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Soluble VCAM-1 Induces Chemotaxis of Jurkat and Synovial Fluid T Cells Bearing High Affinity Very Late Antigen-41

Atsushi Kitani,2* Noriko Nakashima,* Tomomaro Izumihara,‡ Masaki Inagaki,§ Xu Baoui,† Su Yu,* Takemasa Matsuda,‡ and Takami Matsuyama*

It has been shown that cells with high affinity very late Ag (VLA)-integrins have up-regulated expression of a β1-subunit epitope, which is detected by 15/7 mAb. In this study, we demonstrate that soluble VCAM-1 (sVCAM-1) exhibits chemotactic activity of T cells with high affinity VLA-4 against VCAM-1, such as Jurkat T cells and IL-2-dependent T cells. Moreover, we found that T cells in the synovial fluid show high basal migration in the absence of sVCAM-1, compared with peripheral blood T cells in patients with rheumatoid arthritis. Among T cells in the synovial fluid, CD45RO+ memory T cells, in response to sVCAM-1, showed a much higher than basal migratory response when compared with CD45RA+ naive cells, while no significant difference was observed between CD4+ and CD8+ T cells. The chemotactic activity of sVCAM-1 is inhibited in the presence of anti-VCAM-1 and anti-VLA-4, which interfered with the binding between VCAM-1 and VLA-4. Inhibition studies using various kinase inhibitors (C3 exoenzyme, KN62, and H7) show that Rho, Ca2+/calmodulin-dependent kinase II, and protein kinase C are involved in signal transduction in sVCAM-1-induced chemotaxis, respectively, whereas tyrosine kinase seems to play a lesser role, since genistein showed only partial inhibition of T cell chemotaxis. Western blot analysis using an anti-phospho-serine mAb (MO82) reveals that Ser82 in the vimentin is phosphorylated specifically by Ca2+/calmodulin-dependent kinase II. In conclusion, sVCAM-1 contributes to the development of the inflammation of synovial lesion. The Journal of Immunology, 1998, 161: 4931–4938.

Endothelial adhesion molecules play important roles in the tissue recruitment of leukocytes in chronic inflammatory conditions such as rheumatoid synovium (1, 2). In particular, VCAM-1 is abundantly expressed in the lining layer of synovium, and a soluble form of VCAM-1 (sVCAM-1)1 has been found in the synovial fluid (SF) of patients with rheumatoid arthritis (RA) (3, 4). Recently it has been shown that sVCAM-1 induces chemotaxis of human endothelial cells and that the chemotactic activity of rheumatoid SF for endothelial cells is blocked by Abs to either soluble E-selectin or sVCAM-1 (5, 6). We showed that sVCAM-1 inhibited anti-CD3-induced proliferation of T cells from RA SF partially as a result of the reduction in IL-2 production (7). Thus, the soluble form of endothelial adhesion molecules seems to be dynamically involved in chronic inflammatory states and vascular biologic responses.

The adhesive function of very late Ag-4 (VLA-4) (a heterodimer of α4 and β1 subunits), which is known as a counter receptor for VCAM-1, has been shown to vary in the multiple activation states of T cells (8). Chemokines and PMA rapidly induce a high affinity conformation of VLA-4 on T cells in short cultures (9). In a chronically activated state in vivo, the adhesion activity of lymphocytes from RA SF to fibronectin and VCAM-1 are up-regulated via VLA-4/5 with high affinity (7, 10). Studies according to the differential adhesive activity of diverse leukemic cell lines revealed that the epitope of β1-subunit with high affinity conformation of VLA integrins was identified by the 15/7 mAb and a group of HUTS mAb (11, 12).

In this study, we demonstrate that sVCAM-1 exhibits chemotactic activity toward T cells with high affinity VLA-4 such as Jurkat cells and IL-2-dependent T (IL-2 T) cells, which express up-regulated 15/7 mAb epitope. Inhibition of the chemotactic activity of sVCAM-1 for T cells by the addition of anti-VCAM-1 and VLA-4 confirm that the chemotactic activity is mediated by the binding of sVCAM-1 to VLA-4 on the cell surface. Further studies using various kinase inhibitors and Western blot analyses suggest that the chemotactic activity of sVCAM-1 is mediated by Rho, Ca2+/calmodulin-dependent kinase II (CaM kinase II), and protein kinase C (PKC) signaling in T cells.

