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*J Immunol* 2000; 164:5010-5014; doi: 10.4049/jimmunol.164.10.5010
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**Cutting Edge: Stromal Cell-Derived Factor-1 Is a Costimulator for CD4⁺ T Cell Activation**

Toshihiro Nanki,² and Peter E. Lipsky³

Stromal cell-derived factor (SDF)-1 is a chemoattractant for T cells, precursor B cells, monocytes, and neutrophils. SDF-1α was also found to up-regulate expression of early activation markers (CD69, CD25, and CD154) by anti-CD3-activated CD4⁺ T cells. In addition, SDF-1α costimulated proliferation of CD4⁺ T cells and production of IL-2, IFN-γ, IL-4, and IL-10. Stimulation with SDF-1α alone did not induce activation marker expression, proliferation, or cytokine production by the CD4⁺ T cells. SDF-1α-mediated costimulation was blocked by anti-CXC chemokine receptor-4 mAb. RANTES also increased activation marker expression by anti-CD3-stimulated peripheral CD4⁺ T cells, but less effectively than SDF-1α did, and did not up-regulate IL-2 production and proliferation. These results indicate that SDF-1 and CXC chemokine receptor-4 interactions not only play a role in T cell migration but also provide potent costimulatory signals to Ag-stimulated T cells. *The Journal of Immunology, 2000, 164: 5010–5014.*

Mice lacking SDF-1 or CXC chemokine receptor-4 (CXCR4), the unique ligand of SDF-1, exhibited cardiovascular, vascular, and neurologic defects as well as defective B cell lymphopoiesis and a severe impairment of bone marrow myelopoiesis (7–9). SDF-1 is thought to attract progenitor B cells into the microenvironment of stromal cells where growth and differentiation factors are released (4, 7, 10, 11). These results suggest that in certain circumstances SDF-1 and CXCR4 interactions may have other functions than merely their chemoattractant activities.

To examine other specific activities of SDF-1/CXCR4 interactions, we examined the capacity of SDF-1α to stimulate T cell activation. Costimulation with SDF-1α up-regulated the expression of activation markers, proliferation, and cytokine production by anti-CD3-activated CD4⁺ T cells. These findings indicate that besides a role in chemoattraction, SDF-1α may play an important role in costimulating activation of Ag-reactive T cells.

**Materials and Methods**

**Sample**

PBMCs were isolated by Ficoll-Hypaque (Pharmacia Biotech, Piscataway, NJ) gradient centrifugation from healthy donors. CD4⁺ T cells were isolated by negative selection using StemSep columns (Stem Cell Technologies, Vancouver, Canada). Purity of the separated CD4⁺ T cells was more than 95%.

**Culture**

The purified peripheral CD4⁺ cells (2 × 10⁶ cells/ml) were cultured in RPMI 1640 with 10% FCS for 6 h because this induced spontaneous expression of CXCR4, the ligand for SDF-1 on the T cells. For blocking CXCR4, 50 μg/ml anti-CXCR4 mAb (12G5; R&D Systems, Minneapolis, MN) or isotype-matched control mAb (20102.1; R&D Systems) was added for the last hour. Afterward, the cells were incubated in medium supplemented where indicated with SDF-1α or RANTES (R&D Systems) for 2 h. Subsequently, the CD4⁺ T cells were transferred to 96-well microtiter plates with or without anti-CD3 mAb OKT3 (500 ng/ml [American Type Culture Collection, Manassas, VA]). After incubation at 37°C for 8 h, surface activation marker expression was analyzed, cytokine production was analyzed after 8–12 h, and proliferation was determined after 20–60 h.

**FACS analysis**

PE-conjugated anti-CXCR4 mAb (12G5; R&D Systems), FITC- or PE-conjugated anti-CD69 mAb (L78; Becton Dickinson, San Jose, CA), FITC-conjugated anti-CD25 mAb (ACT-1; Dako, Carpinteria, CA), and FITC- or PE-conjugated anti-CD154 mAb (TRAP1, Pharmingen, San Diego, CA; and 89h76, Becton Dickinson; respectively) were used. The stimulated peripheral CD4⁺ T cells were stained with the above mAbs and were analyzed with a FACSscan (Becton Dickinson).

