The β-chemokine receptor CCR5 has been shown to modulate cell migration, proliferation, and immune functions and to serve as a co-receptor for the human immunodeficiency virus. We and others have shown that CCR5 activates related adhesion focal tyrosine kinase (RAFTK)/Pyk2/CAK-β. In this study, we further characterize the signaling molecules activated by CCR5 upon binding to its cognate ligand, macrophage inflammatory protein-1β (MIP1β). We observed enhanced tyrosine phosphorylation of the phosphatases SHP1 and SHP2 upon MIP1β stimulation of CCR5 L1.2 transfectants and T-cells derived from peripheral blood mononuclear cells. Furthermore, we observed that SHP1 associated with RAFTK. However, using a dominant-negative phosphatase-binding mutant of RAFTK (RAFTKm906), we found that RAFTK does not mediate SHP1 or SHP2 phosphorylation. SHP1 and SHP2 also associated with the adaptor protein Grb2 and the Src-related kinase Syk. Pretreatment of CCR5 L1.2 transfectants or T-cells with the phosphatase inhibitor orthovanadate markedly abolished MIP1β-induced chemotaxis. Syk was also activated upon MIP1β stimulation of CCR5 L1.2 transfectants or T-cells and associated with RAFTK. Overexpression of a dominant-negative Src-binding mutant of RAFTK (RAFTKm308) significantly attenuated Syk activation, whereas overexpression of wild-type RAFTK enhanced Syk activity, indicating that RAFTK acts upstream of CCR5-mediated Syk activation. Taken together, these results suggest that MIP1β stimulation mediated by CCR5 induces the formation of a signaling complex consisting of RAFTK, Syk, SHP1, and Grb2.

Chemokines and their receptors play important roles in cell migration, growth, and host inflammatory responses (1–3). These receptors also serve as co-receptors for the human immunodeficiency virus (HIV) (4–6). Recently, chemokines also have been implicated in the development of Kaposi’s sarcoma via Kaposi’s sarcoma herpes virus (human herpes virus (HHV-8)), which encodes for several functional homologues of certain chemokines like MIP and of α-chemokine receptors, including CXCR1, CXCR2, and CXCR3 (7–10).

CCR5 is a G-protein-coupled seven-transmembrane receptor that belongs to the pro-inflammatory β-chemokine receptor family. It is a co-receptor for macrophage-tropic HIV-1 isolates (4, 11, 12). CCR5 is activated by the β-chemokines MIP1β, MIP1α, and RANTES. MIP1β has higher specificity for CCR5 than do MIP1α and RANTES, which also bind to CCR1 and CCR3 (1, 3, 12). Knockout mice lacking CCR5 revealed partial defects in macrophages and also showed enhanced T-cell-dependent immune response and delayed type hypersensitivity reaction, suggesting that CCR5 may play an important role in down-regulating T-cell-dependent immune responses (13).

Despite the emerging role of CCR5 and its ligands in HIV infection and the immune response, relatively little is known about the signaling pathways mediated by this receptor. CCR5 has been shown to induce calcium signals and chemotaxis upon binding to the macrophage-tropic HIV gp160 recombinant envelope protein (14). We (15) and others (16) have also recently shown that CCR5, upon binding to its cognate ligand (MIP1β) or to the HIV-1 envelope glycoprotein from a macrophage-tropic strain, activates a member of the focal adhesion kinase family called related adhesion focal tyrosine kinase (RAFTK; also known as Pyk2 and Cak-β). We further demonstrated that RAFTK associates with the cytoskeletal protein Paxillin upon CCR5 activation (15).

To further elucidate MIP1β-induced CCR5 signaling pathways, we have delineated the roles of the SH2 domain-containing cytoplasmic tyrosine phosphatase subfamily members SHP1 and SHP2 and of the Src-related kinase Syk. SHP1 (also named SHPTP1, PTPK, HC, or SHP) is predominantly expressed in hematopoietic cells, whereas SHP2 (also called SHPTP2, PTP1D, SYP, PTP2C, or SHPTP3) is expressed ubiquitously (17, 18). SHP1 acts as a negative regulator of intracellular signaling (19, 20). In contrast, SHP2 appears to play a positive role in regulating various growth factor-induced signaling pathways (21–24). SH2 domains of SHP1 and SHP2 have been shown to bind to several tyrosine-phosphorylated proteins (25–28). Mice deficient in SHP1 (motheaten or viable motheaten) suffer from several immunological, inflammatory, and hematological abnormalities (29, 30), whereas SHP2 mutant mice possess multiple defects in mesodermal patterning and die at mid-gestation (31). Furthermore, recently, it was shown that chemotactic responses to chemokine stromal cell-derived factor-1α were altered in mature and immature hematopoietic cells derived from motheaten mice (32).