Materials and Methods

Reagents and mAbs

Recombinant sVCAM-1 that was truncated in the EcoRI site in the 5th Ig-like domain and has two binding sites for VLA-4 within its 1st and 4th Ig-like domains was prepared as previously described (7). The following reagents were purchased: H-7, genistein, cytochalasin B (Sigma, St. Louis, MO); C3 exoenzyme (Upstate Biotechnology, Lake Placid, NY); and KN62 (Seikagaku, Tokyo, Japan). The following mAbs were purchased: anti-VLA-4 (HP2/1) and anti-VLA-5 (SAM1) (Immunotech, Marseille, France); anti-CD4 (NU-T H/I), anti-CD8 (NU-T S/C) (Nichirei, Tokyo, Japan).

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3 Abbreviations used in this paper: sVCAM-1, soluble form of VCAM-1; VLA-4, very late antigen-4; CαM kinase II, Ca2+/calmodulin-dependent kinase II; PKC, protein kinase C; IL-2 T, IL-2-dependent T cells; RA, rheumatoid arthritis; SF, synovial fluid; MIP, macrophage inflammatory protein.
Mononuclear cells were isolated from normal volunteers and from the SFs of 12 patients with RA who met the American College of Rheumatology revised criteria for classification of RA (13) by Lymphoprep (Nycomed, Oslo, Norway) gradient centrifugation. After depletion of adherent cells by plastic dishes, T cells were enriched by negative selection using anti-mouse IgG-coated magnetic beads (Dynal, Oslo, Norway) after incubation with an Ab mixture consisting of anti-CD4, anti-CD16, and anti-CD19 mAbs, as previously described (purity was typically 94% CD3-positive) (14). In some experiments, anti-CD4, anti-CD8, anti-CD54, or anti-CD45RA mAb was added to the previous mixture of mAbs to obtain a reciprocal CD8+, CD4+, CD45RA+, or CD45RA−-enriched subpopulation of T cells, respectively. Reanalysis of sorted populations revealed a purity of >94%. IL-2-dependent T cells were obtained by initially stimulating mononuclear cells with PHA, followed by the addition of IL-2 (20 U/ml) and refeeding twice a day in RPMI 1640 medium supplemented with 10% FCS, penicillin (100 U/ml), and streptomycin (100 μg/ml) (all from Life Technologies, Baltimore, MD). Jurkat and H9 T cell leukemic cell lines (Hayashibara Research Institute, Okayama, Japan) were also maintained in RPMI 1640 conditioned medium.

## Chemotaxis assay

Cell migration was measured using 48-well chemotaxis chambers (Neuro Probe, Cabin John, MD) and polyvinylpyrrolidone-free polycarbonate filters (Nucleopore, Pleasanton, CA) with a pore size of 5 μm for PBL and 8 μm for leukemic cells, as previously described, with slight modifications (15). To avoid adsorption of recombinant sVCAM-1 to filters in the migration assay, filters were coated on both surfaces with 1% BSA in Dulbecco's PBS overnight at 4°C, then extensively washed with DPBS followed by distilled water. The filters were subsequently air dried. Triplicates of 106 cells/ml were preincubated with different concentrations of recombinant sVCAM-1 diluted in chemotaxis medium (serum-free RPMI 1640 supplemented with 1 mg/ml BSA) whereas the upper wells were aliquoted with 50 μl of 3 × 106 cells/ml in the chemotaxis medium. Checker board analysis was also performed by varying the concentration of sVCAM-1 in both the lower and upper wells. Where indicated, mAbs and inhibitors were added to the wells at the start of the assay. The assembled chemotaxis chambers were placed at a 5% CO2 incubator at 37°C for 3 h, the top chambers were disassembled, and nonmigrating cells were removed from the upper side of the filters by drawing the filter up over the wiper blade. The filters were fixed and stained with Giemsa. The number of migrated cells was counted by light microscopy in nine high power fields in triplicate. The results are expressed as the mean number (± SD) of cells migrated per high powered field, from triplicate wells.

