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Follicular Dendritic Cells Activate HIV-1 Replication in Monocytes/Macrophages through a Juxtacrine Mechanism Mediated by P-Selectin Glycoprotein Ligand 1

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Follicular dendritic cells (FDCs) are located in the lymphoid follicles of secondary lymphoid tissues and play a pivotal role in the selection of memory B lymphocytes within the germinal center, a major site for HIV-1 infection. Germinal centers are composed of highly activated B cells, macrophages, CD4+ T cells, and FDCs. However, the physiological role of FDCs in HIV-1 replication remains largely unknown. We demonstrate in our current study that FDCs can efficiently activate HIV-1 replication in latently infected monocytic cells via an intercellular communication network mediated by the P-selectin/P-selectin glycoprotein ligand 1 (PSGL-1) interaction. Upon coculture with FDCs, HIV-1 replication was significantly induced in infected monocytic cell lines, primary monocytes, or macrophages. These cocultures were found to synergistically induce the expression of P-selectin in FDCs via NF-κB activation and its cognate receptor PSGL-1 in HIV-1-infected cells. Consistent with this observation, we find that this response is significantly blocked by antagonistic Abs against PSGL-1 and almost completely inhibited by PSGL-1 small interfering RNA. Moreover, a selective inhibitor for Syk, which is a downstream effector of PSGL-1, blocked HIV-1 replication in our cultures. We have thus elucidated a novel regulatory mechanism in which FDCs are a potent positive bystander that facilitates HIV-1 replication in adjacent infected monocytes via a juxtacrine signaling mechanism. The Journal of Immunology, 2009, 183: 524–532.

The natural progression of HIV-1 infection consists of acute and chronic stages (1, 2). In the acute phase of viral infection, an initial peak level of plasma viremia appears within a couple of weeks of transmission. At this early time point in the course of infection, HIV-1 has disseminated to the lymphoid organs and viral reservoirs and latency have been established. The HIV-1 viral load stabilizes at a relatively low level after a period of acute viral infection, defined as the “set point,” during which an immunological activation against HIV-1 is initiated. However, in tandem with seroconversion, HIV-1 production in reservoir or latently infected cells will eventually resume upon specific immunological responses such as host cytokine secretion or cell-mediated immune reactions (3–6).

Lymphoid organs have been proposed to function as a major reservoir for HIV-1 (7). During the course of HIV infection, T cells and macrophages in secondary lymphoid organs also become major reservoir cells for HIV-1 (8). Several in vitro studies have now identified potentially stable reservoirs of inducible latently infected CD4+ cells carrying an integrated form of the viral genome (7–9). In addition to CD4+ T cells, monocytes are thought to be major reservoirs for HIV-1 in vivo, since a number of blood monocytes are maintained in HIV-1-infected patients even during the late disease stages when T cells can be practically undetectable (10, 11). These observations suggest that infected CD4+ T cells and macrophages provide sites as a stable reservoir and producer of HIV-1, causing the persistent production of progeny virus in lymphoid organs. However, it has not been well investigated how these reservoir cells can maintain sufficient levels of viral replication that will retain a sufficient viral load during the long course of this disease.

It is generally believed that the central point in the immune system is the lymphoid organs and germinal centers (GCs) where several immune cell types are localized, although these circulate throughout the whole body (12–14). The GCs of secondary lymphoid tissues are composed of B cells, CD4+ T cells, macrophages, and follicular dendritic cells (FDCs) (15–17). FDCs are characterized by the expression of CD21, CD35, CD40, and specific cell surface adhesion molecules including ICAM-1, VCAM-1, and the surface dendritic cell (DC) markers DC-SIGN and DRC-1 (16, 18–21). The FDCs play an important role in the...

* Abrams used in this paper: GC, germinal center; FDC, follicular dendritic cell; DC, dendritic cell; PSGL-1, P-selectin glycoprotein ligand 1; Syk, spleen tyrosine kinase; LTR, long terminal repeat; MOI, multiplicity of infection; siRNA, small interfering RNA.

