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Effects of Continuous Exposure to Stromal Cell-Derived Factor-1α on T Cell Rolling and Tight Adhesion to Monolayers of Activated Endothelial Cells[1]

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Immobilized stromal cell-derived factor-1α (SDF-1α) has been shown to induce tight adhesion of T cells to purified ICAM-1 in assays done under flow conditions. In this study, we show that soluble SDF-1α induced a rapid (within 20 s) cessation of rolling and tight adhesion of >90% of the rolling T cells on monolayers of activated endothelial cells under similar flow. Within 4 min, the T cells had either started to migrate between the endothelial cells or re-entered the rolling and circulating lymphocyte pool. This deadherence of the firmly bound cells, with either ensuing transmigration or continued rolling, was most likely due to desensitization of lymphocytes to the continuously present SDF-1α. The released rolling lymphocytes could still respond to other activating signals by a second round of tight adhesion. Pretreating the lymphocytes with pertussis toxin almost completely blocked the effect of the chemokine, confirming that the induction of firm adhesion was due to the function of the chemokine on the lymphocytes and not the endothelial cells. Pretreating the endothelium with SDF-1α did not lead to firm adhesion of subsequently added lymphocytes, also indicating that the effect was due to soluble, not endothelially bound, chemokine. Blocking experiments showed that the same molecules mediated rolling before and after SDF-1α-induced tight adhesion. This is the first study to demonstrate the effect of soluble SDF-1α on T cell rolling on an endothelial cell monolayer. The data broaden our understanding of the stimulatory factors directing the firm adhesion and ensuing transmigration of leukocytes into tissues through activated endothelium. ***The Journal of Immunology,*** **2000, 164: 5035–5040.**

Migration of lymphocytes into tissues is mediated by a multistep process involving rolling of the circulating cells along the vessel walls, rapid activation/up-regulation of the adhesion capacity of the rolling cells, tight adhesion, deadhesion, and transendothelial cell migration (1–5). The transition of a rolling cell to one that is tightly adhered to the vessel occurs within seconds in vivo (4). Effective tight adhesion is a reversible process, where detachment is needed for both the progressive migration of the lymphocytes into the tissue or the return of the lymphocytes into the rolling and circulating pools.

Characterization of the molecular pathways involved in rapid adhesion following initiation of rolling has received considerable attention in recent years and is well understood for myeloid cells (6–9). Soluble chemotactic factors (chemokines) trigger a rapid transition of rolling neutrophils to tightly adhered ones in assays done under physiological flow (6, 9). Chemokines have also been shown to increase lymphocyte adhesion (10–12). In an elegant study, Campbell et al. (10) showed that stromal cell-derived factor 1α (SDF-1α),[3] 6-C-kine, and macrophage-inflammatory protein-3β trigger rapid arrest of lymphocytes rolling on purified peripheral node addressin (PNAd) to ICAM-1 coated on the same substrate. Induction of tight adhesion was triggered by immobilizing the chemokine on the same substrate as PNAd and ICAM-1. Pachynski et al. (11) used very similar experimental conditions to show that 6-C-kine (secondary lymphoid-tissue chemokine) triggers rapid adhesion of lymphocytes to recombinant mucosal addressin cell adhesion molecule-1. Piali et al. (12), on the other hand, stimulated HUVECs with IFN-γ and TNF-α and found firm adhesion of incoming lymphocytes in a parallel plate flow assay . This firm adhesion was likely due to the chemokines IFN-γ induced protein-10 (IP10) and monokine induced by IFN-γ (Mig) produced by the endothelial cells.

Although the results outlined above are quite compelling, they do not directly address whether the addition of soluble chemokine induces rapid adhesion under flow. Another important aspect not covered by these studies is what happens to the rolling lymphocytes upon continual exposure to a chemokine. Desensitization of leukocytes to a chemokine has been shown both in firm adhesion of IL-8RA-transfected cells to purified ICAM-1 and IL-8 under flow (13), with IP10 and Mig on immobilized ICAM-1 in a static assay (12) and in chemotaxis assays (14). The latter study also showed that the capacity to respond to other chemotactic factors was retained by the desensitized leukocytes that no longer respond to the original chemokine. Finally, chemokines induce rapid adhesion to an artificial substrate of purified ligands, but what are the consequences of exposure of soluble chemokine to lymphocytes rolling on the complex surface of the vascular endothelium under continuous flow?

