Differential Regulation of CXCR4-mediated T-cell Chemotaxis and Mitogen-activated Protein Kinase Activation by the Membrane Tyrosine Phosphatase, CD45*

Received for publication, November 20, 2002, and in revised form, January 3, 2003
Published, JBC Papers in Press, January 8, 2003, DOI 10.1074/jbc.M211803200

Aaron Z. Fernandis‡, Rama P. Cherla‡, and Ramesh K. Ganju§
From the Division of Experimental Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts 02115

The chemokine receptor CXCR4 and its cognate ligand, stromal cell-derived factor-1α (CXCL12), regulate lymphocyte trafficking and play an important role in host immune surveillance. However, the molecular mechanisms involved in CXCL12-induced and CXCR4-mediated chemotaxis of T-lymphocytes are not completely elucidated. In the present study, we examined the role of the membrane tyrosine phosphatase CD45, which regulates antigen receptor signaling in CXCR4-mediated chemotaxis and mitogen-activated protein kinase (MAPK) activation in T-cells. We observed a significant reduction in CXCL12-induced chemotaxis in the CD45-negative Jurkat cell line (J45.01) as compared with the CD45-positive control (JE6.1) cells. Expression of a chimeric protein containing the intracellular phosphatase domain of CD45 was able to partially restore CXCL12-induced chemotaxis in the J45.01 cells. However, reconstitution of CD45 into the J45.01 cells restored the CXCL12-induced chemotaxis to about 90%. CD45 had no significant effect on CXCL12 or human immunodeficiency virus gp120-induced internalization of the CXCR4 receptor. Furthermore, J45.01 cells showed a slight enhancement in CXCL12-induced MAP kinase activity as compared with the JE6.1 cells. We also observed that CXCR4 treatment enhanced the tyrosine phosphorylation of CD45 and induced its association with the CXCR4 receptor. Pretreatment of T-cells with the lipid raft inhibitor, methyl-β-cyclodextrin, blocked the association between CXCR4 and CD45 and markedly abolished CXCL12-induced chemotaxis. Comparisons of signaling pathways induced by CXCL12 in JE6.1 and J45.01 cells revealed that CD45 might moderately regulate the tyrosine phosphorylation of the focal adhesion components the related adhesion focal tyrosine kinase/Pyk2, focal adhesion kinase, p130Cas, and paxillin. CD45 has also been shown to regulate CXCR4-mediated activation and phosphorylation of T-cell receptor downstream effectors Lck, ZAP-70, and SLP-76. Our results show that CD45 differentially regulates CXCR4-mediated chemotactic activity and MAPK activation by modulating the activities of focal adhesion components and the downstream effectors of the T-cell receptor.

The chemokine CXCL12 and its corresponding receptor CXCR4 play an important role in immune and inflammatory responses, lymphopenia in bone marrow, and in embryonic developmental processes (1–7). Targeted disruption of either CXCR4 or CXCL12 protein leads to severe defects that are embryologically lethal (3–6). CXCR4 has also been shown to act as a coreceptor for the T-cell tropic human immunodeficiency virus (HIV), 1 type 1 strain and to play a crucial role in HIV pathogenesis (8–10).

Although CXCL12 acts as a potent chemoattractant for various cell types including T-cells and regulates the directional movement of these cells, relatively little is known about the signaling pathways that may mediate these effects (1, 11). We and others (12–16) have recently deciphered the molecular mechanisms involved in regulating CXCR4 and CCR5-mediated chemotaxis. We have demonstrated that CXCL12 binding to CXCR4 stimulates multiple signaling pathways including activation of focal adhesion components such as the related adhesion focal tyrosine kinase (RAFTK, also known as Pyk2 or Cak-β), focal adhesion kinase (FAK), paxillin, and p130Cas (12). Furthermore, protein-tyrosine phosphatases SHP1 and SHP2 have also been shown to be involved in CXCR4- or CCR5-mediated chemotaxis (13, 15, 17). Hematopoietic cells derived from mice lacking SHP1 showed altered patterns of chemoattractive response to CXCL12 (17). SHP2 was shown to associate with CXCR4 and to regulate the CXCL12-induced migration of T- and pre-B-cells (15). In the present investigation, we further delineated the role of tyrosine phosphatases and showed that the membrane-bound tyrosine phosphatase CD45 is a key regulator of CXCL12-induced and CXCR4-mediated chemotaxis.