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immune response by interacting with CD4+ T or B cells and in organization of the follicular structure.

In HIV infection, human FDCs can capture and retain infectious HIV particles in a stable manner on their cell surfaces for several months or even years via Fc receptors or other molecules (22–25). Unlike conventional DCs, FDCs are not themselves infected with HIV despite expression of chemokine receptors and DC-SIGN (24). Furthermore, active HIV infection is largely confined to sites surrounding the FDCs (24), suggesting that this microenvironment is highly conducive to infection with this virus. FDCs have also been shown to transmit signals to the GC microenvironment which also appears to increase HIV infection and replication (24, 25).

Our previous study showed that FDCs stimulated virus production in MOLT-4 T cells preexposed to HIV-1(23). Very recently, Thacker et al. (26) also reported that FDCs contributed to virus replication in CD4+ T cells infected with HIV-1 obtained from peripheral blood and GCs by increasing viral transcription mediated by TNF-α upon coculture. However, the role of FDCs in HIV-infected monocytes/macrophages is largely unknown.

We here report that FDCs can activate HIV-1 production in surrounding infected monocytes or macrophages via a cell-cell interaction with a clear mechanistic distinction from CD4+ T cells reported by Thacker et al. (26). This enhancement in monocytic cells was found to be mediated mainly by an association between P-selectin on FDCs, acting as a ligand, and P-selectin glycoprotein ligand 1 (PSGL-1), the cognate receptor, on HIV-1-infected cells. Furthermore, we delineate the biological significance of the PSGL-1/spleen tyrosine kinase (Syk) pathway in the FDCs-mediated switch to induce HIV-1 replication. Our current findings thus shed new light on mechanisms involved in the HIV replication pathway that are mediated through intercellular communication and provide clues for the design of future novel therapeutic interventions against AIDS and related disorders.

Materials and Methods

Cell culture and reagents

Several FDC lines were established from fresh human palatine tonsils and maintained as described previously (23). Briefly, FDCs were isolated from freshly excised tonsils surgically removed. Tonsils were cut into pieces in fresh palatine tonsils surgically removed. Tonsils were cut into pieces in PBS for 2 h were washed three times with PBS and then twice with RPMI 1640 before coculturing.

Isolation of total RNA from cells and quantitative RT-PCR

U1 cells and FDCs were harvested after coculturing and washed three times with PBS. Total RNA was then extracted using Isogen (Nippongene) according to the manufacturer’s instructions. RNA (1 μg) was reverse transcribed using Superscript III (Invitrogen) before semiquantitative RT-PCR, and quantitative RT-PCR was performed using a SYBER Green One-step Real-time PCR kit (Invitrogen) with mRNA-specific primer pairs. Analyzed genes and corresponding primers are listed in supplemental Table I.4

Neutralization assay

HIV-1-infected cells were pretreated with neutralizing Abs (anti-PSGL-1, anti-ICAM-1, or control mouse IgG) for 2 h before and during coculturing. Optimal concentrations were determined by the IC50 values in accordance with the manufacturer’s instructions. Culture supernatants were collected after 3 days and subjected to measurement of HIV-1 p24.

Chemicals and inhibitory assays

BAY11-7082 and JNK inhibitor II were purchased from Merck. The Syk-specific inhibitor ER-27319 (29, 30) was purchased from Sigma-Aldrich. Cells were pretreated with 30 μM ER-27319, 1 μM JNK inhibitor II, 1–2 μM BAY11-7082, or DMSO (Sigma-Aldrich) for 2 h. The inhibitor treated or untreated cells were then cocultured with FDCs in the presence of Syk or NF-κB inhibitor. In small interfering RNA (siRNA) experiments, U1 cells were transfected with control or PSGL-1 siRNA (Santa Cruz Biotechnology) by Nucleofector (Amaza) and then cocultured with FDCs. Lysates and supernatants were collected from these cultures after 3 days for measurement of p24 and Western blotting analysis.

