Monocyte Migration and LFA-1-Mediated Attachment to Brain Microvascular Endothelia Is Regulated by SDF-1□ through Lyn Kinase[1]

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Infiltration of activated monocytes into the brain is a prerequisite for the development of various neurological disorders such as HIV-associated dementia, multiple sclerosis, and other inflammatory processes. In these pathologies, the chemokine SDF-1□ (CXCL12) is over-expressed and might attract monocytes into the CNS. We demonstrate here that SDF-1□ stimulates migration of monocytes through its receptor, CXCR4, and decreases monocyte adherence to surfaces coated with ICAM-1, a ligand for □$_2$ integrins. SDF-1□ also decreases monocyte adherence to brain microvascular endothelial cells (BMVEC) that are activated with TNF-□, IL-1□, or recombinant envelope glycoprotein from HIV-1, which increase BMVEC expression of ICAM-1. The decreased adherence is linked to down-regulation on monocytes of the activation-dependent epitope of the □$_2$ integrin LFA-1 by SDF-1□. Knockdown of Lyn in monocytes using small interfering RNA decreases SDF-1□-mediated migration and prevents the inhibition of monocyte attachment to ICAM-1 and activated BMVEC. Thus, in SDF-1□-stimulated monocytes, Lyn acts as a positive regulator of migration and a negative regulator of adhesion to BMVEC through the LFA-1 integrin. These results provide a novel Lyn-mediated signaling mechanism for the regulation of monocyte movement at the blood-brain barrier. The Journal of Immunology, 2008, 181: 4632–4637.

Infiltration of activated monocytes into the brain is involved in the development of several neurological disorders including HIV-associated dementia (HAD),[3] multiple sclerosis, and others. In HAD, for example, HIV-1 infection within the brain is necessary but not sufficient for progression of disease, and the massive movement of activated monocytes and macrophages into the CNS are believed to secrete toxic metabolites and lead to neuronal injury (1–3). In addition, the blood-brain barrier (BBB) is often altered in inflammatory diseases and is implicated in their pathogenesis (4). Proinflammatory cytokines such as TNF-□ and interleukins, as well as viral proteins such as the HIV-1 envelope glycoprotein gp120, have been shown to contribute to BBB dysfunction (5–7). Moreover, astrocytes and microglia have been shown to produce chemokines and cell migration-inducing cytokines that appear to attract leukocytes across BBB into the brain.

The chemokine SDF-1□ is one of the mediators expressed at increased levels in the CNS of patients with HAD and other neuroinflammatory disorders (8–12). SDF-1□ has been shown to play roles in ontogeny, leukocyte migration, and the pathogenesis of inflammatory diseases, and induces intracellular signaling through its receptor CXCR4. SDF-1□ may recruit monocytes into the CNS but the mechanism by which this chemokine couples to the signaling cascades and modulates monocyte attachment and movement at the BBB remains unclear. We now report that the Src family kinase Lyn is a critical mediator that relays suppressing signals from the chemokine receptor CXCR4 to □$_2$ integrins in human monocytes. Moreover, we further demonstrate that Lyn mediates the monocyte chemotactic response to SDF-1□. The net result of SDF-1□/CXCR4/Lyn signaling is a decrease in monocyte attachment on activated brain microvascular endothelial cells (BMVECs) and an increase in monocyte migration toward SDF-1□ gradient. Our present results provide new insights into the modulation of SDF-1□/CXCR4 signaling in monocyte mobilization and migration across the BBB.

Materials and Methods

Monocytes

Blood was obtained from healthy donors by leukopheresis. Monocytes were isolated and purified by counter current elutriation leading to 99% purity. To verify the quality of the monocyte preparations, FACS analysis was performed to assess the surface markers CD14, CD19, CD3, and CD4. Monocytes were kept in RPMI 1640 supplemented with L-glutamine and 10% FBS. For adhesion assays, monocytes were stained with Calcein according to the manufacturer's protocol (Invitrogen). In brief, 12.5 □M Calcein was added to a cell suspension followed by incubation for 30 min at room temperature. The cells were washed twice before use in adhesion assays. All experiments were conducted using cells from a minimum of three different donors.

