The Rap GTPases Regulate B Cell Migration Toward the Chemokine Stromal Cell-Derived Factor-1 (CXCL12): Potential Role for Rap2 in Promoting B Cell Migration[1]

Sarah J. McLeod, Anson H. Y. Li, Rosaline L. Lee, Anita E. Burgess, and Michael R. Gold[2]

Stromal cell-derived factor-1 (SDF-1) is a potent chemoattractant for B cells and B cell progenitors. Although the binding of SDF-1 to its receptor, CXCR4, activates multiple signaling pathways, the mechanism by which SDF-1 regulates cell migration is not completely understood. In this report we show that activation of the Rap GTPases is important for B cells to migrate toward SDF-1. We found that treating B cells with SDF-1 resulted in the rapid activation of both Rap1 and Rap2. Moreover, blocking the activation of Rap1 and Rap2 via the expression of a Rap-specific GTPase-activating protein significantly reduced the ability of B cells to migrate toward SDF-1. Conversely, expressing a constitutively active form of Rap2 increased SDF-1-induced B cell migration. Thus, the Rap GTPases control cellular processes that are important for B cells to migrate toward SDF-1. *The Journal of Immunology,* **2002, 169: 1365–1371.**

C hemokine-directed migration is essential for B cell development, B cell activation, the differentiation of B cells into Ab-producing cells, and the long-term survival of plasma cells (1–4). The chemokine stromal cell-derived factor-1 (SDF-1),[3] also known as CXC chemokine ligand 12, is involved in all of these processes. First, SDF-1 made by bone marrow stromal cells plays an essential role in retaining B cell precursors in the bone marrow (5), where they can adhere to stromal cells and receive survival and differentiation signals. B cell development is severely impaired in mice lacking either SDF-1 or its receptor, CXCR4 (6–8). SDF-1 is also a potent chemoattractant for both mature B cells and memory B cells (9–11) and may contribute to their ability to migrate into and within lymphoid organs (1), where they can encounter Ag and receive T cell-derived costimulatory signals. Finally, SDF-1 directs plasma cells to the bone marrow (4), where they can survive and produce Abs for long periods of time (12).

Cell migration is a complex process that involves the establishment of a polarized morphology oriented in the direction of movement, actin-dependent formation of membrane processes such as lamellipodia that move the leading edge of the cell forward, transient integrin-dependent adhesion at the leading edge of the cell, and actin/myosin-dependent forces that pull the rear of the cell in the direction of migration (13). The mechanism by which chemokine receptor signaling regulates these processes is not completely understood. Chemokines such as SDF-1 signal via heptahelical receptors. Pertussis toxin-sensitive G proteins couple these receptors to phospholipase C (PLC)-β, resulting in increases in intracellular Ca^{2+} as well as the diacylglycerol (DAG)-dependent activation of protein kinase C (14, 15). SDF-1 also activates the Fyn, Lyn, and Pyk2 tyrosine kinases, the extracellular signal-regulated kinase (ERK), and the phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway (16–18). Although Pyk2, PI3K, and protein kinase C have been implicated in SDF-1-dependent integrin activation and lymphocyte migration (19–21), it is likely that many signaling pathways regulate these processes.

In this report we investigated the role of the Rap GTPases in SDF-1-induced B cell migration. Rap1A, Rap1B, Rap2A, and Rap2B (each encoded by a separate gene and collectively referred to as Rap) belong to the Ras superfamily of GTPases. Rap1A and Rap1B are 97% identical and are assumed to be functionally equivalent. The same is true for Rap2A and Rap2B. Rap1 and Rap2 are more distantly related, with ~60% identity at the amino acid level. Like other GTPases, Rap1 and Rap2 are molecular switches that cycle between an inactive GDP-bound state and an active GTP-bound state. Many receptors have been shown to activate Rap1 (reviewed in Ref. 22). In contrast, few extracellular stimuli that activate Rap2 have been identified (23). Although it is not known to what extent the functions of Rap1 and Rap2 overlap, several lines of evidence suggested that Rap1 could be involved in cell migration. First, activated Rap1 can induce integrin activation (24–28), which is important for adhering the leading edge of the cell to the substrate during cell migration. Second, the putative *Saccharomyces cerevisiae* ortholog of Rap1, BUD1/Rsr1, is involved in bud site selection (29), a cell polarity decision that may be analogous to what occurs when lymphocytes assume a polarized morphology in the direction of a chemoattractant. Finally, in *Drosophila*, loss-of-function mutations in Rap1 are embryonically lethal because multiple cell types fail to migrate to the proper location (30).

We show in this report that SDF-1 activates both Rap1 and Rap2 in B cell lines and that blocking SDF-1-induced activation of Rap1 and Rap2 decreases the ability of these cells to migrate toward SDF-1. Conversely, expressing a constitutively active form of Rap2 in B cells increases their ability to migrate toward SDF-1. This is the first reported function for Rap2 and indicates that Rap1 and/or Rap2 regulate processes that are important for chemokine-induced B cell migration.