Flow cytometry

Cells were washed twice with staining buffer (5% FBS and 0.09% NaN3/FBS) and then stained with PSGL-1-FITC (BD Biosciences) for 1, 3, or 5 days.

Measurement of HIV-1 p24

Cell culture supernatants were collected after centrifugation at 4000 rpm for 5 min at 4°C and then processed for flow cytometry.

Results

FDCs activate HIV-1 production in adjacent HIV-1-infected monocytic cells

To address whether FDCs can also activate HIV-1 replication in the surrounding infected monocytes/macrophages as an effective bystander or stimulator, several primary FDCs were established from fresh palatine tonsils of three healthy human donors (23).
Since each of these established cell lines was very similar in nature, exhibiting typical properties of FDCs (positive for CAN-42, S-100/H9251, CD54, DC-SIGN, and CXCR4; morphological character such as spine-like spiculae and intercellular gap junction), the FDC1 line was mainly used in subsequent experiments. FDCs themselves were not productively infected with HIV-1 (Fig. 1A), consistent with previous reports (22–25).

Initially, the FDCs were cocultured with chronically HIV-1-infected monocytic cell line U1 to examine whether they had the ability to activate HIV-1 replication. After 3 days of growth, HIV-1 production was analyzed for HIV-1 p24. The results showed that coculturing with FDCs significantly induced HIV-1 replication in the two infected cell types tested, whereas no such induction was observed when the U1 cells were cultured with 293T cells (Fig. 1B). A parallel kinetic study further demonstrated that the p24 levels in supernatants and lysates were increased in a time-dependent manner in U1 cells grown under these coculture conditions (Fig. 1C and D). To address whether this trend occurred also in primary cells, FDCs were cocultured with PBMCs from healthy donors after infection with R5 (HIV-1JR-FL) virus. As shown in Fig. 1E, the virus production was considerably augmented in coculture with FDCs. Furthermore, parallel experiments revealed that the virus production in monocytes or macrophages purified from PBMCs was also increased by coculturing with
FDCs (Fig. 1, F and G). These data thus strongly indicate that FDCs can indeed activate viral replication monocytes/macrophages infected with HIV-1.

The enhancement of HIV-1 production by FDCs requires direct cell-cell interactions

To investigate whether this stimulation by FDCs was achieved by direct cell-cell interaction or soluble factors, we used two different cell culture methods for FDCs and U1 cells as follows: 1) FDCs were separately cultured with U1 cells using Transwells and 2) U1 cells were grown in culture medium supplemented with FDC supernatant. Although both culture systems could partially induce HIV-1 replication in U1 cells, these effects were ~20–30% of the full induction of those observed following coculture with FDCs (Fig. 2). This suggested that direct cell-cell interactions might be required for the full induction of HIV-1 replication in monocytic cells, although certain soluble factors may also activate HIV-1 replication to a lesser degree. Furthermore, the fixation of FDCs with paraformaldehyde before coculture completely abrogated the induction of HIV-1 replication in U1 cells, suggesting a requirement for bioactive cell surface molecules in this response.

Taken together, these data indicate that direct interactions via cell surface bioactive molecules are important to fully stimulate HIV-1 replication in monocytic U1 cells by FDCs.

Activation of NF-κB in both FDCs and HIV-1-infected cells following coculture

Our initial analysis demonstrated that FDCs can enhance HIV-1 replication in infected cells via cell-cell interaction. We thus examined whether this induction is initiated by the activation of the HIV-1 long-terminal repeat sequence (LTR). Quantitative and semiquantitative RT-PCR analyses revealed that the levels of HIV-1 mRNA were increased in U1 cells in tandem with increased supernatant p24 levels under coculture conditions with FDCs (Fig. 3, A and B).

HIV-1 replication has been shown to be regulated by host transcription factors such as NF-κB, NF-AT, Sp1, and AP-1 that are...
recruited and bind directly to the HIV-1 LTR (31–33). To determine the identity of the cis-regulatory element(s) within the HIV-1 LTR that are the targets of FDC-mediated transcriptional activation, we examined various 5′-deletion mutants of these regions as described in Fig. 3C (34). Coculturing of U1 cells with FDCs resulted in the activation of CD12 and CD23 reporter constructs.