Brain endothelial BB19 cells

The human brain capillary endothelial BB19 cell line was a generous gift of Drs. J. Joseph (National Institutes of Health, Bethesda, MD) and J. Nelson (Oregon Health Sciences University, Portland, OR). This cell

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[3] Abbreviations used in this paper: HAD, HIV-associated dementia; BMVEC, brain microvascular endothelial cell; BBB, blood-brain barrier; EBM, endothelial basal medium; siRNA, small interfering RNA; CNS, central nervous system; EC, endothelial cells; RFU, relative fluorescence units; PP2, pyrazolopirimidine.

line was derived from primary BMVEC immortalized through transformation with the E6 and E7 genes of the human papilloma virus type 16 (13, 14). BB19 cells were cultured in endothelial basal medium (EBM) supplemented with growth factors (bovine brain extract, hydrocortisone, human EGF, FBS, and gentamicin/amphotericin-B; Cambrex) and were used for a maximum of six passages. Cells were seeded in T-75 flasks precoated with 2 μg/cm^2 fibronectin at a density of 7500 cells/cm^2 and cultured at 37°C with 5% CO_2 and saturated humidity. Cell culture medium was changed every 2 days. Cells attached to the cell culture surface within 1 day and started to grow after the second day of culture. The cells were passaged every 5–6 days or when ~80% confluent. To activate the endothelial cells, IL-1α, TNF-α (5 ng/ml), or gp120 (1 μg/ml) were added to appropriate wells for 4 h at 37°C in a CO_2 incubator.

Reagents

Recombinant TYBE gp120 was provided by Dr. R. Doms (University of Pennsylvania, Philadelphia, PA). Recombinant LAV gp120 was obtained from the National Institutes of Health AIDS Research and Reference Reagent proteins. Other reagents were AMD3100 (Sigma-Aldrich), Calcein (Invitrogen), SDF-1α (PeproTech), PP2 (Calbiochem), and recombinant human ICAM-1 or VCAM-1 (R&D Systems).

Adhesion assay

BB19 cells were seeded in 96-well plates at 5 × 10^4 cells per well and grown for 2–3 days at 37°C in CO_2 incubator with fresh media added after 24 h. When indicated, the endothelial cells were activated as described above. The cells in each well were then washed twice with PBS $Ca^{++}Mg^{++}$. Calcein labeled monocytes, cultured with or without SDF-1α (200 ng/ml), were added to the desired wells and incubated for 45 min at 37°C. The nonadherent cells were washed away and plates were read with a fluorescence plate reader using 485/530 nm excitation/emission filter sets. Average percent adhesion was calculated as: [(relative fluorescence units (RFU) after wash)/(RFU before wash)] × 100. For adhesion assays using recombinant proteins, the 96-well plates were coated with recombinant human ICAM-1 or recombinant human VCAM-1 or purified BSA overnight at 4°C. Calcein-labeled monocytes were incubated in the recombinant protein-coated wells for 90 min at 37°C. The rest of the procedure was followed as described above for endothelial cell adhesion assays.

Migration assay

A total of 1 × 10^5 monocytes cultured in RPMI 1640 with 2% FBS were placed in the upper chamber of a Transwell (Costar). The lower chamber was filled with media containing SDF-1α. Dose response analysis demonstrated a typical U-shaped curve with maximal migration at 200 ng/ml (data not shown) and that concentration was therefore chosen to most effectively carry out the inhibitory studies as well as other experiments. Monocytes were allowed to migrate through the polycarbonated membrane for 2 h at 37°C. The cells in the lower chamber were collected and counted by FACS at top speed for 1 min. The chemotactic index was determined using the formula: chemotactic index = (number of cells migrated in the presence of SDF-1α)/(number of cells migrated in the absence of SDF-1α).

Transendothelial migration

BB19 cells were seeded on 6.5-mm diameter and 5-μm pore size polycarbonate microporous membranes of Transwell chambers (Costar) that were precoated with human fibronectin. A total of 100 μl of EC medium were added to the top chamber, and 600 μl were added to the bottom chamber. After 4 days of culture, the monolayer was assessed for transendothelial electrical resistance using EVOM voltmeter (World Precision Instruments), which showed a resistance pattern in the range of ~60 Ohms, consistent with prior studies of solute transport (14). Before the transendothelial migration, the media was removed and the inserts were transferred to a new 24-well plate containing 600 μl of fresh RPMI 1640 supplemented with 2% FBS. A total of 100 μl of monocytes (1 × 10^5) were added on the monolayer. After 4 h, the media in the bottom chamber containing migrated monocytes were collected and counted by FACS. Also, monocytes attached to the bottom of the Transwells were stained with Diff Quik and counted under the microscope. The chemotactic index was calculated as described above.