Syk, a cytoplasmic protein-tyrosine kinase, plays an important role in the signaling pathways mediated by the B-cell antigen, Fc receptor, T-cell antigen receptor, and integrin receptor and thereby modulates cell growth and chemotaxis (33–}

Received for publication, January 24, 2000
Published, JBC Papers in Press, March 23, 2000, DOI 10.1074/jbc.M000689200

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* This work was supported in part by National Institutes of Health Grants HL53745 and HL61940 (to J. E. G.) and Grant CA76850 (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.

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† The abbreviations used are: HIV, human immunodeficiency virus; MIP, macrophage inflammatory protein; RANTES, regulated on activation normal T-cell expressed; RAFTK, related adhesion focal tyrosine kinase; RAFTKm906, wild-type RAFTK, FACS, fluorescence-activated cell sorter; GST, glutathione S-transferase.

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Syk interacts with various tyrosine-phosphorylated proteins (38, 39). Prior analysis of RAFTK indicated that tyrosine 402 in its N-terminal domain binds to the SH2 domain of Src kinases and activates such Src kinases upon lipopolysaccharide or bradykinin treatment (40).

This study demonstrates that MIP1β stimulation of CCR5 transfectants induces the tyrosine phosphorylation of SHP1 and SHP2 and activates Syk. This induction also results in the formation of a signaling complex consisting of RAFTK, Syk, SHP1, and Grb2. These results indicate that RAFTK acts upstream of Syk and suggest that it does not regulate the SHP1 and SHP2 phosphorylation mediated by CCR5.

**EXPERIMENTAL PROCEDURES**

**Reagents and Materials—**Anti-RAFTK antibodies were generated as described previously (41). This antisera recognized both human and murine forms of RAFTK and did not cross-react with focal adhesion kinase. Antibodies to Syk, SHP1, and SHP2 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Monoclonal anti-phosphotyrosine antibody (4G10) was a generous gift from Dr. Brian Druker (Oregon Health Sciences University, Portland, OR). Electrophoresis reagents and nitrocellulose membranes were obtained from Bio-Rad. The protease inhibitors leupeptin and α,α'-antitrypsin and all other reagents used were obtained from Sigma. Endo-LAM was purchased from Molecular Probes, Inc. (Eugene, OR).

**Construction of CCR5 Stable Transfectants—**CCR5 was transfected into the pre-B lymphoma cell line L1.2 as described previously (42, 43), and transfectants were selected in medium containing mycophenolic acid. FACs analysis was used to monitor the cell-surface expression of CCR5. CCR5 was expressed at a high level in these cells (42). The β-chemokines MIP1α, MIP1β, and RANTES bind with high affinity to the expressed CCR5 receptors. These cells possess properties characteristic of native CCR5, as they bind to macrophage-tropic HIV-1 gp120 in the presence of soluble human CD4 (42).

**Cell Culture—**CCR5 transfectants and mutants were grown in RPMI 1640 medium (containing 10% fetal calf serum, 2 mmol/liter glutamine, 1 mmol/liter sodium pyruvate, 50 μg/ml penicillin, 50 μg/ml streptomycin, and 55 μmol/liter β-mercaptoethanol) supplemented with 100 mmol/liter sodium hypoxanthine, 16 mmol/liter thymidine, 2.5 μmol/liter mycophenolic acid, and 125 μmol/liter xanthine. CCR5 L1.2 transfectants containing RAFTK constructs were grown in mycophenolic acid medium containing 0.8 mg/liter Gentamicin (G418, Life Technologies, Inc.).

**Transfectants—**Transfectants of mutants RAFTK m402, RAFTK m906, and wild-type RAFTK (RAFTKWT) were produced by transfection of the CCR5 L1.2 cells with the RAFTK m402, RAFTK m906, or RAFTKWT construct, respectively (15). pcDNA vector without an RAFTK construct was used as a control. Mutants RAFTK m402 and RAFTK m906 were generated by replacing Tyr402 and Tyr906 with Phe, respectively, by site-directed mutagenesis. Tyr402 of RAFTK is the pS-genetic binding site for phosphatases, and Tyr906 has previously been shown to bind to Src kinases (40). Plasmids carrying RAFTK m402, RAFTK m906, RAFTKWT, or pcDNA control vector were transfected by electroporation into the CCR5 L1.2 cells using Bio-Rad electroporation equipment. The transfectants were selected in medium containing mycophenolic acid and G418. The double mutants expressed equal levels of protein as determined by SDS-polyacrylamide gel electrophoresis and Western blot analysis. Several clones of double transfectants were used in the signaling studies.