In this study, we demonstrate that soluble SDF-1α, when injected into an assay with lymphocytes rolling on an endothelial cell monolayer under continuous flow, induces rapid, transient adhesion of the rolling lymphocytes. Under the continual presence of the chemokine the deadhered and thus desensitized cells are still

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[3] Abbreviations used in this paper: SDF-1α, stromal cell-derived factor-1α; PNAd, peripheral node addressin; IP10, IFN-γ induced protein-10; Mig, monokine induced by IFN-γ; PTX, pertussis toxin.

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capable of firm adhesion upon addition of other stimulants. The molecular mediators of rolling in the desensitized cells and the leukocytes before firm adhesion were similar. These findings emphasize the importance of desensitization to chemokines in lymphocyte homing.

Materials and Methods

Blood samples and γδ T cell purification

Heparinized peripheral blood from 1-wk to 3-mo-old male calves was collected, diluted 1:1 with warm HBSS, and centrifuged through Histopaque 1077 (Sigma, St. Louis, MO) at 800 × *g* for 30 min at room temperature. Mononuclear cells were collected from the Histopaque/plasma interface. Monocytes were removed by adhesion to plastic, and γδ T cells were purified by panning on monolayers of E-selectin cDNA-transfected L cells. After a 30-min incubation at 37°C, the γδ T cells were collected by incubation with 2 mM EDTA, yielding >90% of γδ T cells (Ref. 15 and our unpublished data).

Transmigration assay

Purified γδ T cells were transferred into the upper wells of 3-μm pore 6-well transwell plates (Becton Dickinson Labware, Franklin Lakes, NJ). The cells were allowed to migrate for 4 h at 37°C passively or after the addition of 200 ng/ml of SDF-1α (PeproTech, Rocky Hill, NJ) to the lower well. The migrated cells were collected and the γδ T cells were enumerated with a hemocytometer and FACS analysis of the GD3.8$^+$ (pan-γδ T cell marker; (16)) lymphocytes identified by their distinctive light scatter profile.

Rolling assay

To address the effect of the continuous exposure to a soluble chemokine and possible desensitization, a system in which the rolling of unactivated lymphocytes on endothelial cell monolayers can be reproducibly analyzed over extended periods of time is required. We have previously defined an in vitro system using stimulated endothelial cells grown on the internal surface of glass capillary tubes as the adhesive substrate and bovine peripheral blood γδ T cells as the rolling lymphocyte population (15). The assay employs a circulatory loop; thus, the same population of lymphocytes can be continuously monitored over time. Also, a chemokine added into this assay is continuously presented to the cells. The bovine γδ T cell population can be purified in large numbers and, by isolating the cells from newborn animals, they are homogeneously in an unactivated state. As such, unless an additional stimulus is added to the rolling assays, bovine γδ T cells continuously roll on activated endothelial cells for up to 1 h. Importantly, in this system the receptor-ligand interaction involved in rolling does not induce signaling, leading to firm attachment of the lymphocytes.

For the current study, this previously published capillary tube shear-dependent rolling assay was modified slightly (15). Bovine umbilical cord endothelial cells were grown to confluency on the internal surface of sterile 1.4-mm capillary tubes (Drummond Scientific, Broomall, PA) and stimulated with 50–100 ng of LPS for 4–5 h at 37°C to induce expression of endothelial adhesion molecules. Tubing was attached to each end of the capillary tube to form a closed circulatory system. Within this system, fluid and cells were recirculated by using a variable peristaltic pump (Cole Parmer, Barrington, IL). The capillary tube was mounted on an inverted microscope (Nikon Diaphot; Nikon, Tokyo, Japan) modified for video microscopy (Sony CDD Camera, Sony, Tokyo, Japan). The enriched γδ T cells were incubated for 2–3 h at 37°C and injected into the system at 1–2 × 10^6 cells/ml in DMEM with 10% FBS and 20 mM HEPES. SDF-1α (2–40 ng/ml) was added to the assay at time points indicated in the Figs. 1–5. Each lot of SDF-1α used in the study was tested for activity in the chemotaxis assay, aliquoted, and stored at −80°C until they were used. In some experiments, PMA (100 ng/ml) was added to the assay. To further characterize the effect of SDF-1α, pertussis toxin (PTX; 100 ng/ml) was used to pretreat the lymphocytes for 2 h at 37°C (17). The time needed for the chemokine to disperse throughout the flow system was about 7 s (data not shown). The camera was focused on a field supporting rolling under a shear force of ~2 dynes/cm^2, and the same field was monitored throughout the assay. The number of rolling and firmly adhered γδ T cells within this field of view was determined by analysis of the videotape recording with a macrodriven NIH Image software on a Macintosh 660 AV computer (Apple, Cupertino, CA). Aggregates were excluded from the analyses.