**FIGURE 4.** Involvement of P-selectin/PSGL-1 in reactivation of HIV-1 replication by FDC. A and B, U1 cells (1 × 10⁵ cells/well) were cocultured with FDCs (1 × 10⁴ cells/well) for 3 days. The mRNA levels of the indicated genes were then measured by quantitative RT-PCR. Labels inside parentheses indicate counterpart ligand or receptor molecules. C–E, U1 cells (1 × 10⁵ cells/well) were cocultured with FDCs (1 × 10⁴ cells/well) in the presence of BAY 11-7082 (1 μM) for 3 days and the levels of PSGL-1 in these cells were then analyzed by quantitative RT-PCR (C). Cell surface PSGL-1 was analyzed by flow cytometry using an anti-PSGL-1 Ab (D). M1 denotes the range of positive cell populations. E, P-selectin expression in FDCs analyzed by quantitative RT-PCR. F, U1 cells (1 × 10⁵ cells/well) were untreated or pretreated with either PSGL-1 or ICAM-1 Ab for 1 h. Cells were then cocultured with FDCs (1 × 10⁴ cells/well) for 3 days followed by measurement of p24. G, U1 cells (1 × 10⁵ cells/well) were transduced with either control or PSGL-1 siRNA (final 6 nM) by Nucleofector according to the manufacturer’s instructions. Cells were then cocultured with FDCs (1 × 10⁴ cells/well) for 3 days followed by Western blot analysis with the indicated Abs. Numerical values below the blots indicate p24 signal intensities normalized by α-tubulin intensity derived by densitometry. The data shown are the average ± SD of three independent experiments (*, p ≤ 0.05 and **, p ≤ 0.01 by the Student t test).
that harbor a NF-κB-binding sequence. However, the CD52 and CD54 constructs lacking this NF-κB consensus site were not activated, suggesting the involvement of NF-κB in the HIV-1 replication response (Fig. 3D). Consistent with this notion, the reporter construct CD12 that contains a site-directed mutation within the NF-κB binding site, CD12mK, was not responsive to FDC stimulation. These results together indicate that the stimulation of HIV-1 in infected cells by FDCs is mediated via the activation of the HIV-1 LTR via NF-κB.

To further address this in terms of biological function, cells were treated with the NF-κB inhibitor BAY 11-7082 to further delineate the role of NF-κB in FDC-mediated HIV-1 replication. Treatment with BAY 11-7082 significantly suppressed HIV-1 production from U1 cells, even when growing in coculture with FDCs (Fig. 3E), although the viability of both cell types was not significantly affected by this exposure (data not shown). Taken together, our data thus indicate that intercellular communication pathways trig-

erged by FDCs can promote and augment HIV-1 production in infected cells via NF-κB activation.

We next investigated whether NF-κB is in fact activated in FDCs as well as in U1 cells under coculture conditions. Consistent with our above gene reporter data, NF-κB activation was confirmed in U1 cells as revealed by the phosphorylation status of NF-κB p65 and 1kBo (Fig. 3F). Interestingly, parallel experiments showed NF-κB activation in FDCs also in our coculture system, as revealed by immunoblotting with phospho-spe-
cific Abs (Fig. 3F). Furthermore, fractionation analysis demonstrated that the nuclear p65 (RelA) levels were significantly enhanced in both U1 and FDCs, indicating the nuclear accumulation of activated NF-κB (Fig. 3F). Parallel kinetic analysis revealed that NF-κB activation in U1 cells was initiated at 12 h and persisted for at least 48 h (Fig. 3G). These findings thus support our contention that cell-cell interactions between FDCs and U1 cells results in the constitutive activation of NF-κB in both cell types and that this is likely to be involved in the amplification of HIV-1 replication signals.