CD45 is expressed exclusively on cells of hematopoietic lineage (18–20). It is a key regulator of antigen receptor signaling in T- and B-cells, playing a pivotal role in the activation and development of lymphocytes (18–23). Studies using CD45-deficient mice and cell lines revealed that this phosphatase is very important for thymocyte differentiation (24, 25). CD45 is shown to influence the early events in T-cell activation by operating as a positive, as well as negative, regulator of the Src family kinases, p56Lck and p59Fyn. Recent studies have also identified CD45 as a negative regulator of cytokine-mediated signaling by acting as a JAK tyrosine phosphatase (26). Thus, CD45 plays a crucial role in cytokine receptor-mediated differentiation, proliferation, and anti-viral responses. In addition, CD45 is also required for some integrin-mediated adhesion

1 The abbreviations used are: HIV, human immunodeficiency virus; FAK, focal adhesion kinase; MAP, mitogen-activated protein; MAPK, MAP kinase; MBC, methyl-β-cyclodextrin; RAFTK, related adhesion focal tyrosine kinase; TCR, T-cell receptor; pTyr, phosphorylserine; PBS, phosphate-buffered saline.

* This work was supported in part by National Institutes of Health Grants AI49140 and CA76950 and by a grant from the American Foundation for AIDS Research (to R. K. G). 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.

‡ Contributed equally to this work.

§ To whom correspondence should be addressed: Harvard Inst. of Medicine-BIDMC, 4 Blackfan Circle, Rm. 343, Boston, MA 02115. Tel.: 617-667-0060; Fax: 617-975-5243; E-mail: rganju@caregroup.harvard.edu.
CD45 Regulates CXCR4-mediated Chemotaxis

Although our understanding of the molecular mechanisms of CD45 in regulating TCR and cytokine receptor signaling have increased substantially, its role in chemokine-mediated biological functions has not been explored. In the present study, we have investigated the role of CD45 in regulating CXCL12-induced chemotaxis and MAP kinase activation in T-cells. Our data indicate a prominent role for CD45 in these processes and thus provide new information regarding CXCR4-mediated chemotactic signaling pathways.

EXPERIMENTAL PROCEDURES

Reagents and Materials—Purified antibodies to phosphepolic p44/42 MAP kinase were obtained from New England Biolabs (Beverly, MA). Antibodies to p44/42 protein, phosphotyrosine (pTyr185, 187), and p66Lck were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Phosphospecific antibodies to RAPTK (pY202, 204) and pAK (pTyr185, 187) were obtained from BIOSOURCE International (Camarillo, CA). Phosphotyrosine antibody (2G10), ZAP-70, and SLP-76 were from Upstate Biotechnology (Lake Placid, NY). Paxillin antibody was from Transduction Laboratory (San Diego, CA). Electrophoresis reagents and nitrocellulose membrane were obtained from Bio-Rad. The protease inhibitors leupeptin and antitrypsin, and all other reagents, were obtained from Sigma.

Primary Lymphocyte Culture—Primary lymphocytes were isolated from heparinized venous blood as described before (31). All the cell lines were cultured in RPMI 1640 with 10% fetal calf serum and 100 units/ml penicillin and streptomycin, and 0.1% sodium bicarbonate. The cells were cytolized with 0.1% Triton X-100 and 0.1% sodium citrate for 2 min on ice. Following stimulation, the cells were washed twice with Hank's buffered salt solution at a density of 10^7 cells/ml for 1 h at 37 °C. Serum-starved cells were stimulated with 100 ng/ml CXCL12 at 37 °C for various time periods. Following stimulation, the cells were washed with ice-cold phosphate-buffered saline and fixed in 4% paraformaldehyde for 10 min at room temperature. Next, the cells were permeabilized with 0.1% Triton X-100 and 0.1% sodium citrate for 2 min on ice. The cells were washed and blocked with 5% bovine serum albumin for 30 min at 4 °C. CXCR4 or CD45 was stained with anti-CXCR4 or anti-CD45 antibodies overnight at 4 °C, followed by staining with secondary antibody coupled to horseradish peroxidase for 2 h at room temperature or overnight at 4 °C. The bands were visualized by using the enhanced chemiluminescent system (Amersham Biosciences). The data are representative of findings from three experiments. The activity of the bands was quantified by densitometric analysis using a laser scanner. The mean densities of the bands are represented as the optical density in units/mm².

Flow Cytometry—The CXCR4 or CD45 receptor on the J45.01, J45/CH11, J45/A2, or J45/LB3 cells was stained with phycoerythrin- or fluorescein-labeled anti-CXCR4 or anti-CD45 antibodies overnight at 4 °C, followed by staining with secondary antibody coupled to Texas red (Vector Laboratories) or phospha tidylidoleinamine (Amersham Biosciences). The cells were cytogned on slides, and the expression of these proteins was visualized using a Leica TCS confocal microscope.