Materials and Methods

Cell culture

The WEHI-231 and 2PK3 murine B cell lines were grown in RPMI 1640 supplemented with 10% heat-inactivated FCS, 50 μM 2-ME, 2 mM glutamine, and 1 mM pyruvate. The DT40 chicken B cell line was grown in the same medium with 1% heat-inactivated chicken serum added. To reduce basal signaling caused by serum components, WEHI-231 cells were grown overnight in medium containing 1% FCS before being used for Rap activation assays.

Rap1, Rap2, and Rac1 activation assays

Cells were resuspended to 2.5×10^7 per milliliter in modified HEPES-buffered saline (31) and stimulated with recombinant murine SDF-1 (R&D Systems, Minneapolis, MN) or with phorbol dibutyrate (PdBu; Sigma-Aldrich, St. Louis, MO). Where indicated, the cells were pretreated with the PLC inhibitor U73122 or its inactive structural analog U73343 (BioMol, Plymouth Meeting, PA). The cells were solubilized and assayed for Rap activation as described previously (32). Briefly, a GST-RalGDS fusion protein was used to selectively precipitate the activated GTP-bound forms of Rap1 and Rap2, which were then detected by immunoblotting with anti-Rap1 (Santa Cruz Biotechnology, Santa Cruz, CA) or anti-Rap2 Abs (BD Transduction Laboratories, Lexington, KY). In some experiments the filters were probed with anti-Rap2 Abs, stripped, and then reprobed with anti-Rap1 Abs. For Rac1 activation assays, the cells were solubilized in a buffer containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% Nonidet P-40, 150 mM NaCl, 30 mM MgCl$_2$, 10% glycerol, 1 mM DTT, 1 mM PMSF, 1 mM pepstatin, and 1 mM Na$_3$VO$_4$. A GST fusion protein containing the Rac1-binding domain of Pak1β (a gift from Dr. A. Hall, University College, London, U.K.) was used to selectively precipitate the activated GTP-bound form of Rac1 (33), which was detected by immunoblotting with an anti-Rac1 Ab (Upstate Biotechnology, Lake Placid, NY). Immunoreactive bands were visualized with HRP-conjugated secondary Abs and ECL detection. To quantitate band intensities, films were scanned, saved as TIFF files, and analyzed using ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

Expression of RapGAPII and Rap2V12 in B cell lines

cDNAs encoding FLAG-tagged RapGAPII (34) or Rap2V12 in the pMSCV retroviral vector were gifts from Dr. M. Matsuda (Osaka University, Osaka, Japan). As described (35), these plasmids or the pMSCV vector were transfected into the BOSC23 packaging cell line and the resulting retroviruses were used to infect WEHI-231 cells, 2PK3 cells, or a variant of the DT40 cell line expressing a transfected murine ecotropic retrovirus receptor (35). Puromycin selection was used to obtain bulk populations of stably infected cells. Expression of FLAG-RapGAPII or FLAG-Rap2V12 was detected by immunoblotting with anti-RapGAPII Abs (34) or with the M2 anti-FLAG mAb (Sigma-Aldrich).

ERK and Akt activation assays

Cells were stimulated with SDF-1, washed with PBS, and then solubilized in RIPA buffer (30 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1% Igepal CA-630 (Sigma-Aldrich), 0.5% sodium deoxycholate, 0.1% SDS, 2 mM EDTA, 1 mM PMSF, 10 μg/ml leupeptin, 1 μg/ml aprotinin, 1 mM Na$_3$VO$_4$, 25 mM β-glycerophosphate, 1 μg/ml microcystin-LR). Detergent-insoluble material was removed by centrifugation. Cell extracts (5 μg protein for ERK and 25 μg protein for Akt) were analyzed by immunoblotting with Abs that recognize the phosphorylated, active forms of ERK or Akt (Cell Signaling Technologies, Beverly, MA). The blots were then reprobed with Abs to ERK (Santa Cruz Biotechnology) or Akt (Cell Signaling Technologies).

Flow cytometry

To analyze cell surface expression of CXCR4, cells were stained with 10 μg/ml rabbit anti-human CXCR4 (Santa Cruz Biotechnology) followed by FITC-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, PA). To avoid FcR interactions, 2PK3 cells were preincubated with 100 μg/ml 2.4G2 anti-FcγRII mAb.

Migration assays

Migration assays were performed in 24-well plates using 5-μm polycarbonate Transwell inserts (Costar, Cambridge, MA) as described by Reif and Cyster (36). SDF-1 was diluted in chemotaxis medium (RPMI 1640/10 mM HEPES/0.5% BSA) and added to the lower chamber while 5×10^5 cells in 0.1 ml chemotaxis medium were added to the upper chamber. After 3 h at 37°C, the number of cells that had migrated into the lower chamber was determined using flow cytometry. The medium from the lower chamber was passed through a FACScan for 30 s, gating on forward and side scatter to exclude cell debris. The number of live cells was compared with a 100% migration control in which 5×10^5 cells had been pipetted directly into the lower chamber and then counted on the FACScan for 30 s.