**FDCs activate HIV-1 production via a P-selectin-PSGL-1 interaction**

We were prompted to examine whether NF-κB up-regulates a spec-
cific cell surface ligand and its cognate receptor in FDCs and HIV-
1-infected monocytic cells, eventually contributing to the ampli-
fication of HIV-1 replication signals via NF-κB activation. To this end, we examined the expression of different cell surface ligands and their cognate receptors which are known to be regulated by NF-κB. We chose three ligand/receptor combinations based upon a database search, ICAM-1/CD11b, VCAM-1/CD49d, and P-se-
lectin/PSGL-1, and the expression of these molecules was ana-
yzed by quantitative RT-PCR. Although the mRNA levels of ICAM-1 and VCAM-1 were not significantly altered upon stimulation, transcripts for P-selectin (CD62P/SLBP) were dramatically increased in FDCs (Fig. 4A). Interestingly, transcripts for the cog-
nate receptor for P-selectin, PSGL-1, were found to be signifi-
cantly up-regulated in U1 cells grown in coculture with the FDCs (Fig. 4B), but this was not the case for the CD11b and CD49d receptors. Quantitative RT-PCR and FACS analysis revealed that treatment with the NF-κB inhibitor BAY11-7082 significantly in-
hibited the increase of PSGL-1 mRNA expression and, conse-
quently the cell surface expression of PSGL-1, in U1 cells co-
cultured with FDCs (Fig. 4, C and D). This suggested a crucial role for NF-κB signaling in the induction of PSGL-1 during this co-
culture in HIV-1-infected cells. Likewise, we found that BAY11-
7082 treatment also decreased the induction of P-selectin mRNA in FDCs, indicating that the NF-κB activation in FDCs could play a crucial role in the induction of P-selectin during the coculture with HIV-1-infected monocytic cells (Fig. 4E).

Next, to test the biological significance of a P-selectin-PSGL-1 interaction in terms of HIV-1 induction in our FDC coculture system, U1 cells were pretreated with blocking Ab against PSGL-1 before setting up these cultures. Treatment with PSGL-1 Ab, but not an ICAM-1 Ab, specifically suppressed HIV-1 production in a dose-dependent manner (Fig. 4F). Consistent with this result, tar-
ged disruption of PSGL-1 by specific siRNA significantly decreased HIV-1 production in U1 cells coculturing with FDCs (Fig. 4G). These results together indicate that a juxtacrine signaling mechanism mediated by PSGL-1/P-selectin underlies the activa-
tion of HIV-1 replication in infected monocytic cells stimulated by FDCs.

**Syk acts as a downstream effector of PSGL-1 during HIV-1 replication**

Several previous reports have demonstrated that the cytoplasmic domain of PSGL-1 can directly interact with a Src family kinase, the Syk (35). Syk consists of two N-terminal Src homology 2 domains, which bind phosphorylated ITAM sequences, and a C-
terminal tyrosine kinase domain (35–37). The phosphorylation of Syk acts as a downstream effector of PSGL-1 during HIV-1 replication in infected monocytic cells stimulated by FDCs.

Syk is a mediator of P-selectin/PSGL-1 signaling for HIV-1 replication. To next examine the possible biological functions of Syk during HIV-1 replication, we used a specific inhibitor of the molecule ER-27319 (29, 30) in our FDC cocultures. Treatment with ER-
27319 significantly decreased HIV-1 production and this was ac-
companied by a reduction in the phosphorylated Syk levels in U1

![FIGURE 5.](http://www.jimmunol.org/)

**FIGURE 5.** Syk is a mediator of P-selectin/PSGL-1 signaling for HIV-1 replication in U1 cells. A and B, U1 cells (1 × 10⁶ cells/well) were un-
treated or pretreated with either ER-27319 (30 μM) or JNK inhibitor II (1 μM) for 1 h. Cells were then cocultured with FDCs (1 × 10⁶ cells/well) for 3 days in the presence or absence of inhibitor. Cell supernatants were assayed for measurement of p24 (B). The data shown are the average ± SD of two independent experi-
ments (*, p ≤ 0.05 and **, p ≤ 0.01 by the Student t test).
cells (Fig. 5), whereas JNK inhibitor II had no such effects. These results indicate that the juxtacline signaling between FDCs and HIV-1-infected mononuclear cells mediated by P-selectin/PSGL-1 results in the activation of Syk, which serves as a mediator of the function of NF-κB activation in the HIV-1 replication pathway.