**Primary Lymphocyte Culture—**T-cell-enriched, monocyte-depleted cultures were generated from peripheral blood mononuclear cells as described (15, 44). Briefly, peripheral blood mononuclear cells were separated by Ficoll-Hypaque gradient centrifugation and two rounds of adherence to plastic. Non-adherent cells were stimulated with phorbol ester (10 ng/ml) and ionomycin for 3 days. Cells were removed to fresh medium supplemented with recombinant human interleukin-2 (Advanced Biotechnologies, Columbia, MD). Three-week-old activated T-cells, which were found to be ~35% positive for CCR5 by FACS analysis, were used for further studies.

**Stimulation of Cells—**Cells were washed twice with Hank’s buffered saline solution (Sigma), resuspended at 1 × 10^6 cells/ml in Hank’s buffered saline solution, and then starved of serum for 2 h at 37 °C. The serum-starved cells were stimulated with 200 ng/ml MIP1β at 37 °C for various time periods. Following stimulation, cells were lysed using modified radioimmunoprecipitation assay buffer (50 mmol/liter Tris-HCl, pH 7.4, 1% Nonidet P-40, 150 mmol/liter NaCl, 1 mmol/liter phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 10 μg/ml pepstatin, 10 mmol/liter sodium vanadate, 10 mmol/liter sodium fluoride, and 10 mmol/liter sodium pyrophosphate). Total cell lysates were centrifuged at 10,000 × g for 10 min. Protein concentrations were determined by protein assay (Bio-Rad).

**Immunoprecipitation and Western Blot Analysis—**Immunoprecipitation studies were conducted as described (45). Briefly, identical amounts of protein from each time course were clarified by incubation with protein A-Sepharose CL-4B (Amersham Pharmacia Biotech) for 1 h at 4 °C. Protein A-Sepharose was removed by brief centrifugation, and the supernatants were incubated with different primary antibodies as described below for each experiment for 2 h at 4 °C. Immunoprecipitation of the antibody-antigen complexes was performed by incubation at 4 °C with protein A-Sepharose (10% suspension). Non-specific bound proteins were removed by washing the Sepharose beads three times with modified radioimmunoprecipitation assay buffer and one time with phosphate-buffered saline. Immune complexes were solubilized in 30 μl of 2× Laemmli buffer, and samples were separated on 5 or 12% SDS-polyacrylamide gel and then transferred to nitrocellulose membranes. The membranes were blocked in 5% nonfat milk protein for 1 h and probed with primary antibody for 3 h at room temperature or at 4 °C overnight. Immunoreactive bands were visualized using horseradish peroxidase-conjugated secondary antibody and the enhanced chemiluminescence system (ECL, Amersham Pharmacia Biotech). The densitometric scanning of films was done using a Bio-Rad Model GS-700 imaging densitometer.

**Syk kinase assay—**The Syk kinase assay was performed by first immunoprecipitating lysates with anti-Syk antibody. The immune complexes were then washed twice with radioimmunoprecipitation assay buffer and twice with Syk kinase buffer (20 mm Hepes, 50 mm NaCl, 10 μg/ml NaVO₃, 5 mm MgCl₂, and 5 mm MnCl₂). The complex was incubated in Syk kinase buffer and 5 μCi of [γ-32P]ATP for 20 min at 30 °C. The reaction was terminated by adding 4× SDS sample buffer and boiling the samples for 5 min at 100 °C. Proteins were then separated on 8% SDS-polyacrylamide gel and detected by autoradiography. Rabbit IgG was used as a negative control.

**Glutathione S-Transferase Fusion Protein Binding Studies—**The RAFTK C-terminal domain (amino acids 681–1009)-glutathione S-transferase (GST) fusion protein was produced as described (46). Briefly, the fusion protein was expressed in E. coli and cloned into the pGEX-2T expression vector (Amersham Pharmacia Biotech). The GST fusion protein was produced by 1× isopropyl-β-D-thiogalactopyranoside induction and purified by affinity chromatography on a glutathione-Sepharose column (Amersham Pharmacia Biotech) according to the manufacturer’s recommendations. Grb2-GST fusion proteins were purchased from Santa Cruz Biotechnology. For the immunoprecipitation experiment, GST alone was pre-absorbed to remove phosphatases. The bound proteins were eluted by boiling in Laemmli sample buffer and subjected to 8% SDS-polyacrylamide gel electrophoresis and Western blot analysis.