Results

Because Rap1 plays an essential role in cell migration during *Drosophila* embryogenesis (30), we hypothesized that the Rap GTPases might also be involved in the chemokine-induced migration of B lymphocytes. As an initial test of this hypothesis, we asked whether treating B cells with the chemokine SDF-1 induced the activation of Rap1 or Rap2. We tested this in three different B cell lines that had been reported to migrate in response to SDF-1, the 2PK3 and WEHI-231 murine B cell lines (36) and the DT40 chicken B cell line (11). To assess Rap activation we used a GST-RalGDS fusion protein to selectively precipitate the active GTP-bound forms of Rap1 and Rap2, which could then be detected by immunoblotting. We found that SDF-1 treatment increased the amount of active GTP-bound Rap1 and GTP-bound Rap2 in 2PK3 cells (Fig. 1A), WEHI-231 cells (Fig. 1B), and DT40 cells (see Fig. 3D). SDF-1-induced activation of Rap1 and Rap2 was observed within 2 min of adding SDF-1 to the cells and persisted for at least
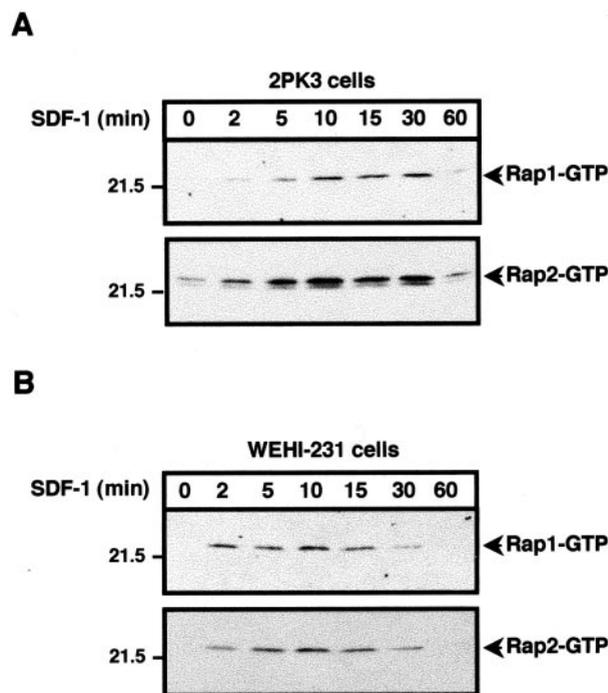

FIGURE 1. SDF-1 activates Rap1 and Rap2 in B cells. 2PK3 cells (*A*) or WEHI-231 cells (*B*) were stimulated with 100 ng/ml (12.5 nM) SDF-1 for the indicated times. A GST-RalGDS fusion protein was used to selectively precipitate the active GTP-bound forms of Rap1 and Rap2, which were detected by immunoblotting with specific Abs. Molecular mass markers (in kilodaltons) are shown to the *left*. For each panel similar results were obtained in at least three independent experiments.
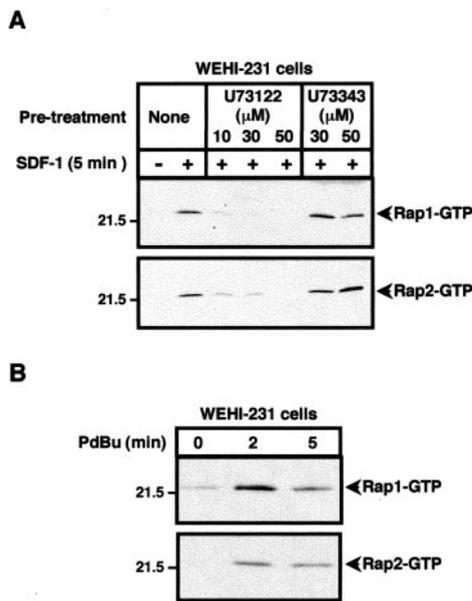
FIGURE 2. Activation of Rap1 and Rap2 via PLC-derived second messengers. *A*, WEHI-231 cells were pretreated with the indicated concentrations of the PLC inhibitor U73122 or the inactive structural analog U73343 for 20 min. The cells were then stimulated for 5 min with 100 ng/ml (12.5 nM) SDF-1. Rap activation was assessed as in Fig. 1. *B*, WEHI-231 cells were stimulated with 100 nM PdBu, a phorbol ester that mimics the PLC-derived second messenger DAG. Rap activation assays were then performed. For each panel similar results were obtained in at least three independent experiments.

15–30 min. This is the first report of receptor-induced activation of Rap2. However, because Rap2 appears to be regulated by the same exchange factors that activate Rap1 (37), it is not surprising that these GTPases are coordinately regulated.