In some rolling experiments, the activated endothelial cells were replaced by E-selectin cDNA-transfected mouse L cells. These transfectants also support lymphocyte rolling (18). In some rolling experiments, a function blocking anti-E- and L-selectin mAb (EL-246) (18), mAb GR113 which blocks γδ T cell rolling on 24-h LPS-activated endothelial cells (15), or a non-blocking E-selectin recognizing mAb EL-81 were injected into the assay at 50 μg/ml for each mAb.

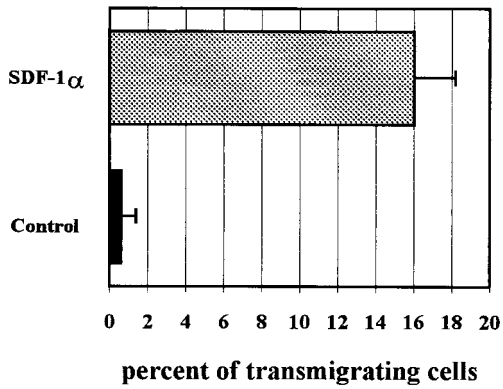

FIGURE 1. SDF-1α-induced cell migration through 3.0-μm micropore filters. Bovine peripheral blood γδ T cells were isolated as described elsewhere (15). Next, the γδ cells were incubated in the upper chambers of 6-well transwell plates for 4 h at 37°C (8 × 10^6 cells/well). After the incubation period, cells in the lower chambers were collected by washing with 5 mM EDTA to loosen adhered cells. The number of γδ T cells was determined by counting the cells with a hemocytometer and FACS analysis of the GD3.8$^+$ (pan-γδ T cell marker (16)) lymphocytes. Black bar shows the proportion of passively migrating cells of all cells inserted in the upper well. Gray bar shows the proportion of cells migrating after the addition of 200 ng/ml of SDF-1α to the lower chamber. Error bars indicate the SD of repeats done in two separate experiments.

Results

Human SDF-1α cross-reacts with bovine γδ T cells

An in vitro chemotaxis assay was used to test whether human SDF-1α cross-reacts with bovine γδ T cells. As shown in Fig. 1, SDF-1α at a concentration of 200 ng/ml induced chemotaxis of purified bovine γδ T cells in a micropore filter assay. In preliminary experiments, the migratory response of bovine lymphocytes was detectable at 50 ng/ml, peaked around 200 ng/ml, and decreased with doses of 400 ng/ml or higher (data not shown). When compared with passive migration, the proportion of γδ T cells migrating from the upper to the lower chamber increased 26-fold when SDF-1α was applied to the lower chamber. When SDF-1α was added to the top chamber with the lymphocytes, thus eliminating the concentration gradient and desensitizing the cells to SDF-1α, the number of lymphocytes migrating through the filter decreased to about 20% of the passively migrating cells (data not shown). Thus, human SDF-1α is chemotactic for bovine γδ T cells.

SDF-1α induces a rapid, transient adhesion of rolling cells to an endothelial cell monolayer

Next, we tested whether SDF-1α could induce rapid adhesion of rolling cells in the capillary tube assay. Rolling of enriched γδ T cells on the LPS-stimulated endothelial cells under continuous flow was established as described in *Materials and Methods*. As shown in Fig. 2*A*, γδ T cells continuously rolled in this assay for over 20 min, with minimal tight adherence to the endothelial cell monolayer. When PMA was infused in the assay, adhesion occurred within 2 min and the cells remained attached for more than 30 min (data not shown; see Fig. 3). As shown in Fig. 2*B*, within 20 s of injecting 20 ng/ml of SDF-1α into the assay, >90% of the rolling cells completely stopped on the endothelial cell monolayer.