### Flow cytometric analysis

Flow cytometry was performed on an EPICS Elite (Coulter) after indirect immunofluorescence staining of the cells using the indicated mAbs. Controls were treated with isotype-matched myeloma proteins (IgG1: MOPC21, IgG2a:UPC10), washed, then stained with FITC-conjugated goat F(ab')2 anti-mouse Ab (Tago, Burlingame, CA).

### Western blot analysis

IL-2-dependent T cells were lysed in immunoprecipitation buffer (PBS (pH7.4), 1% Nonidet P-40 (NP40), 0.01% SDS, 0.5% deoxycholate, 10 μg/ml aprotinin, and 10 μg/ml leupeptin, 1 mM PMSF, 10 mM sodium fluoride, and 1 mM sodium orthovanadate). Lysates were cleared by centrifugation to remove NP40-insoluble protein and saved for tyrosine phosphorylation analysis. The insoluble pellet was solubilized with 1% SDS lysis buffer for vimentin phosphorylation analysis (16). Protein concentrations were determined with the Bio-Rad protein assay, and equivalent amounts of protein were denatured by boiling in sample buffer and then separated by SDS-PAGE (10%) under nonreducing conditions. Proteins were transferred onto nitrocellulose membranes (Hybond-ECL, Amersham, Buckinghamshire, U.K.) and probed with either anti-phosphotyrosine mAb for the NP40-soluble lysates (4G10; Upstate Biotechnology) or with anti-vimentin (1B8) or anti-phosphoephrine mAb (MO82, which specifically reacts with Sez2 in phosphorylated vimentin by calmodulin-dependent kinase II) for the NP40-insoluble protein (17). Peroxidase-conjugated goat anti-mouse Ab (Tago) was then applied, and the detection was conducted using the enhanced chemiluminescence (ECL) detection system (Amersham). The relative intensities of protein bands in the Western blots were determined using an LKB laser densitometer (Uppsala, Sweden).