RESULTS

CXCL12-induced and CXCR4-mediated Chemotaxis Is Reduced in CD45-deficient Cells—CXCL12-induced chemotaxis has been shown to be regulated by tyrosine phosphatases SHP1 and SHP2 (15, 17). We have further extended these studies to evaluate the role of CD45 in CXCR4-mediated and CXCL12-

events (27, 28). It has also been associated with Alzheimer disease and multiple sclerosis in humans (29, 30).

Confocal Microscopy—Confocal microscopy studies were done as described earlier (33). Briefly, Jurkat (JE6.1 clones) cells were washed twice with Hank’s-buffered salt solution (Cellgro) and resuspended in Hank’s-buffered salt solution at a density of 10^6 cells/ml for 1 h at 37 °C. Serum-starved cells were stimulated with 100 ng/ml CXCL12 at 37 °C for various time periods. Following stimulation, the cells were washed with ice-cold phosphate-buffered saline and fixed in 4% paraformaldehyde for 10 min at room temperature. Next, the cells were permeabilized with 0.1% Triton X-100 and 0.1% sodium citrate for 2 min on ice. The cells were washed and blocked with 5% bovine serum albumin for 30 min at 4 °C. CXCR4 or CD45 was stained with anti-CXCR4 or anti-CD45 antibodies overnight at 4 °C, followed by staining with secondary antibody coupled to Texas red (Vector Laboratories) or phosphatidylethanolamine (Amersham Biosciences). The cells were cytogned on slides, and the expression of these proteins was visualized using a Leica TCS confocal microscope.

Flow Cytometry—The CXCR4 or CD45 receptor on the J45.01, J45/CH11, J45/A2, or J45/LB3 cells was stained with phycoerythrin- or fluorescein-labeled anti-CXCR4 or CD45 antibodies for 1 h at 4 °C. For CXCR4 down-modulation analysis, JE6.1 or J45.01 cells were stimulated with CXCL12 (1 μg/ml) or HIV gp120 (1.2 μg/ml) for various time periods (0, 2, 4, 6 h). Following stimulation, the cells were washed with ice-cold PBS and fixed with 2% formaldehyde for 15 min at room temperature. The cells were stained with anti-CXCR4 antibody as discussed above and then washed with PBS, suspended in 1% formaldehyde in PBS, and subjected to flow cytometric analysis. The results are expressed as the mean ± S.D. of data obtained from three or four experiments performed in duplicate or triplicate. The statistical significance was determined by the Student’s t test.

CD45 Regulates CXCR4-mediated Chemotaxis

5937
CD45 Regulates CXCR4-mediated Chemotaxis

Fig. 1. CD45 regulates the chemotactic response induced by CXCL12. A, cells lacking CD45 antigen (J45.01) or the positive variant control (JE6.1) were subjected to chemotaxis assay in the presence of CXCL12 (0–100 ng/ml) as described under “Experimental Procedures.” B, cells expressing the chimeric protein HLA-A2/cytoplasmic domain CD45 (J45/CH11) or control cells expressing HLA-A2 (J45/A2) were subjected to chemotaxis assay in the presence of CXCL12 (0–100 ng/ml). C, J45.01 cells reconstituted with CD45 (J45/LB3) were analyzed for chemotactic response toward varying concentrations of CXCL12 as indicated and compared with the response of the J45.01 cells. D, shows the difference in cell migration in response to CXCL12 between CD45-negative JE6.1 and CD45-positive (J45/01) cells and between transfectants containing the cytoplasmic domain of CD45 (J45/CH11), transfectants lacking the cytoplasmic domain (J45/A2), and the CD45-reconstituted J45.01 cells (J45/LB3). Results presented in the graph are representative of three experiments; p < 0.005.

CD45 Regulates CXCL12-induced Chemotaxis

Therefore, the differences in chemotaxis observed in the various Jurkat clones and transfectants are not because of variation in CXCR4 levels.

CD45 Does Not Regulate CXCR4 Internalization—CXCL12 and gp120 at higher concentrations have been shown to induce CXCR4 receptor internalization (33). CXCR4 trafficking is important in HIV infection and immune regulation. Recently, we have shown that the proteasome pathway regulates CXCL12-induced down-modulation and chemotaxis (33). In Fig. 1, we have shown that CD45 regulates CXCL12-induced chemotaxis. Thus, we next explored the role of CD45 in the ligand-induced down-modulation of the CXCR4 receptor. No significant difference in CXCL12 or HIV gp120-induced down-modulation of CXCR4 was observed between the CD45-positive JE6.1 (Fig. 2A) and CD45-negative J45.01 (Fig. 2B) cells. These results suggest that CD45 does not regulate the CXCL12- or gp120-induced pathway leading to CXCR4 internalization.