siRNA transfection

dsRNA oligonucleotides were purchased from Dharmacon. Four short interfering RNAs targeting human Lyn as well as four nonspecific scrambled siRNA (negative control) were designed by Dharmacon. dsRNA was resuspended at a final concentration of 20 μM in 1× siRNA universal buffer. Aliquots were kept at −80°C. Monocytes were transfected with a nucleofector device type I using Human Monocyte Nucleofection kits and protocols, all provided by Amaxa. In brief, freshly isolated monocytes were suspended in Human Monocyte Nucleofector solution at a final concentration of 6 × 10^6 monocytes/100 μl. For each transfection, 100 μl monocyte solution was subsequently mixed with 5 μg of siRNA and transferred into Amaxa-certified cuvettes. Cells were pulsed using the program Y-01 of the nucleofector-I. Immediately after transfection, 1 ml of prewarmed media was added to the cells. The transfected monocytes were transferred to one well of a 12-well plate using specific pipette tips as provided in the Amaxa kit. The transfected monocytes were allowed to recover for 48 h in a humidified 37°C/5% CO_2 incubator. Such a protocol resulted in ~80% cell survival.

Western blotting

Gene silencing in monocytes transfected with either scrambled siRNA or Lyn-siRNA was analyzed by Western blotting. The cells were washed twice with cold PBS before being lysed in Reporter lysis buffer (Promega). A total of 30 μg of total proteins were loaded per well and separated on a 7% SDS-PAGE for 90 min at 100 volts. Proteins were subsequently transferred to a nitrocellulose membrane (Bio-Rad) overnight at 30 volts. The nitrocellulose membranes were probed with Lyn mAb at a concentration of 1:2000 (Upstate Biotechnology). The secondary Ab was a goat-anti-rabbit Ab conjugated to HRP used at a concentration of 1:5000 (Cell Signaling Technology). The proteins were revealed by chemiluminescence using the ECL kit (Amersham Biosciences). For loading control, the membranes were stripped and reprobed with GAPDH Ab at a concentration of 1:2000 (Cell Signaling Technology). Lyn expression was determined relative to GAPDH, based on densitometry.

Abs

The Abs used for flow cytometry and their suppliers were as follows: mAb24 for the active conformation of human β$_2$ integrins was kindly provided by Dr. N. Hogg (London Research Institute, London, England), IgG1 control mAb (BD Pharmingen), goat F(ab′)2 anti-mouse IgG-PE secondary Ab (Caltag Laboratories), FITC-conjugated anti-human ICAM-1/CD54, and control IgG1 mAbs (R&D Systems). For adhesion blocking, an unconjugated anti-human ICAM-1/CD54 Ab (R&D Systems) was used. For Western blotting, anti-Lyn (Upstate Biotechnology) and anti-GAPDH (Cell Signaling Technology) Abs were used.

FACS analysis

Immunofluorescence staining was performed according to standard procedures. In brief, 2 × 10^5 cells per sample were incubated with mAb24 or control mAb at 37°C in Mg^{++} buffer for 30 min. The cells were then washed with ice-cold PBS 1% FBS, incubated with PE-conjugated goat F(ab′)2 anti-mouse IgG at 4°C for 30 min, washed again, fixed with 1% formaldehyde, and then analyzed on a FACScan flow cytometer. ICAM staining was conducted similarly except that FITC-conjugated primary Abs were used.

Results

We first investigated the effect of SDF-1α on attachment of primary human monocytes to the surfaces coated with ICAM-1 (ligand for β$_2$ integrin), VCAM-1 (ligand for α$_4$ and α$_9$ integrins), or BSA (control) (15). Attachment of monocytes was highest on ICAM-1 and lowest on BSA, with intermediate attachment observed for VCAM-1 (Fig. 1A). Treatment of monocytes with SDF-1α suppressed the increased adhesion to ICAM-1 but had no effect on attachment to VCAM-1. The effect of SDF-1α could be reversed by pretreatment of monocytes with the specific CXCR4 antagonist AMD3100 (Fig. 1A). Furthermore, incubation of anti-human ICAM-1 Ab with ICAM-1-coated surfaces before addition of monocytes blocked attachment, confirming specific binding to ICAM-1 (Fig. 1B). Together, these data indicate that SDF-1α through its receptor CXCR4 down-regulated monocytes adherence to surfaces coated with ICAM-1.