**PSGL-1 and Syk inhibition blocks FDC-induced HIV-1 replication in primary monocytes**

Finally, we addressed whether FDCs can also activate HIV-1 production in infected primary cells via P-selectin/PSGL-1 pathway, in this case human primary monocytes from healthy donors that had subsequently been exposed to HIV-1JRFL. At 24 h after viral infection, the primary monocytes were cocultured with FDCs in the presence or absence of either PSGL-1 Ab or the Syk inhibitor ER-27319. Both of these treatments significantly inhibited HIV-1 production in the primary monocytes in a manner similar to U1 cells (Fig. 6). These results indicate that similar to U1 cells, the PSGL-1/Syk signaling is likely to be a major pathway mediating FDC-induced HIV-1 replication in primary monocytes.

**Discussion**

Previous studies have indicated that HIV-1 infection is largely confined to the GCs of secondary lymph nodes where FDCs commonly reside (15–17). This microenvironment could thus provide the site for highly productive HIV-1 infection whereby FDCs might execute “on-switch” signaling to increase HIV replication. Furthermore, cell–cell infection appears to be far more efficient for viral spread than cell-free virus infection (38, 39). We here report that FDCs can facilitate HIV-1 replication in adjacent infected monocytes/macrophages via a cell-cell interaction mechanism.

FDCs have been shown to interact with B or CD4+ T cells in the GCs of normal lymph nodes (16, 17, 20). It is also reported that in tonsils, CD150 (SLAM) monocytes were localized not only in T cell areas, but also within GCs, suggesting they play a role in B cell activation (40). Moreover, substantial numbers of HIV-infected macrophages were observed in GCs during the course of HIV infection (41). Thus, FDCs can interact with HIV-infected monocytes or macrophages under these conditions during HIV-1 infection. Furthermore, the dysfunctional FDC network is observed in secondary lymph nodes of lymphadenopathy, where the degeneration of the FDC network is usually seen following highly active antiretroviral therapy or administration of therapeutic vaccine in HIV or SIV infection (42–45). One of the most common histological features of HIV-1-associated lymphadenopathy is hyperplastic lymphoid follicles that subsequently undergoes folliculolysis, in which FDCs can be scattered to the extra-GC within the lymph nodes such as cortical sinuses and mantle bodies (46, 47).

Our results with immunohistochemical analysis indicate that FDCs reside with various types of HIV-1-infected cells including monocytes or macrophages in lymphoid organs of HIV-1-associated lymphadenopathy (supplemental Fig. 1). Therefore, our current proposed model for cell-cell interaction between FDCs and HIV-1-infected monocytic cells may reflect the biological or pathological aspects of the natural HIV infection in vivo. However, we could not determine the specific cell surface molecules for activating HIV-1 replication via the cell-cell interaction in vivo. Moreover, it is not well confirmed whether a multitude of other cells, cytokines, and other factors in vivo could influence the cell-cell interaction observed in our in vitro coculture system. Further careful analysis should be performed using human tissues as well as a humanized mouse model inoculated with HIV-1-infected human cells.