Next, we wished to identify the signaling pathway that couples the SDF-1 receptor, CXCR4, to the activation of Rap1 and Rap2. We had previously shown that the B cell Ag receptor activates Rap1 via PLC-γ2-dependent production of DAG (32). Because chemokine receptor signaling leads to the PLC-β-dependent production of DAG, we hypothesized that SDF-1 would activate Rap1 and Rap2 via this pathway. To test this we asked whether the PLC inhibitor U73122 (38) would block SDF-1-induced activation of

Rap1 and Rap2. Indeed, we found that the activation of Rap1 and Rap2 by SDF-1 was significantly inhibited when WEHI-231 cells were pretreated with 10–50 μM U73122 (Fig. 2*A*). In contrast, 30–50 μM U73343 (38), an inactive structural analog of U73122, did not inhibit the activation of Rap1 or Rap2 (Fig. 2*A*). SDF-1-induced activation of Rap1 and Rap2 was also inhibited by U73122, but not U73343, in 2PK3 cells (data not shown). Thus, in B cells, SDF-1-induced activation of Rap1 and Rap2 is dependent on PLC activity. Moreover, treating WEHI-231 cells with the phorbol ester PdBu, which mimics the action of DAG, resulted in the activation of both Rap1 and Rap2 (Fig. 2*B*). Because the other PLC-derived second messenger, Ca^{2+}, does not cause significant activation of Rap1 (32) or Rap2 (data not shown) in B cells, it is likely that SDF-1 activates Rap1 and Rap2 via PLC-β-dependent production of DAG.

To test whether activation of Rap1 and/or Rap2 is important for B cells to migrate toward SDF-1, we used a loss-of-function approach in which we blocked Rap activation by expressing RapGAPII in B cell lines. RapGAPII is a Rap-specific GTPase-activating protein (GAP) that is expressed in the brain (34) but not in B cells (Fig. 3*A*). It promotes the conversion of active GTP-bound Rap1 and Rap2 to the inactive GDP-bound form (34, 37) while having no effect on the activation of other closely related GTPases such as Ha-Ras (34), R-Ras (34), RhoA (39), and Rac1 (see Fig. 4*C*). The use of Rap-specific GAPs has proven to be a powerful tool for elucidating the functions of the Rap GTPases. In particular, inhibiting Rap activation via the expression of RapGAPII has been used to show that Rap activation is important for receptor-induced integrin activation (24–27).

We used retroviral-mediated gene transfer to generate bulk populations of 2PK3, WEHI-231, and DT40 cells that stably express RapGAPII (Fig. 3*A*). Rap activation assays showed that expressing RapGAPII strongly inhibited SDF-1-induced activation of Rap1 and Rap2 in all three of these B cell lines (Fig. 3, *B–D*). Densitometry revealed that SDF-1-induced Rap1 activation was inhibited by >95% while Rap2 activation was inhibited by at least 80% in all three B cell lines. While RapGAPII expression inhibited Rap activation, it did not affect other aspects of SDF-1 signaling. First, we used flow cytometry to show that RapGAPII expression did not down-regulate CXCR4, the receptor for SDF-1 (Fig. 4*A*). The cell surface expression of CXCR4 in RapGAPII-expressing WEHI-231