### Results

Chemotaxis of IL-2-dependent T cells and leukemic cell lines by soluble VCAM-1

The effect of sVCAM-1 on lymphocyte chemotaxis was examined in three cases. sVCAM-1 induced significant levels of migration of IL-2-dependent T cells, but not in unstimulated PBL-T cells (Fig. 1). The chemotactic effect of sVCAM-1 on IL-2-dependent T cells was apparently dose dependent, and the maximal effect was obtained at a concentration of 3.3 nM. A leukemic T cell cell line (Jurkat cells) showed strong migration toward sVCAM-1, whereas another T cell line (H9 cells) showed little migration (Fig. 1). As reported earlier (7), sVCAM-1 does not bind to PBL-T cells, but it does bind to IL-2-dependent T cells via VLA-4 molecules in the presence of divalent cations (Ca2+ and/or Mn2+) from the same donor; moreover, Jurkat cells adhere strongly to immobilized sVCAM-1 whereas H9 adheres to a much lesser extent. Therefore, the differential effect of sVCAM-1-induced chemotaxis on these T cells may be correlated with the variable affinity states of VLA-4 expressed on the T cells. We also confirmed, by checkerboard analysis and by varying the concentrations of chemoattractant in the upper and lower chemotaxis chambers, that, while sVCAM-1 was chemotactic for T cells, it was not chemokinetic for T cells (Table I). These studies also showed that lymphocyte chemotaxis was completely abrogated when sVCAM-1 was at an equal concentration in both lower and upper chambers. Finally, we performed studies to show that the amount of sVCAM-1 absorbed to its filter in our chamber assay was negligible. In these studies the
though total the expression of VLA-4 (HP2/1) is up-regulated in IL-2-dependent T cells from RA patient S.Y. are shown in Figure 2. Representative flow cytometric profiles from normal donor A.K. and patients with RA (RA SF), were studied by flow cytometry. Rep-22resentative flow cytometric profiles from normal donors, and T cells from RA SF, VLA-4 (HP2/1) and, most notably, 15/7 subunit detected by ICAM-1 did not inhibit Jurkat T cell migration toward sVCAM-1. An mAb for β1 subunit (4B4) also showed strong inhibition (85%) of IL-2 T cell migration. As expected, pretreatment of sVCAM-1 with anti-VCAM-1 mAb (2G7) (74% decrease) or pretreatment of IL-2 T cells with anti-FLA-4 mAb (HP2/1) (82% decrease) abrogated IL-2-dependent T cell migration toward sVCAM-1. An mAb for β1 subunit (4B4) also showed strong inhibition (85%) of IL-2 T cell migration, suggesting that αββ5 might have little, if any, role in its migration. Unexpectedly, anti-ICAM-1 mAb considerably inhibited IL-2-dependent T cell migration, suggesting that the signal triggered through ICAM-1 might perturb VLA-4-mediated signaling in IL-2-dependent T cells. Regarding the effect of mAbs on Jurkat T cells, similar inhibition of Jurkat T cell migration was obtained using anti-VCAM-1, anti-VLA-4, and anti-β1 subunit (4B4). However, anti-ICAM-1 did not inhibit Jurkat T cell migration toward sVCAM-1. Collectively, sVCAM-1-induced T cell migration was mediated by its binding to VLA-4 on the cell surface and signaling through VLA-4-triggered cytoplasmic mobility.

**Effects of various mAbs on the chemotaxis of T cells toward sVCAM-1**

The specificity of IL-2 T and Jurkat cell migration by sVCAM-1 was confirmed by various mAbs, including mAbs that blocked cell adhesion between VCAM-1/VLA-4. As shown in Figure 3, anti-CD3 stimulation during the chemotaxis assay period did not enhance sVCAM-1-mediated IL-2 T cell migration. As expected, pretreatment of sVCAM-1 with anti-VCAM-1 mAb (2G7) (74% decrease) or pretreatment of IL-2 T cells with anti-VLA-4 mAb (HP2/1) (82% decrease) abrogated IL-2-dependent T cell migration toward sVCAM-1. An mAb for β1 subunit (4B4) also showed strong inhibition (85%) of IL-2 T cell migration, suggesting that αββ5 might have little, if any, role in its migration. Unexpectedly, anti-ICAM-1 mAb considerably inhibited IL-2-dependent T cell migration, suggesting that the signal triggered through ICAM-1 might perturb VLA-4-mediated signaling in IL-2-dependent T cells. Regarding the effect of mAbs on Jurkat T cells, similar inhibition of Jurkat T cell migration was obtained using anti-VCAM-1, anti-VLA-4, and anti-β1 subunit (4B4). However, anti-ICAM-1 did not inhibit Jurkat T cell migration toward sVCAM-1. Collectively, sVCAM-1-induced T cell migration was mediated by its binding to VLA-4 on the cell surface and signaling through VLA-4-triggered cytoplasmic mobility.

### Table II. Negligible haptotactic effect by sVCAM-1 absorbed to the filter during the IL-2-dependent migration assay

<table>
<thead>
<tr>
<th>sVCAM-1 (3rd Step)</th>
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<tr>
<td>sVCAM-1 (2nd Step)</td>
<td>20 ± 3</td>
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<td>82 ± 14</td>
<td>88 ± 12</td>
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*Migration assay of IL-2-dependent T cell line (A.K.) using a filter treated with three step incubations was performed as follows: 1st step, filters were blocked with 1% BSA overnight; 2nd step, the lower surface of each filter was immersed with sVCAM-1 (3.3 nM) (+) or medium alone (−) for 3 h; 3rd step, each filter was placed in the assembled chemotaxis chamber, then the migration activity of T cells in the presence (+) or absence (−) of sVCAM-1 (3.3 nM), filled in the lower wells in triplicate, was evaluated for 3 h at 37°C. A representative result (±SD) from three independent experiments is shown.*