CXCL12-induced Tyrosine Phosphorylation of CD45 and Its Association with the CXCR4 Receptor—To investigate further CD45-regulated chemotactic signaling mechanisms, we first determined the tyrosine phosphorylation status of CD45 upon CXCL12 treatment in the CD45-positive JE6.1 cells. As shown in Fig. 3A, CXCL12 stimulation induced the increased tyrosine phosphorylation of CD45. This phosphorylation was rapid and reached a maximum level between 0.5 to 2.5 min. Equal amounts of CD45 protein were present in each lane (Fig. 3A, bottom panel). We also investigated whether the CXCR4 recep-
CD45 Regulates CXCR4-mediated Chemotaxis

Tyrosine phosphorylation of CD45 and its association with CXCR4 upon stimulation with CXCL12. CD45-positive variant (JE6.1) cells (A and B) or peripheral blood lymphocytes (C) were either unstimulated (0) or stimulated with CXCL12 (100 ng/ml) for the indicated time periods. Cells were lysed and immunoprecipitated (IP) with CD45 (A) or CXCR4 (B and C) antibody. The immune complexes were separated on 7% SDS-PAGE gel, transferred to nitrocellulose membrane, and immunoblotted with anti-phosphotyrosine antibody (4G10, pTyr99) (A, top panel) followed by anti-CD45 antibody (A, bottom panel). B and C, the blots were probed with anti-CXCR4 antibody. Protein loading was analyzed by running 50 μg of lysates on SDS-PAGE and immunoblotting with anti-actin antibody (bottom panels). D, the CD45-positive Jurkat cell clone (JE6.1) was stimulated with CXCL12 (100 ng/ml) for the indicated time point. The cells were fixed with paraformaldehyde and subjected to confocal microscopic analysis using anti-CD45 (green) and anti-CXCR4 (red) antibodies, as described under “Experimental Procedures.” Yellow represents the colocalization of CD45 and CXCR4. P-Tyrosine, phosphotyrosine; WB, Western blot; TCL, total cell lysates; PBL, peripheral blood lymphocyte; UN, unstimulated.

CD45 Regulates CXCR4-induced Src-related Kinases—Src kinases have been shown to play an important role in cell migration and adhesion (39, 40). p56 Lck, a member of the Src family of protein-tyrosine kinases, is a physiological substrate of CD45 (18–20). It has been shown that CD45-mediated dephosphorylation of Tyr505 (Lck) activates this kinase. Therefore, we compared the CXCL12-induced tyrosine phosphorylation and kinase activity of Lck in CD45-positive and -negative cell lines. As shown, CXCL12 increased the kinase activity of Lck (Fig. 5) as compared with the untreated cells in the CD45-positive cell line. However, no significant change in Lck kinase activity was observed in the CXCL12-stimulated CD45-negative cells. We also observed that Lck protein was hyperphosphorylated at tyrosine residues in the CD45-negative cells as compared with the CD45-positive cells. Equal amounts of Lck were present in the cell lysates.

CXCR4-mediated Tyrosine Phosphorylation of Focal Adhesion Components Is Regulated by CD45—Several components of focal adhesion complexes are known to regulate chemokine-mediated chemotaxis (41, 42). These include RAFTK/Pyk2, FAK, paxillin, and p130Cas. These proteins have also been