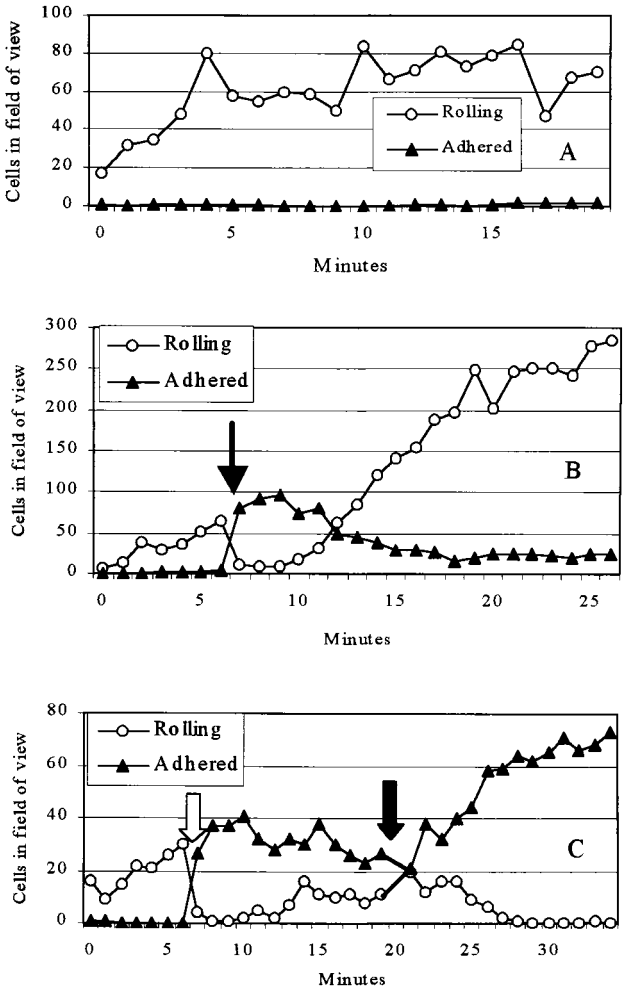

FIGURE 2. SDF-1α induces firm attachment of rolling lymphocytes to endothelial cells. Bovine umbilical cord endothelial cells were grown on the internal surface of capillary tubes and activated with LPS (50–100 ng/ml) for 4 h at 37°C. Purified $\gamma\delta$ T cells were introduced into a circulatory loop with a shear of 2 dynes/cm^2. *A*, $\gamma\delta$ T cells were allowed to roll on the endothelial cells without any treatment for a period of 20 min. *B*, Effects of the addition of SDF-1α (20 ng/ml; arrow). *C*, Addition of SDF-1α (40 ng/ml; open arrow) and subsequent PMA (100 ng/ml; filled arrow). Results presented in *A–C* are representatives of 4, 5, and 2 experiments, respectively.

The bound cells remained tightly adhered for 2–4 min, some flattened and began to migrate in between the endothelial cells, whereas others eventually detached and re-entered the circulation. Once the T cells released and re-entered the circulation, most of them immediately began to roll on the endothelial cell monolayer in the continual presence of activating concentrations of SDF-1α. Thus, they rapidly became desensitized to the induction of firm adhesion by the chemokines. In control experiments, a second injection of SDF-1α following the deadhesion of the adhered cells did not cause new firm adhesion of the cells (data not shown), also speaking for the desensitization of the released cells.

Interestingly, the number of cells rolling after the transient firm adhesion of some of the lymphocytes was higher than before the addition of the chemokine (Fig. 2*B*). To test whether the rerolling lymphocytes could respond to other potential signaling molecules and reinitiate tight adhesion, PMA was injected into the assay. As shown in Fig. 2*C*, PMA caused a rapid induction of tight adhesion of the lymphocytes.

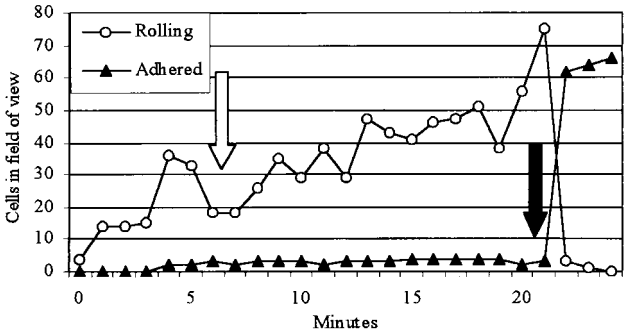

FIGURE 3. Pretreating lymphocytes with PTX inhibits the firm binding of lymphocytes to endothelial cells after SDF-1α treatment. Bovine $\gamma\delta$ T cells were pretreated with PTX (100 ng/ml) for 2 h at 37°C and allowed to roll on bovine umbilical cord endothelial cells at a shear of 2 dynes/cm^2. SDF-1α was added (20 ng/ml; open arrow), followed by PMA (100 ng/ml; filled arrow). A representative of two experiments is shown.