FIGURE 3. RapGAPII expression inhibits SDF-1-induced activation of Rap1 and Rap2. *A*, Retroviruses were used to establish stable bulk populations of 2PK3, WEHI-231, and DT40 cells containing either the empty pMSCV vector (*lane 1*) or pMSCV containing cDNA encoding FLAG-tagged RapGAPII (*lane 2*). The expression of FLAG-RapGAPII was detected by immunoblotting cell extracts with anti-RapGAPII Abs. Similar results were obtained using anti-FLAG Abs. *B–D*, Vector-transfected and RapGAPII-expressing 2PK3 cells (*B*), WEHI-231 cells (*C*), or DT40 cells (*D*) were stimulated with 100 ng/ml (12.5 nM) SDF-1 for the indicated times. Rap activation was analyzed as in Fig. 1. Molecular mass markers (in kilodaltons) are shown to the *left*. For each panel similar results were obtained in at least two independent experiments.
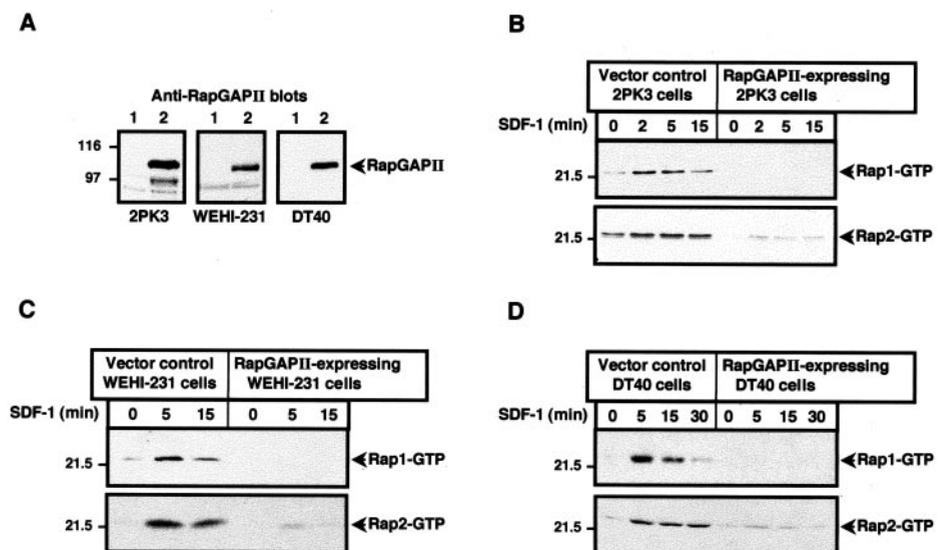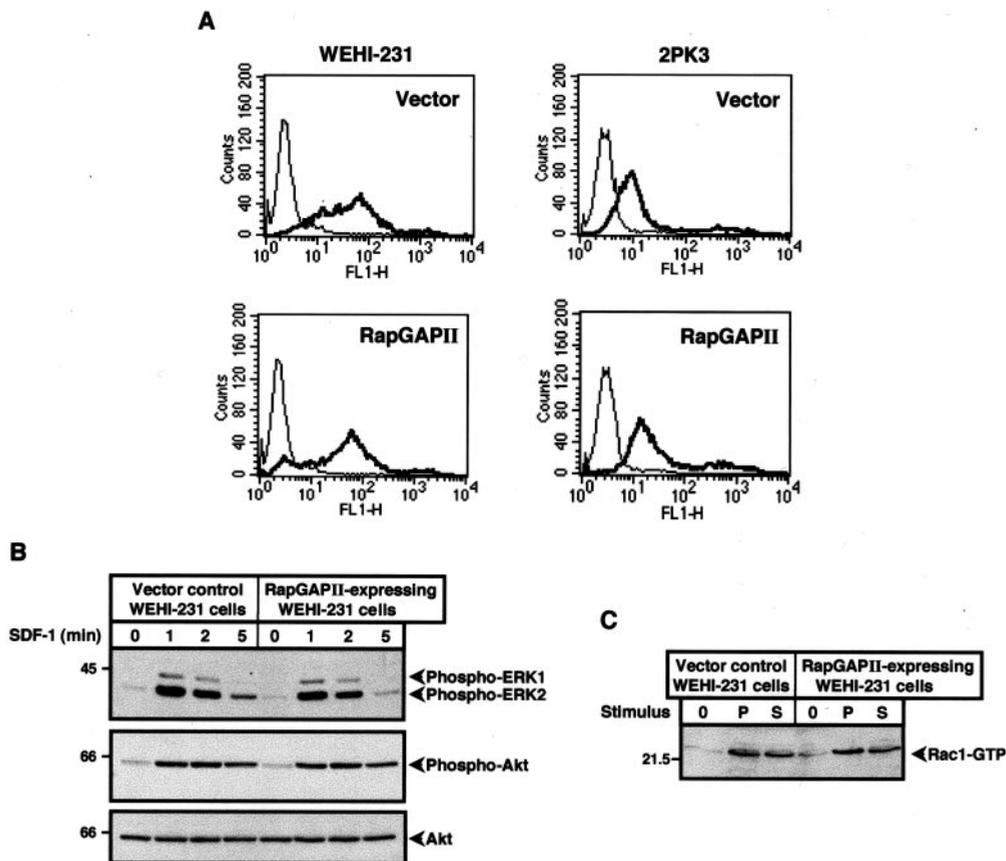
FIGURE 4. RapGAPII expression does not alter other aspects of SDF-1-induced signaling. *A*, RapGAPII expression does not alter cell surface expression of CXCR4. Vector-transfected and RapGAPII-expressing WEHI-231 cells (*left panels*) or 2PK3 cells (*right panels*) were analyzed for CXCR4 expression by flow cytometry. The thin lines represent unstained cells while the thick lines represent cells stained with rabbit anti-CXCR4 plus FITC-conjugated goat anti-rabbit IgG. CXCR4 expression on DT40 cells could not be assessed because Abs to chicken CXCR4 are not available. *B*, RapGAPII expression does not inhibit SDF-1-induced activation of ERK or Akt. Vector-transfected and RapGAPII-expressing WEHI-231 cells were stimulated with 100 ng/ml (12.5 nM) SDF-1 for the indicated times. Activation of the ERK and Akt kinases was analyzed by immunoblotting with Abs that recognize the active, phosphorylated forms of ERK1 and ERK2 (*top panel*) or Akt (*middle panel*). Equal loading was confirmed by reprobing the blots with anti-Akt Abs (*bottom panel*). Other experiments showed that RapGAPII expression did not alter the levels of ERK1 or ERK2 in WEHI-231 cells. *C*, RapGAPII expression does not inhibit SDF-1-induced activation of the Rac1 GTPase. Vector-transfected and RapGAPII-expressing WEHI-231 cells were stimulated with buffer alone (0), 100 nM PdBu (P), or 100 ng/ml (12.5 nM) SDF-1 (S) for 5 min. GTP-bound Rac1 was precipitated using a GST-Pak1β fusion protein and detected by immunoblotting with an anti-Rac1 Ab. For each panel similar results were obtained in at least two independent experiments.