### Up-regulation of β integrin 15/7 epitope expression on IL-2-dependent T cells

In the next study, surface adhesive molecules on PBL-T and IL-2-dependent T cells from normal donors, and RA SF (RA SF) were studied by flow cytometry. Representative flow cytometric profiles from normal donors and RA SF (RA SF) are shown in Figure 2A. In normal donors, the expression of VLA-4 (HP2/1) is up-regulated in IL-2-dependent T cells as compared with PBL-T cells in donors studied. Although total β1 subunit expression detected by 4B4 was not significantly increased in IL-2-dependent T cells in comparison with PBL-T cells, the high affinity epitope of the β1 subunit detected by 15/7 is up-regulated in IL-2-dependent T cells as compared with that in PBL-T, which is consistent with the previous finding of sVCAM-1 binding to IL-2 T cells (7). ICAM-1 expression was not significantly different between PBL-T and IL-2 T cells in normal donors. CD4+ T cells are dominant among PBL-T cells (CD4+ T cells, 42–57%; CD8+ T cells, 24–39%), whereas the CD4+/CD8+ T cell ratio among IL-2 T cells varied (CD4+ T cells, 22–73%; CD8+ T cells, 25–75%) in the three cases studied. With regard to T cells from RA SF, VLA-4 (HP2/1) and, most notably, 15/7 β1 subunit epitope and ICAM-1 expression were up-regulated when compared with levels found in normal PBL-T cells. In leukemic T cells, both Jurkat and H9 T cells in comparison with PBL-T cells showed greater expression of VLA-4 and β1 subunit (4B4). While 15/7 expression was up-regulated in Jurkat cells, it was not up-regulated in H9 T cells (Fig. 2B). It was estimated that, when the mean fluorescence intensity (MFI) was over 2.2 in 15/7 expression, the cells possessed high affinity VLA-4 in parallel assays of cell attachment (data not shown).

### Increased chemotactic activity of T cells in SF from patients with RA

It was reported that T cells in RA SF express increased 15/7 β1 epitope (18). Therefore, it was presumed that chronically activated T cells from RA SF with high affinity VLA-4 may migrate toward sVCAM-1. T cells in RA SF spontaneously migrated without sVCAM-1 (Fig. 4). The numbers of migrated T cells from RA SF donors (48 ± 13 cells/field) were significantly higher than the numbers of migrated T cells from RA PBL (22 ± 5 cells/field) and normal PBL (20 ± 3 cells/field) without the presence of chemotactant. Moreover, RA SF T cells showed significantly increased migration in response to sVCAM-1 (95 ± 21 cells/field), compared with medium without sVCAM-1 (48 ± 13 cells/field). The
presence or absence of sVCAM-1 had no discernable effect on the migration of T cells from both RA PBL (with sVCAM-1, 25 ± 7; without sVCAM-1, 22 ± 5 cells/field) and normal PBL (with sVCAM-1, 21 ± 3; without sVCAM-1, 20 ± 3 cells/field). Furthermore, a subset study using negative selection by magnetic beads showed that both CD4⁺- and CD8⁺-enriched subsets were chemotactic for sVCAM-1 (Fig. 5). Synovial T cells are known to be enriched with CD45RO⁺ memory T cells as compared with PB

FIGURE 2. Surface expression of integrins on (A) normal PBL-T, IL-2-dependent T cells, and RA T cells from SF and (B) Jurkat and H9 leukemic T cells was analyzed by flow cytometer. A representative profile is shown from the studies of three normal individuals (PBL- and IL-2-dependent T cells) and nine patients with RA (RA SF T cells).