To study further the sensitization and desensitization events that occurred following injection of SDF-1α into the rolling assay, a sequential dose-response analysis was done. Rolling interactions were established, as described for Fig. 2, and then SDF-1α was injected at 2 ng/ml, which led to variable induction of firm adhesion, ranging from 5 to 50% of the cells in the field of view. In experiments in which significant tight adhesion was induced (approaching 50% of the rolling cells), deadhesion and rerolling occurred in the same fashion as shown in Fig. 2*B* (data not shown). In experiments in which 2 ng/ml of SDF-1α induced minimal tight adhesion without a reduction of rolling, a second injection of 20 ng/ml of SDF-1α led (Fig. 4) to a rapid induction of tight adhesion and reduction in rolling as seen in Fig. 2*B*. Thus, sensitization and desensitization was dose dependent, with 2 ng/ml of SDF-1α being at the lower threshold needed to induce the effects.

Pretreatment of lymphocytes with PTX blocks SDF-1α-induced firm adhesion

Chemokine signaling has, in many cases, been shown to be mediated through $G_i\alpha$-linked receptors (10, 11). PTX blocks this signal transduction pathway by inactivating $G_i\alpha$ proteins. The effect of SDF-1α was almost completely blocked by pretreatment of the lymphocytes with PTX (Fig. 3). The treatment with PTX, however, did not prevent the capability of the cells to firmly bind to the endothelium, since the addition of PMA to the assay still induced firm binding of the cells. The effect of PTX also showed that the

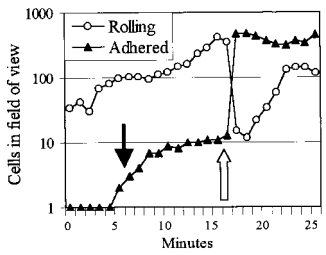

FIGURE 4. A dose-response experiment of the effect of SDF-1α on rolling lymphocytes. Bovine $\gamma\delta$ T cells were introduced into a circulatory loop with a shear of 2 dynes/cm^2. Low-dose SDF-1α was added into the assay (2 ng/ml; arrow). A higher dose (20 ng/ml; open arrow) was added to determine whether the cells were desensitized by the lower dose of SDF-1. Please note the log scale used.

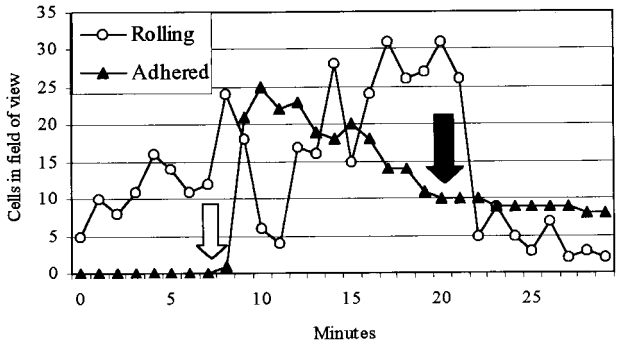

FIGURE 5. Pretreating endothelial cells with SDF-1α does not lead to firm adhesion of freshly injected $\gamma\delta$ T cells. Bovine umbilical endothelial cells were pretreated with SDF-1α by injecting 20 ng/ml into the rolling assay and allowing it to circulate in the tubing for 8 min. The tubing was washed thoroughly with media and bovine $\gamma\delta$ T cells were injected into the assay ($t = 0$ min). A new dose of the chemokine SDF-1α (20 ng/ml; open arrow) and the mAb GR113 (50 μg/ml; filled arrow) were added as indicated. A representative of two experiments is shown.

effect of the chemokine under these conditions is most crucial for the lymphocytes, not for the endothelial cells.