and 2PK3 cells (Fig. 4*A*) was very similar to that in the corresponding control cells transfected with the empty vector. Moreover, RapGAPII expression did not inhibit other SDF-1-induced signaling events, including activation of the ERK mitogen-activated protein kinases (Fig. 4*B*) and activation of Akt (Fig. 4*B*), a kinase that is a target of PI3K signaling. RapGAPII expression also had no effect on the ability of SDF-1 to activate Rac1 (Fig. 4*C*), a GTPase that is involved in cell motility. Thus, RapGAPII expression appears to selectively inhibit the activation of Rap1 and Rap2.

To determine whether blocking SDF-1-induced Rap activation impaired B cell migration toward SDF-1, we performed Transwell migration assays. We found that expressing RapGAPII caused a significant reduction in the ability of 2PK3 cells, WEHI-231 cells, and DT40 cells to migrate toward SDF-1, as compared with cells transfected with the empty vector (Fig. 5). In 2PK3 cells, RapGAPII expression inhibited SDF-1-induced migration by 80 ± 10% (mean ± SD; $n = 5$). Similarly, RapGAPII expression inhibited SDF-1-induced migration by 50–60% in DT40 cells and by 80–90% in WEHI-231 cells. Thus, activation of Rap1 and/or Rap2 is important for SDF-1 to induce B cell migration. Interestingly, the spontaneous migration of 2PK3 cells in the absence of

SDF-1 was also inhibited when Rap activation was blocked. This suggests that Rap activation may play a general role in B cell motility.

To support the idea that Rap activation is important for SDF-1-induced B cell migration, we used a gain-of-function approach in which we expressed a constitutively active mutant form of Rap in B cell lines. Although we were unable to express the activated Rap1V12 protein in B cell lines, perhaps because it has lethal effects, we were able to obtain stable bulk populations of 2PK3 cells expressing a FLAG-tagged version of the activated Rap2V12 protein (Fig. 6*A*). Rap2 activation assays confirmed that this transfected Rap2V12 protein was constitutively active and that the total amount of activated Rap2 in cells expressing this protein was significantly increased (Fig. 6*B*). When we examined the migration of these Rap2V12-expressing 2PK3 cells in Transwell assays, we found that both their spontaneous migration and their SDF-1-induced migration were significantly increased (Fig. 6*C*). The SDF-1-induced migration of Rap2V12-expressing 2PK3 cells was 2.6 ± 0.5 ($n = 13$) times the migration of vector-transfected 2PK3 cells. Thus, increasing the amount of activated Rap2 potentiated B cell migration toward SDF-1. Because SDF-1-induced B cell migration
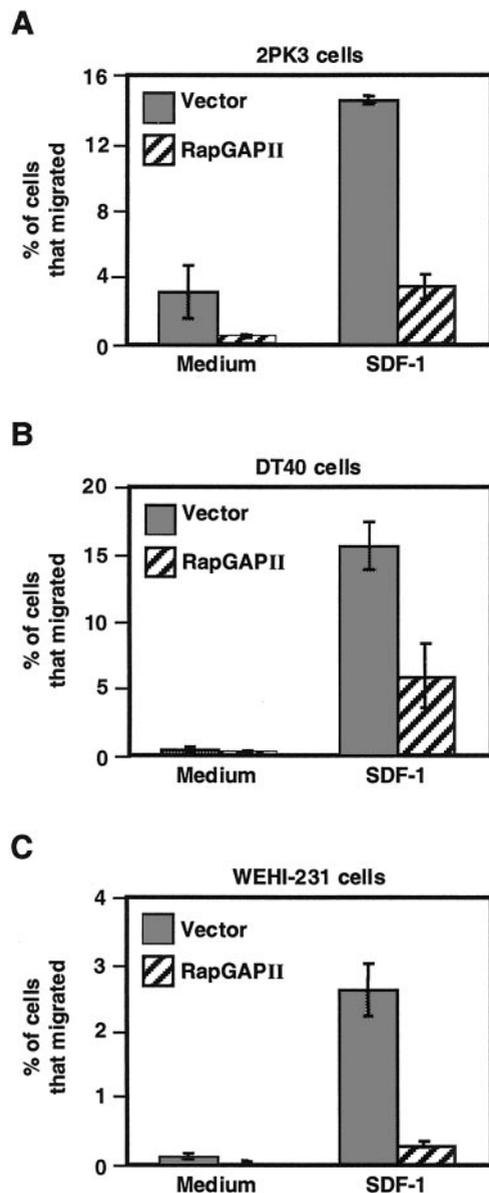
FIGURE 5. RapGAPII expression inhibits B cell migration toward SDF-1. Transwell assays were used to compare the migration of vector-transfected and RapGAPII-expressing 2PK3 cells (*A*), DT40 cells (*B*), or WEHI-231 cells (*C*). The cells were added to the top chamber while the bottom chamber contained either medium alone or medium containing 100 ng/ml (12.5 nM) SDF-1. After 3 h at 37°C, the number of cells that had migrated into the lower chamber was determined. The data are expressed as the percentage of the total input cells that had migrated into the lower chamber. Each point represents the mean $\pm$ SD for triplicate wells in the same experiment. For each panel similar results were obtained in at least three independent experiments.