Effect of Lipid Raft Inhibitor on the Association of CD45 with CXCR4 and CXCL12-induced Chemotaxis—Plasma membranes of many cell types, including T-cells, contain microdomains referred to as lipid rafts (34–36). These domains are rich in sphingolipids and cholesterol, which form a lateral assembly in a saturated glycerophospholipid environment. The domains are known to serve as moving platforms on the cell surface and are more ordered and resistant to detergents like Triton X-100. The domains also act as good sites for cross-talk between various proteins. These include cytoskeletal proteins, Src family kinases, protein kinase C, actin-binding proteins, G proteins, and various molecules involved in TCR signaling (35, 36). Lipid rafts have also been shown to be important for T-cell polarization and chemotaxis (37, 38). To characterize the role of lipid rafts in CXCR4-mediated signaling, we examined the effect of the lipid raft inhibitor, methyl-β-cyclodextrin (MBC), on the association of CXCR4 with CD45. As shown in Fig. 4A, pre-treatment of JE6.1 cells with MBC (10 μM) inhibited the CD45 and CXCR4 association, as detected by immunoprecipitation followed by Western blotting. An equal amount of protein was present in each lane as detected by immunoblotting the lysates with anti-actin antibody (Fig. 4A, bottom panel). Furthermore, we observed that MBC treatment attenuated CXCL12-induced chemotaxis in a dose-dependent manner in medium without serum (Fig. 4B). The maximum inhibition observed was at a 10 μM concentration. However, no effect on chemotaxis at various MBC concentrations was observed in medium containing 2.5% serum (Fig. 4C). The lack of effect may be because of cholesterol replenishment under this condition.
shown to be involved in integrin-triggered cell adhesion and cell spreading. Therefore, we examined the importance of CD45 in regulating the tyrosine phosphorylation of these molecules upon stimulation with CXCL12 by using phosphorylation site-specific antibodies. As shown in Fig. 6A, the CXCL12-induced tyrosine phosphorylation of RAPTK at tyrosine residues 402 and 881 was slightly reduced in the CD45-negative cells (J45.01) as compared with the CD45-positive cells (JE6.1). Equal amounts of RAPTK were present in each sample. Similarly, the tyrosine phosphorylation of FAK was slightly reduced at tyrosine residue 397 in the CD45-negative cells as compared with the CD45-positive cells (Fig. 6B). Furthermore, we found that tyrosine phosphorylation of other focal adhesion components, paxillin (Fig. 6C) and p130Cas (Fig. 6D), was also reduced in the CD45-negative cells as compared with the CD45-positive cells.

CD45 Regulates CXCR4-mediated Chemotaxis

**Fig. 4.** Lipid raft inhibitor disrupts the interaction between CD45 and CXCR4 and blocks CXCL12-induced chemotaxis. A, JE6.1 cells, untreated or pretreated with MBC (10 mM) for 1 h, were stimulated with CXCL12 (100 ng/ml) for the indicated time periods. The cells were lysed, and the lysates were subjected to immunoprecipitation (IP) with anti-CXCR4 antibody. The immunoprecipitates were separated on 7% SDS-PAGE followed by immunoblot analysis with anti-CD45 antibody. Protein loading was analyzed by running 50 μg of lysates on SDS-PAGE and immunoblotting with anti-actin antibody (bottom panel). B and C, JE6.1 cells were preincubated with MBC at different concentrations (mM) in the absence (B) or presence (C) of 2.5% serum. The chemotactic activity of the treated cells toward CXCL12 (100 ng/ml) was monitored as described under “Experimental Procedures”; p < 0.005. TCL, total cell lysates; WB, Western blot.

**Fig. 5.** CD45 modulates CXCL12-induced Lck kinase activity. Lysates obtained from unstimulated (0) or CXCL12-stimulated (100 ng/ml) CD45-negative and -positive cells were immunoprecipitated (IP) with anti-Lck antibodies. The immune complex was subjected to an in vitro kinase assay, as described under “Experimental Procedures” (upper panel) by using enolase (acid-denatured) as a substrate. The immune complex was also separated on SDS-PAGE and immunoblotted with anti-phosphotyrosine antibody (4G10) (middle panel). The same blot was stripped and reprobed with anti-Lck antibody (bottom panel). C, antibody control; P-Tyrosine, phosphotyrosine.

**Fig. 6.** CD45 regulates the tyrosine phosphorylation of focal adhesion components. Lysates, obtained from CD45-negative (J45.01) or -positive (JE6.1) cells unstimulated (0) or stimulated with CXCL12 (100 ng/ml) for the indicated time periods, were run on SDS-PAGE and subjected to serial immunoblotting with phosphospecific antibodies of RAPTK pTyr402, pTyr881, or anti-RAFTK antibody (A) or phosphospecific FAK pTyr397 or anti-FAK antibody (B). The immunoblots were stripped and rebotted with RAPTK or FAK, respectively. Cell lysates prepared from unstimulated (0) or CXCL12-stimulated (100 ng/ml) cells for the indicated time periods were immunoprecipitated (IP) with anti-paxillin (C) or anti-p130Cas (D) antibodies. The immune complexes were separated on SDS-PAGE and immunoblotted with anti-phosphotyrosine antibody (4G10) (C and D, upper panels). The same blots were stripped and reprobed with anti-paxillin (C, bottom panel) or anti-p130Cas (D, bottom panel) antibody. P-RAPTK or P-FAK, tyrosine phosphorylation at the indicated site; C, antibody control; TCL, total cell lysates; P-Tyrosine, phosphotyrosine; WB, Western blot; MW, molecular weight.
CD45 Regulates CXCR4-mediated Chemotaxis

