanding of how chemokine receptors can act as HIV-1 coreceptors, as well as providing a biological basis for HIV-1 infection of PBMC. In addition, these types of reagents may have therapeutic applications.

Except in a few cases addressing the in vivo biological relevance of CCR5 (12, 25, 32, 33), most studies showing CCR3 or CCR5's coreceptor role in HIV infection were performed using the chemokine receptor expressed in transfected cell lines (the human cervical carcinoma cell line HeLa; the murine embryo fibroblast 3T3; Cos monkey kidney cells; the quail fibrosarcoma cell line QT6; and the Cf2th canine thymoma). In all but one of these (7), in which CCR5 receptor–transfected Jurkat T cells were used, expression was transient. Furthermore, system read-out in most of these experiments is by syncytium formation, fusion reaction, or *env*-mediated reaction between transiently expressing CCR5 receptor cells and cells infected with a recombinant vaccinia virus carrying the *env* glycoprotein. We used PBL as HIV-1 infection targets and measured viral replication by determining the amount of p24 present in cell cultures. In no other instance were PBL used as target cells, except in the original paper by Cocchi et al. (6); interestingly, these authors tested the MCP-1 effect using not PBL but rather the PM1 cell line as target cells, and no effect was observed. It is convenient to recall the misinterpretations in HIV research based on the overuse of cell lines as targets for HIV-1 infection. In light of previous data showing that CCR5 is the major coreceptor for M-tropic HIV-1 strains such as BaL, our results can be interpreted in several ways. Inhibition of BaL replication in CD4+ T cells by MCP-1 or the MCP-1R02 antibody might be achieved in two ways. First, and we believe most likely, we have shown that during MCP-1–triggered activation and chemotaxis, leukocytes polarize in such a way that chemokine receptors migrate toward the leading edge of the cell (34). The polarization of chemokine receptors is quite probably related to their subsequent endocytosis, and may thus take part in preventing HIV-1 infection of the chemokine-targeted cell. As a second possibility, when MCP-1 and the agonist mAb MPC-1R02 interact with the CCR2 receptor, they trigger macrophage and T cell activation; this is measured by the products of soluble mediators, some of which may be implicated in HIV-1 replication control. And third, the ability of a virus to use a coreceptor molecule does not preordain that the virus can use that molecule regardless of the cell type on which it is expressed. Posttranslational modification of coreceptors, for instance, may dispose them to cell infection only by certain HIV-1 strains. Circumspection has been advised with reference to the results that chemokine coreceptors accurately reflect the cellular tropism of the virus (35).

Given the lack of appropriate reagents, previous studies indeed required specific chemokine receptor expression. These results therefore complement earlier work, as we have generated these reagents; we can now identify the role of these receptors in normal leukocytes. Using our anti-CCR2B mAb panel, the ability of each mAb to recognize the transfected CCR2B receptor was compared in several cell lines; surprisingly, variation in the antibody reactivity pattern was detected. The reason for this is not yet understood, but warns that caution is needed when receptors are expressed in different cell lines and functional assays are used.

The mechanisms through which chemokines exert their inhibitory effects are complex, and may include receptor blockade, desensitization, sequestration, or internalization. Since the

antibody promoting HIV-1 suppressive activity also triggers chemokine responses, HIV-1 may be inactivated through the chemokine receptor–triggered signal transduction pathway, an interconnected cascade of G proteins and cell-specific catalytic enzymes which involves G protein activation, the MAPK cascade, and the serine/threonine and tyrosine kinases (27). Finally, HIV-1 tropism may be a result of the differential expression of chemokine receptors on leukocytes. The T-tropic virus thus usually fails to enter macrophages, while M-tropic viruses generally do not enter transformed T cell lines. Since our antibodies recognize the chemokine receptor on T cells as well as on macrophages, they may inhibit both macrophage-tropic and T cell–tropic HIV-1 virus and therefore be effective therapeutic instruments for use against HIV-1 infection.

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