is enhanced by Rap2V12 and inhibited when the activation of both Rap1 and Rap2 activation is blocked by RapGAPII, it indicates that Rap activation is an important determinant of B cell migration.

Discussion

In this report we have shown that activation of the Rap GTPases is important for B cells to migrate efficiently in response to SDF-1, a chemokine that plays an important role in B cell development, trafficking, and activation. We found that SDF-1 activates both Rap1 and Rap2 in B cells. Moreover, blocking Rap activation reduced the ability of B cells to migrate toward SDF-1, while
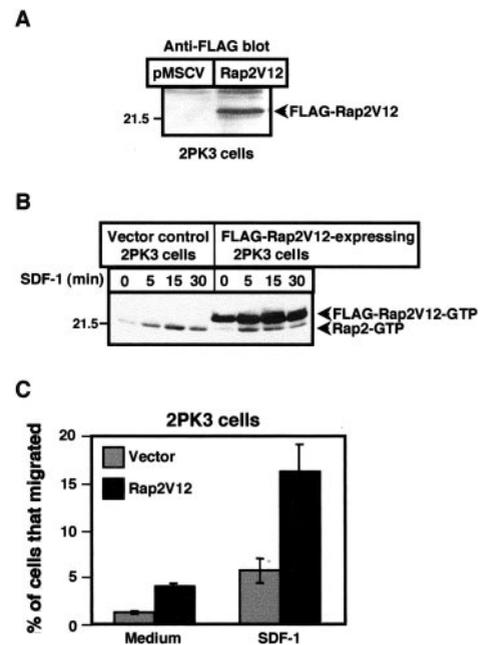

FIGURE 6. Rap2V12 expression increases B cell migration toward SDF-1. *A*, Retroviruses were used to establish stable bulk populations of 2PK3 cells containing either the empty pMSCV vector or pMSCV containing cDNA encoding FLAG-tagged Rap2V12. Expression of FLAG-Rap2V12 was detected by immunoblotting cell extracts with the M2 anti-FLAG mAb. *B*, SDF-1-induced Rap2 activation was assessed in vector-transfected and Rap2V12-expressing 2PK3 cells as in Fig. 1. The active GTP-bound forms of the endogenous Rap2 as well as the transfected FLAG-Rap2V12 are indicated by the arrows. *C*, Transwell assays were used to compare the migration of vector-transfected and Rap2V12-expressing 2PK3 cells. The cells were added to the top chamber while the bottom chamber contained either medium alone or medium containing 100 ng/ml (12.5 nM) SDF-1. After 3 h at 37°C, the number of cells that had migrated into the lower chamber was determined. The data are expressed as the percentage of the total input cells that had migrated into the lower chamber. Each point represents the mean $\pm$ SD for triplicate wells in the same experiment. For each panel similar results were obtained in at least three independent experiments.

expressing the constitutively active Rap2V12 enhanced SDF-1-induced B cell migration.

Although Rap1 is activated by many receptors, including the T and B cell Ag receptors, growth factor receptors, and cytokine receptors (reviewed in Ref. 22), this is the first report that a chemokine receptor which regulates cell migration activates the Rap GTPases. Rap2 has previously been shown to be activated only by cell adhesion (40) and by phorbol ester treatment (23). Thus, this is the first demonstration of receptor-induced activation of Rap2. In terms of the signaling pathway by which the SDF-1 receptor CXCR4 activates the Rap GTPases, we found that SDF-1-induced activation of Rap1 and Rap2 was dependent on PLC. The PLC inhibitor U73122 effectively blocked the ability of SDF-1 to activate both Rap1 and Rap2. It is likely that the SDF-1-induced Rap activation is mediated by the DAG produced by PLC-β. We found that phorbol esters that mimic DAG could activate both Rap1 and Rap2 in B cells. In contrast, the Ca^{2+} arm of the PLC pathway does not appear to contribute significantly to Rap activation in B cells because Ca^{2+} ionophores do not activate Rap1 or Rap2 in B cells and do not potentiate phorbol ester-induced Rap activation (Ref. 32 and our unpublished observations). Because most chemokines stimulate PLC-β-dependent production of DAG (14), it is likely that other chemokines that regulate B cell migration will

also activate Rap GTPases in B cells. Whether Rap activation is important for these chemokines to stimulate B cell migration remains to be determined.