**Chemotaxis Assay—**The assay was performed in 24-well plates containing 5-μm porosity inserts (Costar Corp., Cambridge, MA) as described (45). Briefly, cells were resuspended at 6.8 × 10⁶/ml in RPMI 1640 medium containing 2.5% bovine serum albumin. 50 ng of MIP1β were added to the bottom wells, and 150 μl of cells (1 × 10⁶) untreated or pretreated with different concentrations of sodium orthovanadate were loaded onto the inserts. Cells migrating to the bottom wells were collected, centrifuged, and counted. The results shown are representative of findings from three independent experiments.

**RESULTS**

**SHP1 and SHP2 Are Tyrosine-phosphorylated upon MIP1β Stimulation—**Cytoplasmic tyrosine phosphatases have been shown to be important positive and negative regulators of signaling pathways. To characterize the role that phosphatases play in CCR5-mediated signal transduction, the CCR5 transfectants were stimulated with MIP1β, and lysates were analyzed for SHP1 and SHP2 tyrosine phosphorylation. As shown in Fig. 1 (A and C, respectively), MIP1β stimulation of CCR5 transfectants resulted in an increase in tyrosine phosphorylation of SHP1 and SHP2. Equal amounts of SHP1 and SHP2 proteins were present in each lane (Fig. 1, A and C, lower
The TCL lanes A immunoprecipitates with control IgG (lated with MIP1 and cell lysates (A control IgG (C lanes RAFTK (SDS-polyacrylamide gel electrophoresis and immunoblotted with photophosphotyrosine antibody (upper panels), followed by anti-SHP1 antibody (A and B, lower panels) or anti-SHP2 antibody (C and D, lower panels). The TCL lanes represent 30 μg of total cell lysate. Fold increase represents values calculated after densitometric scanning of gels. WB, Western blot.

Fig. 1. Tyrosine phosphorylation of SHP1 and SHP2 upon MIP1β stimulation. Unstimulated (0 lanes) or stimulated CCR5 L1.2 cell lysates (A and C) and T-cells (B and D) were immunoprecipitated (IP) with anti-SHP1 (A and B) or anti-SHP2 (C and D) antibody or control IgG (C lanes). The immune complexes were run on SDS-polyacrylamide gel and subjected to serial immunoblotting with anti-phosphotyrosine antibody (upper panels), followed by anti-SHP1 antibody (A and B, lower panels) or anti-SHP2 antibody (C and D, lower panels). The TCL lanes represent 30 μg of total cell lysate. Fold increase represents values calculated after densitometric scanning of gels. WB, Western blot.

Tyro11n phosphorylation in MIP1β-stimulated T-cells as compared with the similarly stimulated CCR5 L1.2 transfectants (15). SHP1 and SHP2 Associate with Various Signaling Molecules—Tyr906 of RAFTK may act as a putative binding site for SHP proteins. Therefore, we examined the association of RAFTK with SHP1. As shown in Fig. 2A, SHP1 associated with RAFTK constitutively; this association was modestly increased upon MIP1β stimulation. To further confirm this association, we used a GST fusion protein containing the C-terminal domain of RAFTK. As shown in Fig. 2B, SHP1 was observed to associate with this fusion protein. SHP1 also associated with Grb2 upon MIP1β stimulation (Fig. 2C). Since SHP2 was also tyrosine-phosphorylated following MIP1β stimulation, we studied whether it associated with the adaptor protein Grb2. As shown in Fig. 2D, SHP2 associated with Grb2, and this association increased upon chemokine stimulation.

RAFTKm906 Has No Effect on the SHP1 or SHP2 Phosphorylation Induced by MIP1β—Since SHP1 was shown to associate with RAFTK upon MIP1β stimulation, we were interested in whether RAFTK modulated the MIP1β-stimulated phosphorylation of SHP1. To address this question, we created double-transfected L1.2 cells that expressed human CCR5 and RAFTKm906 in which Tyr906 was replaced with Phe. Tyr906 of RAFTK is a putative binding site for phosphatases. As shown in Fig. 3 (A and B, respectively), there was no significant increase in tyrosine phosphorylation of SHP1 and SHP2 in the RAFTKm906 transfectants as compared with the pcDNA transfectants. An equal amount of SHP1 and SHP2 proteins was present in all samples (Fig. 3, A and B, lower panels).