FIGURE 3. Effect of various mAbs on the chemotaxis of (left) IL-2-dependent T cell line and (right) Jurkat T cells toward sVCAM-1 (3.3 nM). Cells were preincubated with medium, OKT3 (anti-CD3, 100ng/ml), HP2/1 (anti-VLA-4, 10 μg/ml, blocks between VCAM-1/VLA-4), 4B4 (anti-CD29, 1 μg/ml), and YH-370 (anti-ICAM-1, 10 μg/ml) for 30 min before chemotaxis at room temperature. sVCAM-1 (3.3 nM) was preincubated with medium or anti-2G7 (anti-VCAM-1, 10 μg/ml, blocks interaction between VCAM-1/VLA-4) for 30 min before chemotaxis at room temperature. Results are expressed as the mean number (±SD) of cells migrated per high powered field in triplicate wells and are representative of three separate experiments.
Furthermore, this study showed that a CD45RO-enriched memory T cell subset from RA SF exhibited the most notable migratory response to sVCAM-1, while the CD45RA enriched T cell subset showed less migration than unseparated T cells from RA SF.

**Effects of various inhibitors on T cell-chemotaxis toward sVCAM-1**

In the next series of studies, we examined the signal transduction pathways involved in sVCAM-1-induced chemotaxis using various inhibitors. Since the cell permeability of C3 exoenzyme is quite low, cells were preincubated with C3 exoenzyme at the concentration of 25 \( \mu \text{g/ml} \) overnight as previously reported (21).

Other inhibitors were added at the indicated concentrations 30 min before the chemotaxis assay. sVCAM-1-induced chemotaxis in both IL-2-dependent and Jurkat T cells were significantly inhibited by C3 exoenzyme (Rho inhibitor), H7 (protein kinase C inhibitor), and KN62 (CaM kinase II inhibitor) (Fig. 6). These results suggest that the GTP-binding protein Rho, protein kinase C, and CaMII kinase activation pathways were involved in sVCAM-1-induced chemotaxis. Although the inhibitory effect of genistein (45% inhibition in IL-2-dependent T cells; 21% inhibition in Jurkat cells) was not as great an effect as that observed with other inhibitors, such as C3 exoenzyme, H7, and KN62, the tyrosine kinase pathway may as well be involved in sVCAM-1-induced signaling. As predicted, cytochalasin B, which inhibits actin polymerization and cell motility, completely abolished IL-2-dependent T and Jurkat T cell migration. In addition, the complete abrogation of T cell chemotaxis in the presence of EDTA confirmed that the binding of sVCAM-1 to VLA-4 was divalent cation dependent.

**CaM kinase II is activated through VLA-4 by sVCAM-1 binding**

Several recent studies have established that the clustering of \( \beta_1 \)-integrins at focal adhesion sites results in increased tyrosine phosphorylation of pp125FAK (22, 23). However, substrates immunoprecipitated with anti-FAK showed that sVCAM-1 did not induce phosphorylation of pp125FAK in IL-2-dependent T cells (data not shown). It was recently shown that phosphorylated peptide-specific Abs enabled several kinase-specific activations, such as cdc2, MAPK, and CaM kinase II (17, 24). Using MO82 mAb, which detects phosphorylated Ser\(^{82} \) by activated CaM kinase II in vimentin, CaM kinase II activity was assessed in sVCAM-1-induced T cell chemotaxis. Western blot analysis with an anti-vimentin mAb showed that vimentin (58 kDa) is constitutively synthesized in IL-2-dependent T cells (Fig. 7A). In regard to the Ser\(^{82} \) phosphorylation site in vimentin, a time-dependent increase in phosphorylation was observed (from 5 to 30 min) in the presence of sVCAM-1 (Fig. 7B). Thus, the CaM kinase II activation pathway is involved in sVCAM-1 signaling which induces T cell-chemotaxis.