Since ICAM-1 is a ligand for the β$_2$ integrin LFA-1 (16), it seemed plausible to examine the expression of an activation-dependent epitope of β$_2$ integrin in SDF-1α-stimulated and unstimulated monocytes. Therefore, we used FACS analysis using mAb24, which recognizes activated β$_2$ integrins (17). Our results show that activation state-dependent conformational epitope for β$_2$ integrin expression was decreased in SDF-1α stimulated cells (Fig. 1C).

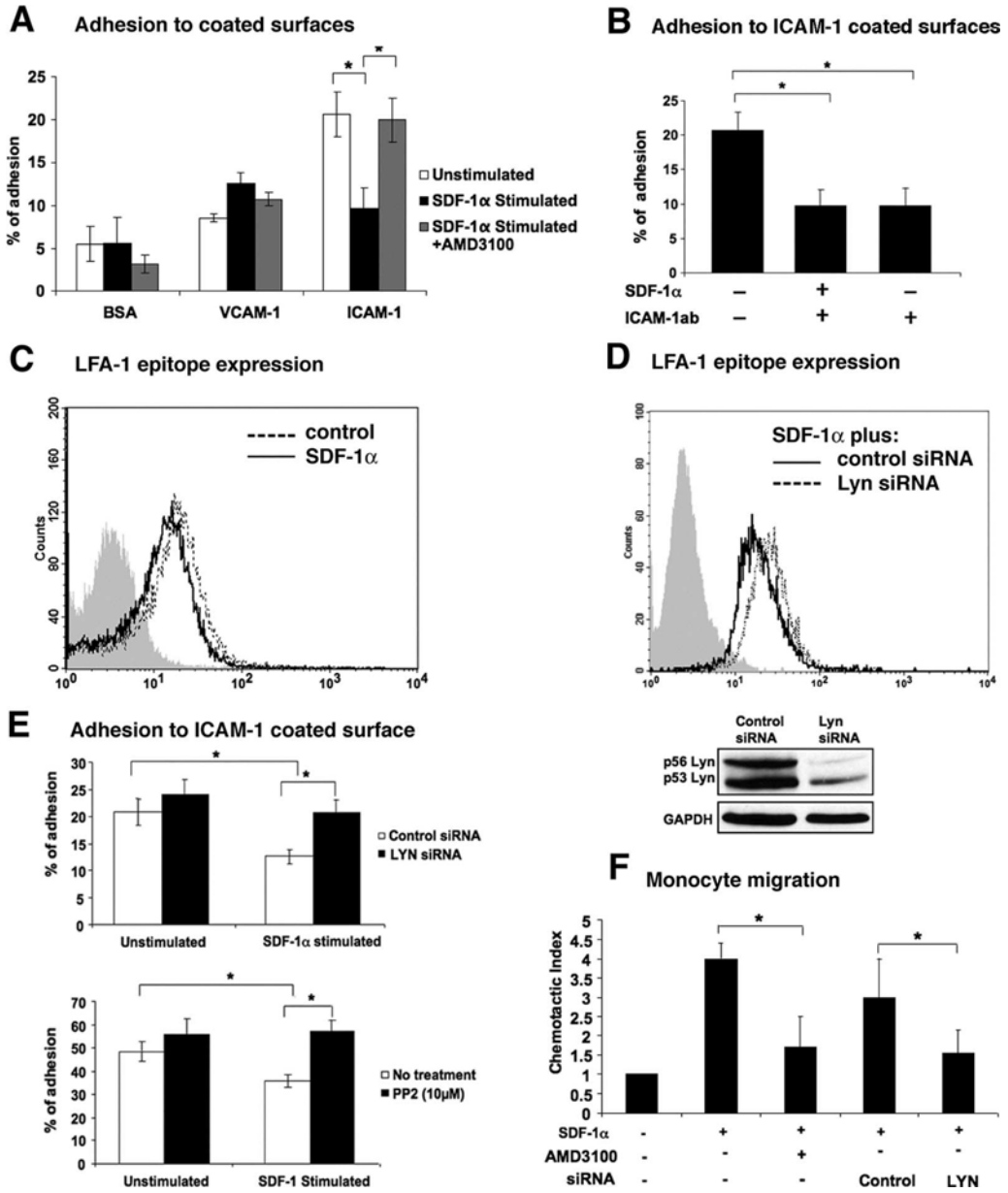

FIGURE 1. CXCR4-Lyn signaling axis is a positive regulator of monocyte migration and a negative regulator of LFA-1-mediated attachment to surfaces coated with ICAM-1. A, Effect of SDF-1□ stimulation on the attachment of human monocytes to surfaces coated with ICAM-1, VCAM-1, or BSA (control) was assessed using adhesion assay. Pretreatment of monocytes with AMD3100, a specific antagonist for the SDF-1□ receptor CXCR4, was used as control. B, Anti-ICAM-1 mAb inhibits monocyte attachment to surfaces coated with ICAM-1. ICAM-1-coated wells were preincubated for 1 h using 25 □g/ml Ab before adding monocytes. C, FACS analysis of □$_2$ integrin-activation epitope expression in unstimulated (dotted line) and SDF-1□-stimulated monocytes (solid line). Cells were stained with anti-integrin mAb24 or with isotype control (gray histogram). D, FACS analysis of □$_2$ integrin-activation epitope expression in SDF-1□-stimulated monocytes that were transfected with either control siRNA (solid line) or Lyn siRNA (dotted line). Cells were stained with anti-integrin mAb24 or isotype control (gray histogram). Lower panel, Western blot showing the expression levels of Lyn kinase in monocytes at 48 h following