Effect of Phosphatase Inhibitor on MIP1β-induced Migration of CCR5 Cells—Tyrosine phosphatases have been shown to

Fig. 2. SHP1 associates with RAFTK and Grb2 upon MIP1β stimulation. CCR5 L1.2 cell lysates unstimulated (0 lanes) or stimulated with MIP1β for different time periods were immunoprecipitated (IP) with anti-SHP1 (A), RAFTK C-terminal domain-GST fusion protein (B), or Grb2-GST (C and D). The immune complexes were subjected to SDS-polyacrylamide gel electrophoresis and immunoblotted with RAFTK (A), SHP1 (B and C), or Grb2 (D). The C lanes represent immunoprecipitates with control IgG (A) or GST fusion protein (B–D). The TCL lanes represent 30 μg of total cell lysate. WB, Western blot.
were stimulated with MIP1 and adhesion. To characterize the role of Syk in CCR5-mediated
migration—
The Src-related kinase Syk has been shown to partici-

play an important role in chemotaxis. To determine the poten-
tial role of phosphatases in MIP1β-stimulated chemotaxis, we
examined MIP1β-induced migration in the presence of differ-
ent concentrations of the phosphatase inhibitor orthovanadate.
As shown in Fig. 4 (A and B, respectively), orthovanadate
treatment attenuated the MIP1β-induced migration of CCR5
L1.2 cells and activated T-cells in a dose-dependent manner.
100 μM orthovanadate resulted in ~85% inhibition of CCR5
L1.2 and ~70% inhibition of T-cell chemotaxis. No effect on cell
survival or growth was observed under similar conditions.

Syk Is Phosphorylated and Activated upon MIP1β Stimulation—The Src-related kinase Syk has been shown to partici-
pate in various signaling pathways regulating cell growth and
adhesion. To characterize the role of Syk in CCR5-mediated
signal transduction pathways, CCR5 transfectants or T-cells
were stimulated with MIP1β, and the lysates were analyzed for
Syk kinase activation. As shown in Fig. 5A (upper panel),
MIP1β stimulation of the CCR5 L1.2 transfectants resulted in
enhanced tyrosine phosphorylation of Syk. Equal amounts of
Syk protein were present in each lane (Fig. 5A, lower panel).
In addition, MIP1β stimulation induced Syk-autophosphorylating
activity in CCR5 L1.2 transfectants (Fig. 5B) and, to a lesser
degree, in T-cells (Fig. 5C). Similar to SHP1 and SHP2 phos-
phorylation, Syk-autophosphorylation activity was reduced in
T-cells as compared with the CCR5 L1.2 transfectants upon
MIP1β stimulation.

Syk Associates with RAFTK, Grb2, SHP1, and SHP2—To

further characterize the role that Syk might play in CCR5-
mediated signaling, we sought to identify proteins that associ-
ate with Syk upon MIP1β stimulation. We observed a constit-
tive association of Syk with RAFTK, an association that was
enhanced upon MIP1β stimulation (Fig. 6A). RAFTK has pre-
viously been shown to be phosphorylated and activated by
MIP1β (15). Furthermore, Syk was also shown to associate
with the SH2 domain of Grb2. This association was enhanced
upon MIP1β stimulation (Fig. 6B). Since we observed that Syk
was activated and associated with RAFTK upon MIP1β stimu-
luation, we subsequently studied the association of Syk with
SHP1. As shown, Syk also associated with SHP1 (Fig. 7), and
this association was enhanced upon MIP1β stimulation.

Syk Stimulation by MIP1β Is Mediated by RAFTK—Since
RAFTK associated with Syk, we wanted to see whether this
association was important for Syk activation. To address this
question, we created double-transfected L1.2 cells that ex-
pressed human CCR5 and RAFTKWT, or dominant-negative
mutant RAFTKm402 as compared with transfectants
expressing the pcDNA vector (Fig. 8A). We also observed an ~2-fold decrease in Syk activity in the CCR5 L1.2 transfectants overexpressing the dominant-negative mutant RAFTKm402 as compared with transfectants
expressing the pcDNA vector (Fig. 8B). These studies suggest
that RAFTK may regulate Syk kinase activation in these cells.