9541

Fig. 7. CD45 modulates ZAP-70 and SLP-76 phosphorylation. Lysates, prepared from unstimulated (○) or CXCL12-stimulated (100 ng/ml) CD45-negative or -positive variant cells for the indicated time periods, were immunoprecipitated (IP) with anti-ZAP-70 (A) or anti-SLP-76 (B) antibody. The immune complexes were separated on SDS-PAGE and immunoblotted with anti-phosphorysosine antibody (4G10) (upper panels). The same blot was stripped and reprobed with anti-ZAP-70 antibody (A) or anti-SLP-76 antibody (B) (bottom panels). C, antibody control; TCL, total cell lysates; P-Tyrosine, phosphorysosine; WB, Western blot; MW, molecular weight.

Chemotaxis and transendothelial migration were shown to be regulated by ZAP-70 (44). Therefore, we examined the CXCL12-induced transactivation of ZAP-70 kinase (Fig. 7A) and SLP-76 (Fig. 7B) in CD45-positive cells as compared with CD45-negative cells. As shown, the phosphorylations of ZAP-70 and SLP-76 were impaired in the CD45-negative cells in comparison to the positive variants. No change in ZAP-70 and SLP-76 protein levels was observed in the CD45-positive and CD45-negative cells (Fig. 7, A and B, bottom panels). These results are in correlation with the observation that CD45 regulates ZAP-70 activity, which in turn regulates SLP-76 phosphorylation.

The Role of CD45 in CXCL12-induced Mitogen-activated Protein Kinase (MAPK) Activation—CXCR4 has been shown to activate the MAPK pathway (12). Thus, we examined the role of CD45 in CXCL12-induced p44/42 MAP kinase activation. The activation of MAP kinase was determined by examining the phosphorylation of p44/42 components using phosophospecific (Tyr202 of p44 and Tyr324 of p42) monoclonal antibodies. As shown in Fig. 8, A and B, absence of CD45 in the J45.01 and J45/A2 cells moderately increased the CXCL12-induced phosphorylation of p44/42 MAPK at early time periods. Equal amounts of p44/42 MAPK were present in each sample (Fig. 8, A and B, lower panels).

DISCUSSION

This study indicates the central role of the membrane-bound tyrosine phosphatase, CD45, in CXCL12-induced and CXCR4-mediated chemotactic signaling, which plays a critical role in the immune system by regulating the trafficking and positioning of lymphocytes (11–17, 45). CXCR4 and its cognate ligand, CXCL12, have also been shown to play an important role in HIV gene product nef-mediated chemotaxis and breast cancer metastasis (46, 47). However, CXCR4-mediated chemotactic mechanisms are complex and have not been completely defined. We and others (12–17, 44, 45) have shown that CXCR4-mediated chemotaxis involves activation of multiple signaling molecules including tyrosine phosphatases SHP1 and SHP2, focal adhesion components, Src-related kinases, and the T-cell activating molecule ZAP-70. In the present study, we have shown that another important component of the T-cell receptor signaling complex, the membrane-bound tyrosine phosphatase, CD45, also regulates CXCL12-induced and CXCR4-mediated chemotaxis. We observed reduced migration of CD45-negative T-lymphocytes in response to optimal concentrations of CXCL12 (10–100 ng/ml). However, migratory response was less significant at higher concentrations of CXCL12 (500–1000 ng/ml) (data not shown). The effects observed at higher CXCL12 concentrations were similar to those observed by other investigators (48). Reconstitution of full-length CD45 into J45.01 cells almost completely restored the chemotactic response induced by CXCL12. Furthermore, transfection of the cytoplasmic domain of CD45 (containing tyrosine phosphatase activity) into CD45-negative cells was also able to moderately restore the migratory response, suggesting that CD45 phosphatase activity is important for mediating CXCL12-induced chemotactic signaling. The cytoplasmic domain of CD45 has also been shown to be required for TCR-mediated signaling events (22, 23). The role of CD45 in cell spreading and chemotaxis is controversial. One report (49) indicates that in T-cells, the presence of CD45 prevents cell spreading in response to the binding of CD44, a cell adhesion molecule. However, other studies (27, 50) indicate that CD45 positively regulates integrin-mediated adhesion and spreading in macrophages and the chemotaxis of neutrophils.