Endothelial cells do not present previously bound SDF-1 to the rolling cells

Chemokines have been shown to bind to endothelial cell proteoglycan (19, 20), which contributes to the presentation of the chemokine to the leukocytes. To determine whether the activated endothelial cells used in our rolling assay participated in the induction of firm adhesion, we injected the chemokine into the assay before the addition of the $\gamma\delta$ T cells and allowed it to interact with the endothelial cells for 7–8 min under flow conditions. The tubing was washed by injecting 30 ml of fresh media through the tubing at approximately the same flow rate as in the rolling assay. The $\gamma\delta$ T cells were then added to the assay. Induction of tight adhesion was not seen, the T cells continually rolled as in the injection of cells to an assay before the addition of the chemokine (Fig. 5). The addition of SDF-1α into the assay induced firm adhesion of the cells, thus confirming that the injected cells were capable of responding to SDF-1α (Fig. 5). The fact that, upon injection of SDF-1α to the assay with rolling lymphocytes, a single wave of firm adhesion was seen showed that the soluble chemokine controls the formation of firm adhesion in this assay.

The same adhesion molecules support lymphocyte rolling before and after SDF-1α-induced firm adhesion

The rolling of leukocytes on endothelial cells has been shown to be mediated by multiple adhesion molecules including selectins, α_4 integrins, and CD44 (for a review, see Ref. 5). To study whether the desensitized cells utilized the same adhesion molecules in their rolling as before the addition of the chemokine, we used E-selectin cDNA-transfected L cells to support $\gamma\delta$ T cell rolling as described previously (18). This approach limits rolling to a single molecule. Enriched $\gamma\delta$ T cells were allowed to roll on the transfectants, and after the addition of SDF-1α, firm adhesion of the rolling cells to the L cells was induced (data not shown). As compared with the effect of SDF-1α on $\gamma\delta$ T cells rolling on activated endothelial cells, induction of firm adhesion to the L cells was slower (peak accumulation at 8–10 min) and involved a smaller proportion of the rolling cells ($\approx$15% vs >90%). As published earlier, this rolling could be blocked by the addition of EL-246 into the assay (18). Also, when EL-246 was injected into the assay after SDF-1α when the desensitized cells continued to roll, virtually all rolling was inhibited within 2 min upon the addition of the mAb (data not shown). In Ab-blocking experiments on bovine endothelial cells, >90% of $\gamma\delta$ T cell rolling is blocked within 3 min by the mAb GR113, whereas control mAb EL-81 and EL-246 have no effect (see fig. 1*A* in Ref. 15). When GR113 was injected into the $\gamma\delta$ T cell/endothelial cell rolling assay after the desensitization of the lymphocytes to SDF-1α, similarly almost all rolling was prevented (Fig. 5). Thus, lymphocytes that become desensitized to SDF-1α retain the capacity to roll on the same molecule as they did before SDF-1α treatment.

Rolling speeds of adhering cells are heterogeneous

The rolling speeds of the $\gamma\delta$ T cells were heterogeneous (10.9 $\pm$ 5.3 μm/s in a representative experiment, $n = 64$ cells). Also, the rolling speeds of the cells before the induction of firm adhesion showed variation, some cells rolling and then skipping up to 50 μm before firmly adhering, some rolling continuously until adhering, and some slowing their rolling speed just before stopping (data not shown). By comparison, the rolling speed on E-selectin-transfected L cells was slower (6.0 $\pm$ 7.3 μm/s; $n = 20$). Thus, a specified rolling velocity did not seem to be a prerequisite for firm adhesion.

Discussion

Understanding how lymphocytes tightly adhere to the vascular endothelium is important to our overall understanding of lymphocyte trafficking. Although it was proposed in the original presentation of the multistep model of lymphocyte extravasation that soluble chemokines might induce the transition of a rolling cell to one that is tightly adhered (1), demonstration of this role has been elusive. Indeed, the lack of success in earlier experiments testing a variety of chemokines led investigators to propose that other signaling molecules, such as the molecules mediating the rolling itself, could be important in inducing rapid, tight adhesion of lymphocytes under flow (1, 8, 9). The reports by Campbell et al. (10) and Pachynski et al. (11) clearly showed that some chemokines induce rapid lymphocyte adhesion to purified ligands. This report shows that rapid adhesion can be induced by a soluble chemokine and, importantly, can occur on endothelial cell monolayers. This might reflect the situation in vivo better than an artificial layer of purified ligand, which may or may not reflect the densities of the various ligands on cells in vivo.