**Proliferation assay**

To analyze CD4⁺ T cell proliferation, the MTT assay (Cell Proliferation Kit I [MTT]; Roche, Indianapolis, IN) was used according to the manufacturer’s protocol, and the data reported as OD units.
Cytokine concentrations in the culture supernatant were assayed with ELISA kits for IL-2 (R&D Systems), IFN-γ (R&D Systems), IL-4 (R&D Systems), and IL-10 (BioSource International, Camarillo, CA). Cells were cultured for 8 h to assess IL-2 and IFN-γ production and for 12 h to determine IL-4 and IL-10 production.

Statistics
To compare proliferation and cytokine expression, the Student t test was used.

Results
CXCR4 expression by peripheral CD4⁺ T cells and cultured CD4⁺ T cells
Purified peripheral CD4⁺ T cells expressed low levels of CXCR4 (Fig. 1). Culture with medium for 6 h up-regulated surface CXCR4 expression. Stimulation with anti-CD3 mAb for 8 h did not further up-regulate CXCR4 expression beyond that induced by culture in medium alone.

Costimulation of CD4⁺ T cell function by SDF-1α
Anti-CD3 induced expression of CD69, CD25, and CD154, whereas SDF-1α alone did not (Fig. 2). The combination of anti-CD3 + SDF-1α increased expression of all activation markers beyond that induced by anti-CD3 alone.

Similarly, anti-CD3 stimulation induced proliferation of peripheral CD4⁺ T cells, whereas SDF-1α alone did not (Fig. 3). However, SDF-1α significantly enhanced proliferation of anti-CD3-stimulated CD4⁺ T cells in a concentration-dependent manner. Finally, SDF-1α costimulated cytokine production by anti-CD3-stimulated CD4⁺ T cells (Fig. 4). Although SDF-1α alone failed to induce cytokine production by CD4⁺ T cells, it caused significant concentration-dependent increases in production of all cytokines measured by anti-CD3-stimulated CD4⁺ T cells.

The SDF-1α-mediated enhancement of CD154, CD69, and CD25 expression by anti-CD3-stimulated peripheral CD4⁺ T cells was blocked by anti-CXCR4 mAb (Fig. 5A and data not shown). In addition, the enhancement of IL-2 production was blocked by anti-CXCR4 mAb (Fig. 5B).

RANTES also increased CD154, CD69, and CD25 expression by anti-CD3-stimulated peripheral CD4⁺ T cells (Fig. 6A and data not shown). However, the degree of the enhancement by RANTES was smaller than that by SDF-1α. Moreover, costimulation with RANTES did not up-regulate IL-2 production and proliferation by SDF-1α.

**FIGURE 1.** CXCR4 expression of peripheral CD4⁺ T cells and cultured CD4⁺ T cells. CXCR4 expression was analyzed by FACS. The data shown are from CD4⁺ T cells stained with an isotype-matched control mAb (A), stained with anti-CXCR4 mAb (B), from CD4⁺ T cells cultured with medium for 6 h (C), or stimulated with anti-CD3 for 8 h (D) and stained with anti-CXCR4 mAb. Representative data from one of three independent experiments are shown.

**FIGURE 2.** SDF-1α up-regulates activation marker expression by anti-CD3-stimulated CD4⁺ T cells. Peripheral CD4⁺ T cells were cultured with medium for 6 h and then were stimulated in medium supplemented with 500 ng/ml SDF-1α for 2 h. Subsequently, the T cells were transferred to microtiter plates with or without coating with anti-CD3 mAb and were cultured for 8 h. Expression of CD69 (A), CD25 (B), and CD154 (C) was measured by FACS. Representative data from one of three independent experiments are shown. Presence of SDF-1α or anti-CD3 mAb is indicated.

**FIGURE 3.** SDF-1α up-regulates CD4⁺ T cell proliferation. Peripheral CD4⁺ T cells were cultured with medium for 6 h, and then the T cells were stimulated in medium supplemented with SDF-1α for 2 h. Subsequently, the T cells were transferred to microtiter plates with or without coating with anti-CD3 mAb and were cultured for 20 (A), 40 (B), and 60 h (C). The proliferation was measured by MTT assay and reported as OD units. Representative mean data from one of three independent experiments analyzed in triplicate are shown. Concentration of SDF-1α (ng/ml) and the presence of anti-CD3 mAb are indicated. *, p < 0.05; **, p < 0.01.
anti-CD3-stimulated peripheral CD4$^+$ T cells (Fig. 6B and data not shown).