By using a combination of loss- and gain-of-function approaches we showed that activation of the Rap GTPases promotes B cell migration. In three different B cell lines we found that Rap-GAPII expression inhibited SDF-1-induced activation of Rap1 and Rap2 as well as SDF-1-induced migration in Transwell assays. The simplest interpretation of this data is that Rap activation is important for B cell migration. RapGAPII appears to be a selective inhibitor of Rap activation because it blocked Rap activation but did not inhibit the activation of other targets of SDF-1 signaling, including ERK, Akt, and Rac1. To support the idea that Rap GTPases promote B cell migration, we expressed the constitutively active Rap2V12 protein in the 2PK3 B cell line and showed that it enhanced the ability of these cells to migrate toward SDF-1. Interestingly, we found that the spontaneous migration of 2PK3 cells in the absence of SDF-1 was also dependent on Rap activation because it was inhibited by RapGAPII and enhanced by Rap2V12. This suggests that Rap activation is important for B cell motility in general. This is the first direct demonstration that activation of the Rap GTPases promotes cell motility and migration in mammalian cells. Rap1 had previously been shown to be required for the proper migration of pole cells and mesodermal cells during *Drosophila* embryogenesis (30). In mammalian cells, overexpression of the Rap activator C3G had been shown to enhance integrin-dependent migration of the Ba/F3 hematopoietic cell line (41), but the role of the Rap GTPases in this process was not analyzed.

A key question is whether B cell migration is regulated by Rap1, Rap2, or both of these GTPases. The inhibition of SDF-1-induced B cell migration by RapGAPII was associated with the inhibition of both Rap1 and Rap2 activation. This indicates that Rap1 and/or Rap2 are involved in B cell migration. Because the activated Rap1V12 protein appears to have lethal effects on B cell lines, we cannot directly assess the role of Rap1 in promoting B cell migration. However, we were able to show that expressing the activated Rap2V12 protein enhanced both SDF-1-induced and spontaneous migration in the 2PK3 B cell line. This is the first reported function for Rap2. While the Rap2V12 experiments show only that Rap2 is capable of promoting B cell migration, our preliminary data suggest that activation of endogenous Rap2 does in fact contribute to SDF-1-induced B cell migration. In 2PK3 cells in which we expressed another Rap-specific GAP called SPA-1 (42), Rap1 activation was almost completely inhibited (>95% inhibition) while Rap2 activation was only partially inhibited (~40% inhibition) and SDF-1-induced B cell migration was only slightly inhibited (~15% inhibition). This suggests that the amount of Rap2 activation that still occurred in these SPA-1-expressing cells was capable of mediating SDF-1-induced B cell migration, even in the absence of Rap1 activation. However, we cannot rule out a role for Rap1. Because activated Rap1 and Rap2 bind many of the same effector proteins (43), it is possible that activation of either Rap1 or Rap2 is sufficient to allow B cells to migrate toward SDF-1. Unfortunately, expressing dominant-negative forms of Rap1 and Rap2 would not allow us to determine the relative roles of Rap1 and Rap2 in B cell migration because dominant-negative GTPases act by sequestering upstream activators and Rap1 and Rap2 share the same upstream activators. Nevertheless, we have shown for the first time that Rap2 can regulate cell migration.

We are currently investigating how the Rap GTPases regulate B cell migration. Cell migration is a complex process in which cells assume a characteristic polarized morphology, extend membrane processes at the leading edge of the cell, and then pull the rear of the cell forward. This involves reorganization of the cytoskeleton,

integrin-mediated adhesion at the leading edge of the cell, and actin/myosin-based contractile forces. The Rap GTPases could regulate one or more of these processes. In particular, there is considerable evidence that Rap1 regulates integrin activation. Expression of the activated Rap1V12 protein in T cells and in myeloid cell lines converts the LFA-1 and very late Ag-4 integrins to their high-avidity forms that are capable of binding ligands (24–28). LFA-1 and very late Ag-4 are the major integrins expressed on B cells. Our preliminary data suggest that Rap2 can also regulate integrin activation, because expressing the activated Rap2V12 protein in WEHI-231 cells enhances their PdBu-induced adhesion (S. J. McLeod and M. R. Gold, unpublished observations). Rap activation could also control other aspects of cell motility such as the reorganization of the actin cytoskeleton. Consistent with this idea, our preliminary work has shown that PdBu-induced cell spreading and extension of membrane processes is impaired in A20 murine B lymphoma cells expressing RapGAPII (S. J. McLeod and M. R. Gold, unpublished observations). Although further work is needed to elucidate the role of the Rap GTPases in B cell migration, we have shown in this report that the Rap GTPases are key regulators of this process.

Note added in proof. We have now shown that SDF-1 also activates Rap1 and Rap2 in murine splenic B cells.

Acknowledgments

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