transfection with Lyn siRNA or control siRNA. E, Attachment of SDF-1□-stimulated or unstimulated monocytes to surfaces coated with ICAM-1 at 48 h post transfection with Lyn siRNA or control siRNA (upper panel). Attachment of PP2-treated and untreated monocytes in the presence or absence of SDF-1□ to surfaces coated with ICAM-1 (lower panel). F, Chemotaxis assay showing the migration of monocytes toward SDF-1□ gradient. Monocytes were pretreated with or without AMD3100 or transfected with control or Lyn siRNA. All data show means □ SEM of three independent experiments using cells from different donors, or FACS profiles or Western blots representative of three independent experiments. □, p □ 0.05.

The Src family kinase Lyn was previously identified as a mediator of inside-out signaling pathway that relays signals from the chemokine receptor CXCR4 to □$_2$ integrin LFA-1 in hematopoietic stem/progenitor cells (18). Therefore, we examined whether Lyn was involved in SDF-1□-mediated down-regulation of LFA-1 in human primary monocytes. Using siRNA approach, we were able to achieve □80% reduction in Lyn expression in primary human monocytes as shown by Western blot (Fig. 1D, lower panel). Evaluation of □$_2$ integrin expression in monocytes following SDF-1□ stimulation showed that Lyn depletion led to increased expression of the activation state-dependent conformational □$_2$ integrin epitope (Fig. 1D, upper panel). In parallel, we also tested the adherence of Lyn-depleted cells to surfaces coated with ICAM-1 (Fig. 1E, upper panel). Control siRNA-transfected monocytes showed down-regulation of adhesion following SDF-1□ stimulation (white bars). The loss of Lyn expression in monocytes,