Leukocyte subsets have the capacity to respond to a multitude of chemokines and/or chemotactic factors (14). Furthermore, many of these factors of leukocyte migration may be concomitantly present in tissues recruiting leukocytes. Recent studies have demonstrated the simultaneous production of multiple chemokines by stimulated endothelial cells (12, 20). Similarly, in an infection and at sites of inflammation, chemoattractants are produced by host endothelial, epithelial, and stromal cells and by activated leukocytes within the tissue. Thus, the migrating lymphocytes encounter a multitude of gradients of chemotactic factors. SDF-1 has been shown to be a highly efficacious chemoattractant for human lymphocytes, including both T cells (21) and B cells (22). SDF-1 has two isoforms, SDF-1α and SDF-1β, that differ from one another by four additional amino acids on the carboxyl-terminal of the latter (23). The genes of both SDF-1 isoforms and its only known receptor, CXCR4, are all conserved in the human and mouse with >90% similarity between the species (24–27) and thus made the experiments of cross-reactivity in bovine feasible.

This study shows that lymphocyte sensitization to SDF-1α followed by desensitization can occur within minutes on the surface of the endothelium, yet these cells can still respond to other activating agents, leading to tight adhesion. This finding is of special importance since it shows that desensitized cells are likely still able to respond to other factors within the same or different tissues at the vessel surface. The desensitization process is likely required for the lymphocytes to release and migrate through the vessel or re-enter the blood flow while continuously exposed to the original chemokine. The multitude of chemotactic factors that exist in the body ensures that once desensitization to one factor takes place, there are many others that can regulate the subsequent steps of the extravasation process. Together with the various patterns of adhesion molecules expressed by lymphocyte subsets, chemokines, through sensitization, as originally proposed, and now desensitization, as described here, provide exquisite regulation of the multistep process of lymphocyte homing.

As compared with purified ligands, the endothelial cell monolayer used in our assay and the endothelium in vivo simultaneously provide various densities of multiple ligands for rolling and firm adhesion. Leukocyte subsets could show different usage of these ligands, possibly as a function of their status of activation. Furthermore, the firm adhesion of the first cells upon chemokine stimulus alters the adhesive potentials of the vascular cell wall by allowing leukocyte-leukocyte rolling. This could be one way of increasing the capacity of leukocytes to migrate into the tissue in vivo.

The chemotactic effects of chemokines are known to depend on concentration gradients (14). On the other hand, some chemokines have been shown to be presented by the endothelial proteoglycans (19, 20) and to induce firm adhesion without the formation of a gradient. In vivo, when chemokines are produced by various cells in tissues and enter the vascular lumen, they are either bound by the endothelium or enter the flow of blood. Both forms, immobilized and soluble, have biological activity. For example, Weber et al. (20) demonstrated stopping of rolling monocytes by proteoglycan-bound growth-related oncogene α and subsequent shape changes by soluble monocyte chemoattractant protein-1 on TNF-α-stimulated endothelial cells in assays done under flow. Firm adhesion of rolling lymphocytes on endothelium has been previously shown by Piali et al. (12), who found that stimulation of the endothelial cells with IFN-γ and TNF-α led to the production of the chemokines IP10 and Mig, and that IL-2-stimulated T cells firmly bound to these cells under flow conditions. By injecting the soluble chemokine directly into the assay, the present study confirms that the induction of firm adhesion by SDF-1α is due to the injected chemokine and not other possible factors secreted by the stimulated endothelium. Our findings suggest that neither a concentration gradient nor endothelial presentation is required for SDF-1α-induced firm adhesion. Since a gradient is not required, soluble chemokine released by the tissue could have biological significance at the vessel surface with respect to inducing rapid tight adhesion of rolling lymphocytes. In this setting, upon encountering a chemokine released in the blood at a specific site on a vessel, the rolling lymphocyte will be continually exposed to the chemokine as it is carried with the blood flow. The rolling event, which greatly slows the movement of the lymphocyte within the blood, likely provides a sufficient length of time for exposure to induce tight adhesion (the chemokine acts within seconds).

This study does not address the importance of SDF-1α in vivo, only that a soluble chemokine can induce rapid tight adhesion of rolling lymphocytes. However, as discussed by Bleul et al. (21), SDF-1α is constitutively expressed in a broad range of tissues. It is possible that soluble SDF-1α contributes to basal lymphocyte migration into a variety of tissues. Furthermore, even though SDF-1α production is not increased in inflammation (21), it may also contribute to the recruitment of inflammatory lymphocytes in some tissues. In this setting, the inflammation-induced recruitment would be controlled by the up-regulation of the adhesion molecules required for rolling. Once rolling was initiated, lymphocytes could respond to the basal levels of SDF-1α.