**Discussion**

In the present study, we demonstrate that a soluble form of the adhesion molecule VCAM-1 induces T cell chemotaxis. Analysis of 15/7 \( \beta_1 \) integrin epitope expression revealed that the affinity of VLA-4 for VCAM-1 expressed on T cells is a critical parameter for sVCAM-1 induction of T cell chemotaxis. However, exposure to sVCAM-1 or LVD peptide at the concentration of more than 10 \( \mu \text{M} \) induces 15/7 expression on “resting” T cells (11). Therefore,
we graded the concentration of sVCAM-1 up to 10 μM and found that sVCAM-1 induced “resting” T cell migration at our highest concentration of 10 μM (data not shown). It should be noted, however, that this concentration is a thousand times higher than the physiologic concentration of sVCAM-1 in plasma (9.8 ± 2.8 nM) so that it was unlikely that sVCAM-1 itself initiates chemotaxis in unstimulated cells. While truly resting T cells do not express the 15/7 β1 integrin epitope, it is possible that “partially” activated T cells express the 15/7 epitope as a result of exposure to numerous costimulatory and adhesion molecules such as CD28, CD44, and various integrins (18, 25–27). In fact, the above-mentioned chemokines are known to up-regulate the β1 integrin affinity rapidly, as seen in PMA stimulation through PKC (6, 9). Thus, it is possible that sVCAM-1 acts as a chemotactic factor for partially activated T cells exposed to these substances as they pass through the synovium.

T cells from RA synovium were found to be enriched in CD4+ memory T cells that exhibited an enhanced intrinsic capacity for transendothelial migration as assayed in a HUVEC monolayer over collagen gel (20). In another report, RA T cells from SF and synovial tissue were found to be markedly enriched in CD45RAdim, CD45RO+, CD45RBdim, and CD27− mature memory cells, had low proliferative ability, but exhibited potent helper activity for B cell Ig production (28). We found that RA SF T cells were spontaneously (intrinsically) chemokinetic without chemotactant and showed a significantly higher chemotactic response to sVCAM-1. The observed high chemokinesis in the absence of chemotactant seems partly due to the binding of sVCAM-1 to the cell surface of RA SF T cells (7). We further demonstrate that CD45RO-enriched SF T cells are most notably chemotactic toward sVCAM-1. These results indicate that the migration of SF T cells mediated by VLA integrin may be involved in a memory T cell state that is characteristic for T cells responsive to CC chemokine, RANTES, or MIP1α and -β as a result of exposure to numerous costimulatory and adhesion molecules such as CD28, CD44, and various integrins (18, 25–27). In fact, the above-mentioned chemokines are known to up-regulate the β1 integrin affinity rapidly, as seen in PMA stimulation through PKC (6, 9). Thus, it is possible that sVCAM-1 acts as a chemotactic factor for partially activated T cells exposed to these substances as they pass through the synovium.

A number of soluble forms of adhesion molecules have been identified in SF, e.g., soluble intercellular adhesion molecule-1 (sICAM-1), sE-selectin, and sL-selectin, in addition to sVCAM-1, identifying inflammatory interactions between endothelial cells, leukocytes, and other synovial cells in the diseased joint (32–34). These soluble adhesion molecules may modulate the recruitment and retention of inflammatory cells into SF as well as tissue. In fact, there is a gradient of sVCAM-1 concentration between plasma (9.8 ± 2.8 nM) and SF (17.3 ± 4.4 nM) in patients with RA (n = 11, p < 0.005) consistent with an earlier report (35). These concentrations of sVCAM-1 are within the range of eliciting chemotactic activity in T cells. In this regard, it was also shown that the chemotactic activity of rheumatoid SF for endothelial
cells, and its angiogenic activity, were blocked by Abs to either sVCAM-1 and sE-selectin (5).

It was recently reported that the interaction between LFA-1 and ICAM-1 decreases T cell adhesion mediated by $\alpha_4\beta_2$ and $\alpha_5\beta_1$, suggesting that the integrin cross-talk decreases $\alpha_5\beta_1$ integrin-mediated binding of T cells to fibronectin and VCAM-1 (36). This cross-talk between $\beta_1$ and $\beta_2$ integrins may be the reason that anti-ICAM-1 ligation has an inhibitory effect on the chemotaxis of IL-2-dependent T cells induced by sVCAM-1. The same inhibition does not apply to the Jurkat T cell line, because this line is known to have a defect in LFA-1 signaling (37).