Our data show that CXCL12 treatment increases the tyrosine phosphorylation of CD45. Similarly, T-cell activation has been shown to result in the phosphorylation of CD45 on tyrosine and serine residues located in its cytoplasmic domain (51, 52). Phosphorylation of CD45 might regulate its functions by altering its phosphatase activity or by providing docking sites for its interaction with other proteins (52). In the present studies, we observed by immunoprecipitation that CXCL12 treatment induced the association of CD45 with CXCR4. This result was further confirmed by confocal microscopy, which showed that the CXCR4 receptor colocalized with CD45. CD45 has been shown to interact with other cell surface molecules such as CD2, CD4, and TCR (53). We obtained a somewhat
CD45 Regulates CXCR4-mediated Chemotaxis

...tigated in CXCL12-induced MAP kinase activity and had no effect on CXCR4 internalization. HIV gp120-induced CXCR4 internalization was also not affected by the absence of CD45. These results suggest that CD45 is linked more specifically and positively to the CXCL12-induced chemotactic signaling pathway, has a slight negative effect on the MAP kinase pathway and no effect on CXCR4 internalization processes. In our previous studies, we found that CXCL12-induced chemotaxis was not related to MAPK activation (16). However, thrombin-induced MAP kinase activation was shown to be negatively regulated by CD45 in T-cells (59).

Taken together, our studies demonstrate a novel function of CD45 in regulating chemokine-induced T-cell chemotaxis. These findings also provide new information on the possible cross-talk between TCR and CXCR4-mediated pathways. Specifically, these pathways modulate T-cell chemotaxis through the regulation of various shared signaling substrates such as CD45, Lck, RAFTK, and FAK. Thus, the molecular mechanisms that regulate T-cell activation and migration may involve common signaling molecules, and hence the coordinated integration of both pathways is likely to play an important role in immune regulation and inflammation.

Acknowledgments—We thank Dr. Gary A. Koretzky (University of Pennsylvania School of Medicine) and Dr. Eric J. Brown (University of California, San Francisco, CA) for the generous gift of CD45 transfec-
tants. We also thank Janet Delahanty for editing the manuscript.

REFERENCES

...celli, E., and Thomas, M. L. (1999) Nature 393, 591–594
5. Zou, Y. R., Kottmann, A. H., Kuroda, M., Taniuchi, I., and Littman, D. R. (1998) Nature 393, 595–599
6. Nagasawa, T., Hirotta, S., Takashima, T., Takakura, N., Nishikawa, S., Kitamura, Y., Yoshida, N., Nishikawa, S., Kishimoto, T., and Nagasawa, T. (1996) Nature 383, 591–594
7. Thomas, M. L., and Brown, E. J. (1999) J. Exp. Med. 189, 1215–1225
8. Thomas, M. L., and Brown, E. J. (1999) J. Exp. Med. 189, 1203–1214
9. Thomas, M. L., and Brown, E. J. (1999) J. Exp. Med. 189, 1201–1202
10. Thomas, M. L., and Brown, E. J. (1999) J. Exp. Med. 189, 1199–1200
11. Thomas, M. L., and Brown, E. J. (1999) J. Exp. Med. 189, 1197–1198
CD45 Regulates CXCR4-mediated Chemotaxis