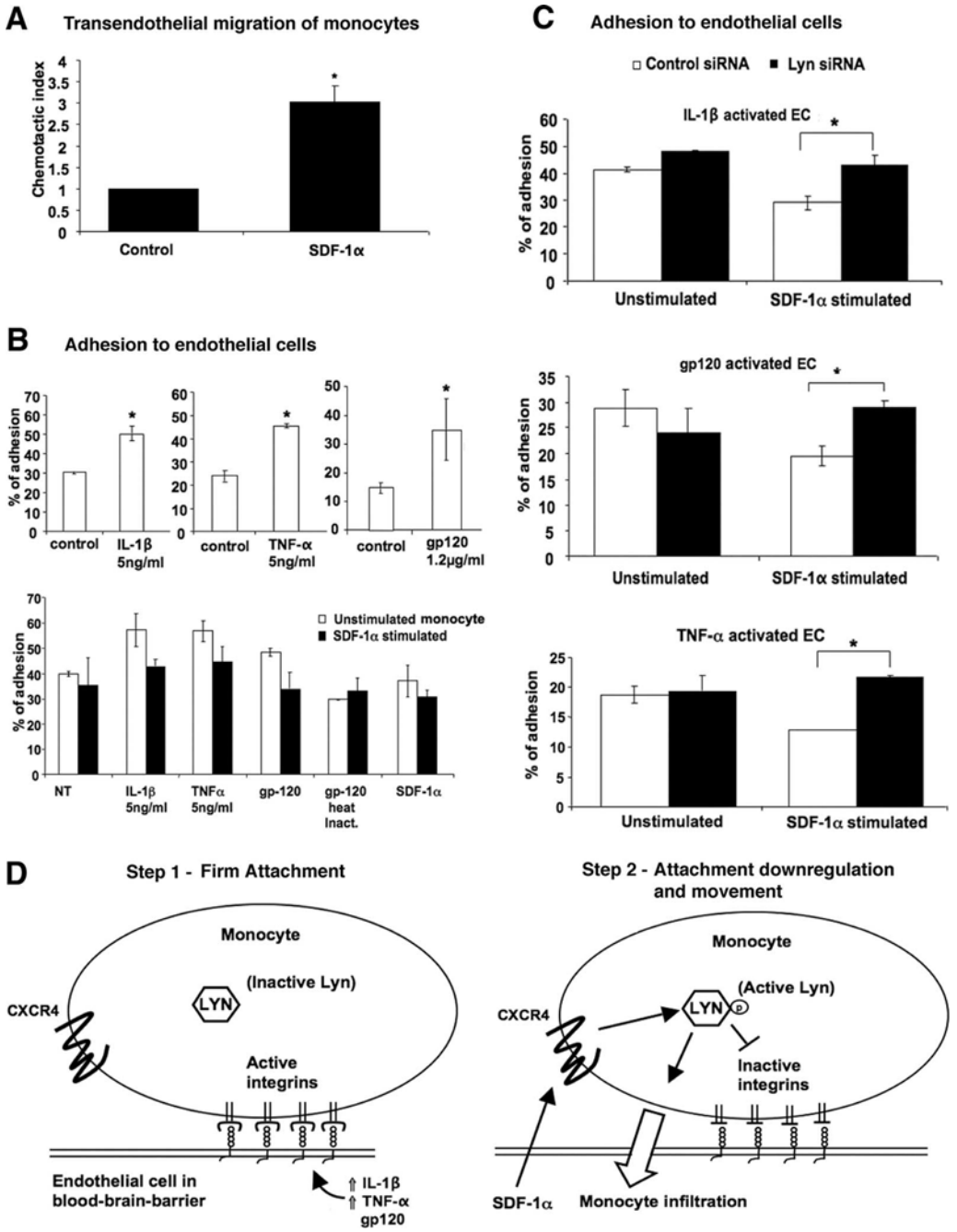

FIGURE 2. CXCR4-Lyn signaling inhibits attachment of monocytes to activated BB19 BMVECs and promotes transendothelial migration toward SDF-1□. A, Transendothelial migration assay showing the migration of monocytes toward SDF-1□ gradient through monolayers of unstimulated BB19 cells. B, Attachment of unstimulated or SDF-1□-stimulated monocytes to BB19 cells activated with IL-1□, TNF-□, or HIV-1 gp120 was performed as described in Materials and Methods. C, Attachment of SDF-1□-stimulated or control monocytes to activated BB19 was conducted 48 h after transfection with either Lyn siRNA or control siRNA. A–C, Represent means □ SEM of three independent experiments using cells from different donors; □, p □ 0.05. D, A two-step model for the increased infiltration of monocytes across inflamed BBB in response to SDF-1□, in the setting of neuroinflammatory disorders. Left panel, Up-regulation of ICAM-1 expression on BMVEC due to activation of these cells with IL-1□, TNF-□, or gp120 promotes arrest and firm attachment of circulating monocytes via $□_2$ integrin/ICAM-1 interactions. Right panel, SDF-1□ activates Lyn through CXCR4 on monocytes. Lyn activation mediates monocyte chemotaxis; in addition, $□_2$ integrin-mediated adhesion is transiently destabilized through inside-out signaling, thereby loosening tight monocyte attachment and facilitating transendothelial migration through BBB.