Signal transduction in sVCAM-1-induced T cell chemotaxis should be considered as two components, 1) outside-in signaling triggered by VLA-4 generated through the binding of sVCAM-1 in the fluid phase, which induced only VLA receptor occupancy, not receptor clustering, and exhibits incomplete signaling when compared with the signaling typically generated in cell adhesion; and 2) a prerequisite signal for cell migration due to cytoplasmic mobilization induced by a G-protein-coupled receptor such as a chemokine receptor. With regards to outside-in signaling, most studies premised immobilized ligands such as fibronectin and anti-VLA mAbs (38–40). Adhesion-induced integrin engagement induced tyrosine phosphorylation of pp125FAK, followed by the subsequent phosphorylation and assembly of various signaling and cytoskeletal proteins that form a focal adhesion structure (22, 23, 41, 42). In contrast, we found that the signal triggered by soluble VCAM-1 in the fluid phase did not effectively induce tyrosine phosphorylation of pp125FAK. In addition, T cells migrated toward sVCAM-1 even in the presence of genistein. Thus, tyrosine phosphorylation of pp125FAK might not be a prerequisite for T cell migration.

With regard to a prerequisite signal for cell migration, our finding that sVCAM-1-induced migration is related to signaling by Rho may well be interpreted by the observation of Laundanna et al. (43). They found that IL-8-stimulated guanine nucleotide exchange on RhoA induces $\alpha_5\beta_2$ adhesion to VCAM-1 in the Jurkat cells transfected with IL-8R and that adhesion was blocked by C3 exoenzyme, which blocked Rho activation. Their transfection experiment coupling chemotactic receptor-triggered signaling to the adhesion-inducing pathway indicated that Rho participates in both cell migration and adhesion signaling pathways. This notion is consistent with our finding that T cell chemotaxis in response to sVCAM-1, an adhesion molecule adhered to by T cells in the fluid phase, is inhibited by C3 exoenzyme. The roles of Rho in adhesion triggering and chemotaxis are also explained by the finding that Rho-microinjected Swiss 3T3 fibroblasts induce phosphorylation of pp125FAK, p130, and paxillin, which are known to localize to focal adhesion and formations of stress fiber. Also, Rho-induced phosphorylation of pp125FAK, p130, and paxillin is observed in the absence of stress fiber formation and is, therefore, independent of Rho-induced actin polymerization (42, 44).

In regard to migration involved in protein kinase C activation, haptotaxis of the Ag-specific human T cell line and PBL-T cells triggered by immobilized anti-VLA-4 was inhibited by calphostin, an inhibitor of PKC, while the effect of tyrosine kinase inhibitor on the haptotaxis depended on the cell type (40).

CaM kinase II was first demonstrated to be required for the migration of vascular smooth muscle cells (45). We found that blocking the activation of CaM kinase II by KN62 inhibited T cell chemotaxis in response to sVCAM-1 by more than 80%, as well as inhibition of protein kinase C activation by H7. Using mAb specific for phosphorylated Ser47 in the vimentin by CaM kinase II, we showed that IL-2 T chemotaxis in response to sVCAM-1-generated CaM kinase II activation. Integrin-mediated elevation of intracellular Ca$^{2+}$ levels in Jurkat T cells was recently described (46), and activation of CaM kinase II by calmodulin may follow after intracellular Ca$^{2+}$ signaling. Moreover, it was reported that Jurkat T cells transfected with a constitutively active mutant of CaM kinase II showed a transcripational block of IL-2 reminiscent of T cell anergy (47). In this respect, the rheumatoid synovium has been proposed as a model of T cell anergy (48), and we previously reported that the T cells in RA SF were bound by sVCAM-1 and anergic in T cell proliferative responses (7).

Finally, two distinct features, highly chemotactic and anergic responsiveness of T cells in RA SF, have been characterized. sVCAM-1 generated in the lining layer of rheumatoid synovium might participate in the acquisition of these functional features of T cells in RA SF.

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References