9543

28. Shenoi, H., Seavitt, J., Zheleznyak, A., Thomas, M. L., and Brown, E. J. (1999) J. Immunol. 162, 7120–7127
29. Licastro, F., Mallory, M., Hansen, L. A., and Masliah, E. (1998) J. Neuroimmunol. 88, 105–110
30. Ballerini, C., Rosati, E., Salvetti, M., Ristori, G., Cannoni, S., Biagioli, T., Massacesi, L., Sorbi, S., and Vergelli, M. (2002) Neurosci. Lett. 328, 325–327
31. Munshi, N., Groopman, J. E., Gill, P. S., and Ganju, R. K. (2000) J. Immunol. 164, 1169–1174
32. Ballerini, C., Rosati, E., Salvetti, M., Ristori, G., Cannoni, S., Biagioli, T., Massacesi, L., Sorbi, S., and Vergelli, M. (2002) Neurosci. Lett. 328, 325–327
33. Ganju, R. K., Dutt, P., Wu, L., Newman, W., Avraham, H., Avraham, S., and Groopman, J. E. (1998) Blood 91, 791–797
34. Ballerini, C., Rosati, E., Salvetti, M., Ristori, G., Cannoni, S., Biagioli, T., Massacesi, L., Sorbi, S., and Vergelli, M. (2002) Neurosci. Lett. 328, 325–327
35. Janes, P. W., Ley, S. C., Magee, A. I., and Kabouridis, P. S. (2000) Semin. Immunol. 12, 23–34
36. Su, M. W., Yu, C. L., Burakoff, S. J., and Jin, Y. J. (2001) J. Immunol. 166, 3975–3982
37. Dianzani, U., Bragardo, M., Funaro, A., Portoles, P., Rojo, J., Malavasi, F., and Pileri, A. (1995) Eur. J. Immunol. 25, 1306–1311
38. Peacock, J. W., and Jirik, F. R. (1999) J. Immunol. 162, 215–223
39. Li, R., Wong, N., Jabali, M. D., and Johnson, P. (2001) J. Biol. Chem. 276, 28767–28773
40. O'Th Educational Program, B., Radosevic, N., Taylor, M. L., Shivakrupa, DeBerry, C., Metcalfe, D. D., Zhou, M., Lowell, C., and Linnekin, D. (2001) Blood 98, 343–350
41. Su, M. W., Yu, C. L., Burakoff, S. J., and Jin, Y. J. (2001) J. Immunol. 166, 3975–3982
42. Schaller, M. D., and Parsons, J. T. (1995) Mol. Cell. Biol. 15, 2635–2645
43. Samuelson, L. E. (2002) Annu. Rev. Immunol. 20, 371–394
44. Ottoson, N. C., Prbila, J. T., Chan, A. S., and Shimizu, Y. (2001) J. Immunol. 167, 1857–1861
45. Tischioni, M., Charvet, C., Noraz, N., Lamy, L., Steinberg, M., Bernard, A., and Deckert, M. (2002) Blood 99, 3111–3118
46. Cho, E. Y., Schoenberger, E. S., Groopman, J. E., and Park, I. W. (2002) J. Biol. Chem. 277, 46039–46044
47. Muller, A., Homey, B., Soto, H., Ge, N., Catron, D., Buchanan, M. E., McManus, T., Murphy, E., Yuan, W., Wagner, S. N., Barrera, J. L., Mohar, A., Verastegui, E., and Zlotnik, A. (2001) Nature 410, 56–56
48. Peacock, J. W., and Jirik, F. R. (1999) J. Immunol. 162, 215–223
49. Li, R., Wong, N., Jabali, M. D., and Johnson, P. (2001) J. Biol. Chem. 276, 28767–28773
50. Harvath, L., Balle, J. A., Christiansen, N. P., Russell, A. A., and Skubitz, K. M. (1991) J. Immunol. 146, 949–957
51. Kang, S., Liao, P., Gage, D. A., and Esselman, W. J. (1997) J. Biol. Chem. 272, 11,018–11,025
52. Autero, M., Saharinen, J., Pessa-Morikawa, T., Soula-Rothhut, M., Oetken, C., Gassmann, M., Bergman, M., Alitalo, K., Barn, P., and Galmberg, C. G., et al. (1994) Mol. Cell. Biol. 14, 1359–1321
53. Dianzani, U., Bragardo, M., Funaro, A., Perteles, P., Rojo, J., Malavasi, F., and Pileri, A. (1995) Eur. J. Immunol. 25, 1306–1311
54. Su, M. W., Yu, C. L., Burakoff, S. J., and Jin, Y. J. (2001) J. Immunol. 166, 3975–3982
55. Isbiri, T., Ohnuma, K., Murokami, A., Takasawa, N., Kobayashi, S., Dang, N. H., Schlossman, S. F., and Morimoto, C. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 12138–12143
56. Inggerdippingen, M., Torgersen, K. M., and Maghazachi, A. A. (2002) Blood 99, 4318–4325
57. Dutt, P., Wang, J. F., and Groopman, J. E. (1998) J. Immunol. 161, 3652–3658
58. Gundersen, H. L., Lou, O., Arendt, C. W., and Berg, N. N. (1998) J. Biol. Chem. 273, 5692–5696
59. Maulon, L., Guerin, S., Ricci, J. E., Farah,Far, D., Breitmayer, J. P., and Auberg, P. (1998) Blood 91, 4232–4241
Differential Regulation of CXCR4-mediated T-cell Chemotaxis and Mitogen-activated Protein Kinase Activation by the Membrane Tyrosine Phosphatase, CD45

Aaron Z. Fernandis, Rama P. Cherla and Ramesh K. Ganju

J. Biol. Chem. 2003, 278:9536-9543.
doi: 10.1074/jbc.M211803200 originally published online January 8, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M211803200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 59 references, 37 of which can be accessed free at http://www.jbc.org/content/278/11/9536.full.html#ref-list-1