DISCUSSION
In our earlier studies using CCR5-transfected murine pre-B
lymphoma L1.2 cells as a model, we have shown that MIP1β
stimulation results in activation of RAFTK/Pyk2 (15). Further-
more, HIV gp120 binding to CCR5 and chemokine stimulation have also been shown to induce RAFTK/Pyk2 phosphorylation (16, 47). In the present study, we have further characterized the role of RAFTK and investigated the possible involvement of the phosphatases SHP1 and SHP2 and the Src-related kinase Syk in signaling pathways mediated by CCR5. Cytoplasmic tyrosine phosphatases and Src-related kinases are known to modulate regulatory pathways of cell spreading, migration, and cytoskeletal organization (48–52).

MIP1β stimulation enhanced tyrosine phosphorylation of the phosphatases SHP1 and SHP2. Both of these phosphatases have been shown to play a role in various receptor-mediated pathways. SHP1 has been shown to act as a negative regulator of signaling pathways, whereas SHP2 appears to function as a positive mediator (17, 18, 21–24). Recent studies of hematopoietic cells from motheaten mice indicate that SHP1 plays an important role in the regulation of the stromal cell-derived factor-1-induced signaling pathway (32). We have observed that SHP1 is associated with RAFTK. However, RAFTK does not appear to mediate the MIP1β-stimulated phosphorylation of SHP1 or SHP2 since overexpression of a dominant-negative phosphatase-binding mutant of RAFTK had little effect on MIP1β-stimulated SHP1 or SHP2 phosphorylation.

We also observed that chemokine stimulation resulted in the enhanced association of SHP1 and SHP2 with Syk and Grb2. In different signaling pathways, SHP1 and SHP2 are known to associate with various signaling molecules, including Vav, Grb2, SOS, and SLP-76 via their SH2 domains (53–56). In addition to their role as phosphatases, SHP1 and SHP2 may act as adaptor proteins by providing docking sites for the recruitment of downstream signaling molecules. The present study indicates that tyrosine phosphatases may play an important role in the regulation of MIP1β-induced migration, as orthovanadate treatment markedly attenuated MIP1β-stimulated chemotaxis. Recently, platelet-derived growth factor-induced migration was shown to be regulated by SHP2 (48).

MIP1β stimulation also induced activation of Syk, a Src-related kinase. Syk has been shown to play a role in signal transduction pathways mediated by B- and T-cell antigen receptors, the Fc receptor, various growth factor receptors, and integrin receptors (33–37). However, the G-protein-coupled m1 muscarinic receptor does not activate Syk. Tyr402 (autophosphorylation site) of RAFTK has been shown to bind to Src kinases, which results in their activation (40). In the present study, we observed that Syk associated with RAFTK and that this association was enhanced upon MIP1β stimulation. RAFTK association with another Src-related kinase, Fyn, has been shown to play an important role in mediating T-cell receptor signal transduction (46, 57). Furthermore, RANTES has been shown to induce ZAP-70 activity and its association with focal adhesion kinase in T-cells (58). In this study, RAFTK appeared to partially mediate Syk activation, as overexpression of a Src-binding mutant of RAFTK resulted in the reduced activation of Syk, whereas overexpression of wild-type RAFTK enhanced Syk activity. Recently, Syk was shown to be upstream of RAFTK/Pyk2 phosphorylation in Fce receptor-1-induced tyrosine phosphorylation in mast cells, whereas Pyk2 phosphorylation by thrombin and the adenosine G-protein-coupled receptor was independent of RAFTK/Pyk2 in these cells (59).

We also observed that Syk associated with SHP1 and that this association was enhanced by chemokine stimulation. Tyrosine phosphatases can cause activation of Src kinases (60). SHP1 has been shown to regulate Syk activity, as overexpression of SHP1-inactive mutants in B lymphoma cell lines results in enhanced Syk kinase activity (61).

Taken together, our results provide new information regarding various downstream signaling molecules involved in CCR5-mediated signaling pathways. We have found that MIP1β stimulation of CCR5 activates Syk and increases the tyrosine phosphorylation of the SH2-domain containing phosphatases SHP1 and SHP2. This results in the formation of a multimeric complex consisting of RAFTK, Syk, Grb2, and SHP1. RAFTK...
was shown to partially mediate the activation of Syk, but had no significant effect on SHP1 or SHP2 tyrosine phosphorylation. These results suggest that RAFTK differentially regulates several downstream signaling targets that are activated upon CCR5 stimulation.

Acknowledgments—We are grateful to Walter Newman and Lijun Wu (LeukoSite, Inc.) for providing the CCR5 L1.2 transfectants. We also thank Janet Delahunty for editing, Nancy DesRosiers and Dan Kelley for preparation of the figures, and Simone Jaudusihg for typing the manuscript.

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