A recent study by Derdeyn et al. (28) revealed significant variation in the levels of SDF-1α in the blood of healthy individuals. In 31 volunteers, the mean SDF-1α ($\pm$SD) concentration was 25 $\pm$ 34.7 ng/ml, with a range of 0.28–106.5 ng/ml. It seems plausible that the variation in our results with induction of firm adhesion with the low doses (2 ng/ml) of SDF-1α could be a reflection of similar interindividual variation in the levels of SDF-1 that each animal's cells were used to in the circulation. Thus, the lower dose would show more variation, whereas the higher dose would be inducing sensitization, and desensitization, of a higher proportion of the cells. Also of importance is the cell surface expression of the CXCR4 receptor for SDF-1α that has been shown to be increased in short periods of culture on human cells (29). Similarly, we have found increased cell surface expression of CXCR4 on bovine lymphocytes under conditions identical to those used to purify and pretreat the cells in the current study (J. M. Kantele and M. A. Jutila, manuscript in preparation). Thus, it is important to recognize that in this study SDF-1α was used to induce firm adhesion to deepen our understanding of the basic mechanisms of lymphocyte homing. The finding that concentration levels lower than those found in vivo were able to induce firm adhesion of the rolling cells could mean that at least part of the leukocytes in the circulation could be desensitized to SDF-1α. This in turn could require changes in the cells' expression of CXCR4 by other stimuli to make them responsive to SDF-1α. However, we do not know the amount of SDF-1α in the FBS that was a part of the media in our assay nor whether the age of an animal would have an effect on the circulatory levels of SDF-1α.

The presence of multiple ligands for rolling presented by the LPS-activated endothelial cells prompted the question whether the mechanisms supporting rolling of lymphocytes before the addition of SDF-1α and after their desensitization to the chemokine were the same. To simplify this question, the assay was done with E-selectin cDNA-transfected mouse L cells that had previously been shown to support bovine $\gamma\delta$ T cell rolling (18). As on endothelial cells, SDF-1α was able to induce firm adhesion of the rolling lymphocytes. The finding of slower and less extensive binding could be due to differences between the expressions of adhesion molecules on the transfectants and the activated endothelial cells. When the blocking experiments were repeated on activated endothelial cells, the mAb GR113 was found to block the rolling of both unstimulated and desensitized lymphocytes. Combined, these experiments showed that $\gamma\delta$ T cell rolling was supported by similar mechanisms before and after SDF-1α-induced firm adhesion of the lymphocytes.

Strikingly, the number of rolling cells increased after the peak response of firm adhesion following injection of SDF-1α into the rolling assay (see Fig. 2*B*), although rolling speeds were essentially unchanged. These results are in contrast to the finding of Campbell et al. (10), who in their assay with PNAd-coated plates found increased rolling speeds of lymphocytes in the presence of SDF-1α. Moreover, reduced rolling speeds of lymphocytes on endothelial cells upon addition of IP10 and MIP-1β has been shown (30). Slower rolling could allow more efficient sampling of locally produced chemokines and lead to the firm attachment of the cells at lower concentrations of chemokines. The combined effects of multiple chemokines, some inducing slower rolling while others

triggering tight adhesion, during endothelial transmigration of leukocytes could be physiologically advantageous and lead to more effective regulation of lymphocyte extravasation. Importantly, desensitization to some chemokines could also be a major regulatory determinant in lymphocyte migration and deserves to be a focus of further studies.

In conclusion, our study demonstrates the striking effect of soluble SDF-1α on the binding of $\gamma\delta$ T cells to a monolayer of endothelial cells under flow conditions. We show that within 20 s upon contact with soluble SDF-1α, >90% of the rolling lymphocytes firmly attached to the endothelium. Peak binding was seen at 2–3 min, which was followed by either permanent or transient adhesion and continued rolling on the endothelium. The cells that deattached exhibited an increased capacity to roll on the endothelium and were still capable of firmly binding to the endothelium following the addition of PMA to the system. The same adhesion molecules that supported the rolling before the addition of the chemokine were also supporting the rolling of the desensitized lymphocytes. The firm adhesion in response to SDF-1α was due to the effect of the chemokine on the lymphocytes, since it could be blocked by pretreating the lymphocytes with PTX. These results further enhance our understanding of the multistep process of lymphocyte homing and emphasize the importance of homotypic desensitization to activating chemokines in this process.

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