We clearly demonstrated here that FDCs, derived from human tonsils, can enhance HIV-1 production in infected monocytes in a coculture system. This enhancement requires direct cell–cell interactions via a juxtacline signaling pathway that is mediated by P-selectin/PSGL-1. Our results are summarized as follows: 1) FDCs can activate HIV-1 replication in infected cells through cell-cell interactions; 2) HIV-1 replication is activated at the transcriptional level and is accompanied by the activation of the HIV-1 LTR through NF-κB; 3) P-selectin expression in FDCs and the up-regulation of its cognate receptor PSGL-1 in HIV-1-infected monocytes cells are facilitated via NF-κB activation; 4) the pathways leading to HIV-1 induction in cell lines also function in human primary monocytes and macrophages infected with HIV-1; and 5) selective inhibitors of PSGL-1 or Syk can efficiently block HIV-1 production in U1 and primary monocytes. These data altogether indicate for the first time that FDCs are a potent inducer of HIV-1 replication in surrounding infected monocytes and macrophages and that PSGL-1/Syk signaling plays a crucial role in this induction of HIV-1.

Very recently, Thacker et al. (26) reported a similar but distinct role of FDCs in the induction of HIV-1 replication in CD4+ T cells obtained from PBMCs and GCs. We also confirmed that FDCs could stimulate HIV-1 replication in MOLT-4 T cells (23) as well as in primary CD4+ T cells (data not shown). However, FDCs-induced HIV-1 replication in CD4+ T cells might be mediated by a distinct mechanism from HIV-1-infected mononuclear cells since the involvement of the PSGL-1/Syk pathway in CD4+ T cells was found to be not prominent (K. Ohba, A. Ryo, and N. Yamamoto, unpublished observation). Therefore, the molecular mechanism for
FDCs to stimulate HIV-1 replication in surrounding infected cells could be attributable to cell type specific.

Intercellular interactions via a ligand/receptor juxtacrine signaling system has been implicated in several virus infections. Tsukamoto et al. (48) reported that the juxtacrine function of the IL-15/IL-15 receptor system in human B cell lines might play a role in the infectivity of EBV (48). Pilotto et al. (49) have demonstrated a crucial protective role for CCL3L1/CCL3 (MIP-1α/pLD78α) signals in both HIV infection and subsequent disease progression. These intercellular communication processes may play an important role in the sustained infection of viruses in different microenvironments within lymphoid organs. Further careful analyses will be required in the future to elucidate the variety of intercellular communication systems that may operate during HIV-1 infection.

There is now some evidence for a role of PSGL-1 as a signal-transmitting receptor in neutrophils (50), monocytes (51), and T lymphocytes (52). This molecule has been reported to associate with Syk through its interaction with moesin and promotes the tyrosine phosphorylation and thus the activation of Syk (35). In addition, signals elicited through PSGL-1/Syk can induce the activation of downstream effectors such as ERK, c-Fos, and NF-κB (53). The activation of NF-κB via PSGL-1 has also been demonstrated in platelet-stimulated monocytes, although the details of the molecular pathways leading to NF-κB activation in this manner have not yet been elucidated (51). Consistent with this result also, we found from our current analyses that PSGL-1/Syk signaling can activate NF-κB. This observation suggests a linkage between PSGL-1 signaling and HIV-1 replication through the activation of NF-κB.

Recently, Gilbert et al. (54) have reported that Src and Syk tyrosine kinases play important roles in the spread of HIV-1 from immature monocyte-derived DCs to CD4^+ T cells. They found that these kinases play a suppressive role in virus transfer in vitro probably by inhibiting the formation of the virological synapse. However, it has not been well characterized whether these signaling molecules contribute to the cell-cell interaction between HIV-1-infected cells and adjacent noninfected cells for virus replication. We showed in this current study that the activation of Syk through the PSGL-1 positively regulates HIV-1 replication in infected monocyctic cells. Thus, Syk could be involved at multiple points in HIV-1 infection and its role could be dependent on each step of HIV-1 life cycle.

In summary, we demonstrate in our current study that FDCs are a potent activator of HIV-1 replication in surrounding infected monocyctic cells. Furthermore, the PSGL-1/Syk pathway is important for this activation of HIV-1 replication. These results shed valuable new light on our understanding of the natural progression of HIV-1 infection over the long term and could provide a means for designing novel therapeutic interventions against AIDS and related disorders.

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Disclosures
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