Discussion

The current data show that SDF-1$\alpha$ is a costimulator of peripheral CD4$^+$ T cell activation. SDF-1$\alpha$ up-regulated cell surface activation marker expression, proliferation, and cytokine productions by anti-CD3-stimulated CD4$^+$ T cells. Stimulation with SDF-1$\alpha$ alone did not up-regulate any of these functions. It is important to note that resting peripheral CD4$^+$ T cells express CXCR4, the ligand for SDF-1 (36–53% positive; $n = 3$), and can respond to SDF-1$\alpha$ by migration (data not shown). However, SDF-1$\alpha$ enhanced activation of CD4$^+$ T cells only when they were also stimulated with anti-CD3. These results suggest that SDF-1 may play an important role not only in T cell migration but also in the effective activation of Ag-stimulated T cells.

Recently, it was shown that SDF-1 also binds a non-CXCR4 receptor (12) and could associate with heparan sulfate (13), suggesting that CXCR4 may not be a unique ligand for SDF-1. However, the current data showed that anti-CXCR4 mAb blocked the

![FIGURE 4](image-url)  
**FIGURE 4.** SDF-1$\alpha$ up-regulates cytokine production. Peripheral CD4$^+$ T cells were cultured with medium for 6 h and then were stimulated in medium supplemented with SDF-1$\alpha$ for 2 h. Subsequently, the T cells were transferred to microtiter plates with or without coating with anti-CD3 mAb and were cultured for 8 h for IL-2 and IFN-$\gamma$ production or for 12 h for IL-4 and IL-10 secretion. IL-2 (A), IFN-$\gamma$ (B), IL-4 (C), and IL-10 (D) concentration in the culture supernatant was analyzed by ELISA. Representative mean data from one of three independent experiments analyzed in triplicate are shown. Concentration of SDF-1$\alpha$ (ng/ml) and the presence of anti-CD3 mAb are indicated. *, $p < 0.05$; **, $p < 0.005$.

![FIGURE 5](image-url)  
**FIGURE 5.** Anti-CXCR4 mAb blocks enhancement of CD154 expression and IL-2 production by SDF-1$\alpha$. Peripheral CD4$^+$ T cells were cultured with medium for 5 h to up-regulate expression of CXCR4 and then were incubated with isotype-matched control mAb or anti-CXCR4 mAb for 1 h. Subsequently, they were stimulated in medium supplemented with 100 ng/ml SDF-1$\alpha$ for 2 h. The T cells were transferred to microtiter plates coated with anti-CD3 mAb and cultured for 8 h. A. Expression of CD154 was assessed by FACS. The data obtained from the cells incubated with isotype-matched control mAb (upper panels) and from the cells incubated with anti-CXCR4 mAb (lower panels) are depicted. Representative data from one of two independent experiments are shown. Presence of SDF-1$\alpha$, control mAb, or anti-CXCR4 mAb is indicated. B. IL-2 concentration in the culture supernatant was analyzed by ELISA. Representative mean data from one of two independent experiments analyzed in triplicate are shown. Presence of SDF-1$\alpha$, control mAb, or anti-CXCR4 mAb is indicated.

![FIGURE 6](image-url)  
**FIGURE 6.** Comparison of costimulatory efficacy of SDF-1$\alpha$ and RANTES. Peripheral CD4$^+$ T cells were cultured with medium for 6 h and then were stimulated in medium supplemented with 50 or 500 ng/ml SDF-1$\alpha$ or RANTES for 2 h. Subsequently, the T cells were transfected to microtiter plates coated with anti-CD3 mAb. After an 8-h incubation, expression of CD154 was assessed by FACS (A). The data obtained from the cells incubated without chemokines (left panel), from the cells costimulated with SDF-1$\alpha$ (center panel), and from the cells costimulated with RANTES (right panel) are depicted. Representative data from one of two independent experiments are shown. After 60 h of culture, proliferation was measured by MTT assay and reported as OD units (B). Representative mean data from one of two independent experiments analyzed in triplicate are shown. Concentrations of SDF-1$\alpha$ and RANTES are indicated. *, $p < 0.01$. 

![Diagram](image-url)
costimulation of anti-CD3-activated CD4⁺ T cells by SDF-1, establishing that CXCR4 is necessary for the costimulation by SDF-1.

In the periphery, mainly naïve CD4⁺ T cells express CXCR4 (14–16), although a substantial percentage (28–37%; n = 3) of memory T cells also express CXCR4. SDF-1 and CXCR4 interactions have been thought to be primarily involved in normal homeostasis by playing a role in the homing of naïve T cells to secondary lymphoid organs (17). The current data suggest that SDF-1 and CXCR4 interaction could also play a role in costimulating Ag-activated CD4⁺ T cells during immune responses in secondary lymphoid organs. Cytokine productions by the CD4⁺ T cells along with CD154 (CD40 ligand) expression were up-regulated by SDF-1 costimulation. The expressed cytokines and CD40 ligand could activate B cells and DCs in lymphoid organs. Thus, SDF-1 may not only attract T cells to secondary lymphoid organs but also may play an important role in costimulating their ability to effect a successful immune response.