however, prevented down-regulation of cell adherence to ICAM-1 in response to SDF-1□ (black bars). To further address the question of the involvement of Lyn kinase activity in CXCR4-regulated inhibition of attachment to ICAM-1, we examined the effect of PP2 on SDF-1□ induced inhibition of monocytes attachment of ICAM-1 (Fig. 1E, lower panel). PP2 inhibits Src family kinases (19) and has been shown to completely inhibit tyrosine phosphorylation of Lyn at the concentration of 10 □M (20). Pretreatment of monocytes with PP2 prevented the down-regulation of adhesion to ICAM-1 in response to SDF-1□ (black bars) as compared with the untreated monocytes (white bars). Combined, these data indicate that SDF-1□/CXCR4 signaling axis inhibits LFA-1 mediated adhesion of monocytes to ICAM-1 through Lyn kinase.

We then examined whether SDF-1□ could elicit chemotactic response in monocytes. Concomitant increase in the chemotactic index toward SDF-1□ gradient was observed (Fig. 1F), which could be blocked by treatment with AMD3100. Furthermore, the selective depletion of Lyn by siRNA potently inhibited SDF-1□-induced cell migration (Fig. 1F). We also observed inhibition of migration toward SDF-1□ following treatment with PP2 (data not shown). Together, these results support the role of SDF-1□ as a positive regulator of monocytes migration and a negative regulator of monocyte adhesion via the Lyn kinase.

CXCR4 also serves as an entry coreceptor for X4 strains of HIV-1 but, in addition to supporting viral entry, CXCR4 ligation by the HIV-1 envelope glycoprotein gp120 can activate intracellular signals in macrophages and trigger inflammatory mediator release (21, 22). We hypothesized that X4 gp120 might also elicit chemotaxis in monocytes through CXCR4-induced signaling pathway. Interestingly, we failed to see any increase in chemotaxis of monocytes in response to recombinant X4 gp120 or in response to whole virions, nor did X4 gp120 affect the attachment of monocytes to ICAM-1 or VCAM-1 (data not shown).

To further extend the data obtained above, we then tested the transendothelial migration of monocytes across a layer of BMVEC, using the transformed BB19 cell line. Monocytes were allowed to migrate across the endothelial monolayers for 4 h in the presence of SDF-1□. Approximately 3-fold increase in the migration of monocytes across endothelial monolayers was observed in response to SDF-1□ gradient (Fig. 2A). Next, we examined the adhesion of monocytes onto endothelial cells. It has been shown that expression of ICAM-1 is up-regulated on the surface of BMVEC cells upon activation with proinflammatory cytokines or with recombinant HIV-1 gp120 (23, 24). In agreement with the data obtained using purified substrate, untreated monocytes showed increased attachment on the activated BB19 cells by □2-fold as compared with the unactivated endothelial cells (Fig. 2B, upper panel). Stimulation of monocytes with SDF-1□ decreased attachment to activated BMVEC (Fig. 2B, lower panel). As control, monocytes attachment to SDF-1□-treated BMVEC was also analyzed, but SDF-1□ activation of BMVEC did not affect the attachment of monocytes. We then showed that the inhibition of attachment resulting from monocyte treatment with SDF-1□ was clearly due to the activation of Lyn kinase, as cells depleted of Lyn by siRNA did not show a reduction of adhesion to BMVEC following SDF-1□ treatment (Fig. 2C). This indicates that Lyn, when activated by SDF-1□ through CXCR4, acts as a negative regulator of monocytes attachment on activated BMVEC that express a high level of ICAM-1. These results are consistent with the data obtained with the purified ICAM-1 substrate (Fig. 1).

Discussion

The present study indicates that the Src family kinase Lyn is an important part of a regulatory network that couples SDF-1□/CXCR4-induced monocyte chemotactic signals with down-regulation of □$_2$ integrin/LFA-1-dependent adhesion to activated BMVECs (Fig. 2D). SDF-1□ triggers monocyte chemotaxis and decreases □$_2$ integrin-mediated attachment to activated BB19 BMVEC, whereas blockade of Lyn expression with siRNA in monocytes results in increased attachment and decreased monocyte movement following SDF-1□ stimulation. Thus, we demonstrate that these cellular effects are associated with Lyn-dependent inside-out LFA-1 integrin signaling. Consistent with these findings, it has been previously shown that Lyn can relay signals from CXCR4 to LFA-1 in normal hematopoietic cells (18) and that the BCR-ABL oncoprotein can disrupt this signaling pathway in malignant leukemic progenitors (25).

Our results suggest a mechanism by which SDF-1□ could play an important role in attracting monocytes into the CNS. The chemokine SDF-1□ is over-expressed in astrocytes and neurons in patients with HAD and certain other neurological disorders (8–11). Moreover, in many neuroinflammatory processes, over-expression of the proinflammatory cytokines IL-1□ and TNF-□ is seen as well (3, 26). Stimulation of BMVECs with these proinflammatory cytokines, as well as with HIV-1 gp120, results in the increased expression of adhesion molecules such as ICAM-1 on these cells (23, 24). We confirmed that IL-1□, TNF-□, and gp120 increase ICAM-1 expression on the surface of BB19 cells (data not shown). More importantly, even though monocyte donor-to-donor variation led to differences in the absolute level of monocyte adhesion (e.g.; Fig. 2, B vs C), the adhesion was consistently enhanced by endothelial cell activation, and this was down-regulated by monocyte treatment with SDF-1□ through pathways involving Lyn.

In the absence of endothelial cells to which monocytes may attach (Fig. 1F), or if endothelial cells are present and not activated (Fig. 2A), SDF-1□ acts as a chemoattractant. If endothelial cells are present and activated, increased monocyte adherence will occur (Fig. 2B). In this setting, for SDF-1□ to be effective in recruiting monocytes, it is likely that both chemoattraction and detachment from the activated endothelial cells are necessary. We suggest that in the setting of inflammation and SDF-1□-mediated chemoattraction, whereby circulating blood monocytes attach through the LFA-1 integrin to activated BMVEC expressing high levels of ICAM-1, the SDF-1□/CXCR4/Lyn-mediated down-regulation of □$_2$ integrin affinity represents one mechanism by which monocytes are aided in their migration across the brain microvascular endothelial barrier. When Lyn is activated through SDF-1□/CXCR4 activation, □$_2$ integrin-mediated monocyte adhesion is transiently destabilized through inside-out signaling, thereby loosening attachments and facilitating movement across the endothelial cell barrier. Of note, signaling through chemokine receptors has been suggested to result in preferential localization of activated Src kinases to the leading edge of the cells (27), likely explaining the observation that only a small decrease in the expression of activation-dependent epitope LFA-1 was detected by FACS in our experiments.

In addition to SDF-1□, multiple other chemokines are over-expressed in neuroinflammatory disorders including MCP-1, MIP-1□, and others (28, 29), and pathogenic roles are supported by studies using animal and in vitro model systems (30–32). Whether the mechanism identified here, by which Lyn mediates SDF-1□/CXCR4 regulation of monocyte attachment and migration, is involved in responses to other chemokines will require further study. Similarly, the precise molecular mechanism that links Lyn to the conformational activity of LFA-1 in SDF-1□/CXCR4-stimulated monocytes remains to be defined. Inside-out regulation of integrins is typically mediated through Rap 1 regulation of talin binding to the integrin cytoplasmic tail, but the specific upstream linkages can differ among various agonists that regulate inside-out integrin affinity (33–36). Finally, the reason that X4 gp120 failed to modulate adhesion and migration in primary human monocytes is uncertain but is consistent with previous observations by others (37, 38).

Based on these observations, we propose a two phase model for the regulation of monocyte movement across the BBB in response to SDF-1□ in the setting of neuroinflammatory disorders (Fig. 2D). In the first phase, the monocytes circulating in the blood may become firmly attached through the LFA-1 integrin to the activated BMVEC expressing high levels of ICAM-1. In the second phase, SDF-1□ activation of CXCR4 leads to decreased ICAM-1-binding activity of monocyte LFA-1 through inside-out signaling mediated

by Lyn kinase, along with monocyte chemotaxis toward the SDF-1□ gradient, enabling migration across the BBB into the brain.

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Disclosures

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