Recently, we found that SDF-1 and CXCR4 interaction might be important for accumulation of CD4⁺ T cells in rheumatoid arthritis synovium. The current data showed that SDF-1 could also be a costimulator of CD4⁺ T cell activation. Thus, SDF-1 and CXCR4 interactions might act not only to stimulate accumulation of CD4⁺ T cells in the rheumatoid arthritis synovium but also to costimulate their activation and thereby the capacity to influence the ongoing inflammation in the tissue.

The mechanism by which SDF-1 costimulates anti-CD3-induced T cell activation is not known. Previous data suggested that SDF-1 inhibited anti-CD3-stimulated phosphorylation of the TCR signaling molecules ZAP-70, SLP-76, and pp36 in Jurkat cells, suggesting that SDF-1 could down-regulate T cell activation (18). However, the current data showed that SDF-1 stimulation up-regulated anti-CD3-mediated activation of peripheral CD4⁺ T cells. The explanation for this discrepancy is currently unknown but could relate to different signaling capabilities of Jurkat cells and peripheral CD4⁺ T cells. Previous data have also indicated that SDF-1 stimulation induces phosphorylation of Pyk2 via a G-coupled protein pathway (19, 20). Activated Pyk2 is known to activate mitogen-activated protein (MAP) kinases, including p44/42 MAP (extra-cellular signal-related kinases 1 and 2) (19–21), c-Jun amino-terminal kinase, and p38 MAP kinase (22, 23). In addition, SDF-1 stimulation increased NF-κB activity (21). These signal pathways may be important in the capacity of SDF-1 to costimulate anti-CD3-induced T cell activation. It is noteworthy that SDF-1 alone did not activate peripheral CD4⁺ T cells, suggesting that signaling induced via Pyk2 activation may be insufficient to activate CD4⁺ T cells without costimulation by engagement of the CD3-TCR complex. In this regard, phosphorylation of Pyk2 has been shown to be induced by engagement of another costimulatory molecule, αβ1 (very late Ag 4) (24), which also is unable to stimulate T cell activation without engagement of the CD3-TCR complex. Therefore, Pyk2 might be an important molecule for costimulation by both SDF-1 and very late Ag 4, although it is likely that different proximal signaling pathways are involved. One of these could involve phosphoinositide 3-kinase, which has been shown to be involved in SDF-1 signaling by a number of cell types (25, 26).

It was previously shown that RANTES, macrophage inflammatory protein (MIP)-1α, MIP-1β, and monocyte chemotactic protein-1 could enhance CD25 expression, IL-2 production, and proliferation of anti-CD3-stimulated peripheral CD3⁺ T cells (27), and high concentrations of RANTES stimulated T cells without anti-CD3 stimulation (28). The current data confirmed that RANTES enhanced early activation marker expression by anti-CD3-stimulated CD4⁺ T cells. However, the effect of RANTES appeared to be considerably weaker than that of SDF-1α in that SDF-1α but not RANTES also enhanced proliferation and IL-2 production by anti-CD3-activated CD4⁺ T cells. Therefore, SDF-1 appears to have a greater potential as a costimulator of peripheral CD4⁺ T cells. It is unlikely that the results reflect the frequency of RANTES ligand expressing peripheral CD4⁺ T cells because RANTES can bind to numerous receptors, including C-C chemokine receptors 1, 3, 5, and 9. Although previous studies reported that RANTES enhanced IL-2 production and proliferation by anti-CD3-stimulated peripheral CD3⁺ T cells (27), the current data showed that RANTES did not enhance IL-2 production and proliferation by anti-CD3-stimulated peripheral CD4⁺ T cells. Therefore, it is possible that CD8⁺ T cells or γδ T cells are necessary for up-regulation of IL-2 production and proliferation by RANTES. Recently, it was reported that MIP-3β up-regulated IL-10 production by peripheral T cells stimulated with anti-CD3 and anti-CD28 (29). However, MIP-3β did not up-regulate CD69 and CD25 expression. These results suggest that other chemokines may also have the potential to costimulate T cell activation, although the pattern of activation may differ for different chemokine-chemokine receptor interactions.

References


