Spatial distribution of L-selectin (CD62L) on human lymphocytes and transfected murine L1-2 cells

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Summary

We have examined the topographical distribution of L-selectin on surface membrane domains of human lymphocytes and murine L1-2 cells transfected to express human L-selectin. L-selectin was immunolocalized using murine monoclonal DREG 200 Fab antibody and a 12 nm colloidal gold-conjugated secondary antibody. Cell surface morphology and surface distribution of gold-labelled L-selectin were visualized using backscatter electron images obtained by high-resolution, field emission scanning electron microscopy. The topographical morphologies of lymphocytes of both types were complex. The surface of human lymphocytes was composed of both microvilli and ruffles; that of the murine cells was composed of long microvilli and few, if any, ruffles. L-selectin on human lymphocytes was observed primarily as focal clusters on the apical surfaces of ruffles and microvilli. Similarly, on the transfected murine cells, L-selectin was detected predominantly on the apical surface of microvilli. We conclude that L-selectin has a common spatial distribution and clustered organization on all leukocytes examined to-date, and that these features of receptor expression likely facilitate rolling of circulating leukocytes on the endothelial surface.

Introduction

Cell adhesion molecules (CAMs) serve a multitude of important roles critical to host defense functions of leukocytes. These roles include emigration of leukocytes to sites of inflammation and recirculation of lymphocytes to secondary lymphoid organs. Other roles of CAMs include adherence reactions of leukocytes with cellular and noncellular substrates involved in their migration, and binding of leukocytes to each other, accessory cells, target cells, or pathogens. Emigration and recirculation of leukocytes are initiated by CAMs that mediate their rolling on microvascular endothelial cells. For neutrophils and some lymphocytes, this function is served by L-selectin (CD62L) expressed constitutively on their surface (von Andrian et al., 1991; Ley et al., 1991). Other CAMs then become subsequently involved, step-wise, in completion of the adhesion cascade (Butcher, 1991; Kishimoto, 1991; Springer, 1994). Although many of the CAMs involved in leukocyte emigration and recirculation are known, the mechanisms that control the sequence of their involvement have not been defined. One such mechanism was suggested to involve differences in spatial distribution of CAMs on the leukocyte surface (Picker et al., 1991; Erlandsen et al., 1993; von Andrian et al., 1993).

Leukocytes are most often depicted as simple spheres, but their surface morphology is much more complex: ruffles, microvilli, or combinations of these structures project from the cell surface of the unactivated cell. This complexity may be significant because receptor availability could be controlled by positioning of CAMs either on the apices of these
structures, or at their base (Picker et al., 1991; Erlandsen et al., 1993; von Andrian et al., 1993).

CAMs on the leukocyte surface can be easily detected by transmission or scanning electron microscopy using immunolabeling with antibody conjugated to colloidal gold (Erlandsen et al., 1993). Colloidal gold has numerous advantages as an opaque marker for electron microscopy, including a distinct shape and small size to allow precise localization (±30 nm) of antigenic sites. In this report we describe the spatial distribution and staining pattern of L-selectin on human lymphocytes and murine L1-2 cells by taking advantage of the high resolution backscatter electron imaging of field emission scanning electron microscopy (FESEM) to visualize simultaneously both cell surface topography and immunogold localization of this cell adhesion molecule.

**Materials and methods**

**Isolation of human leukocytes**

Approval for obtaining human blood specimens by venipuncture was obtained from the Human Subjects Committee at the University of Minnesota. Heparinized venous blood (10 U ml⁻¹) obtained from six different donors was layered over Ficoll-Hypaque (sp. gr. 1.119 gm dl⁻¹) and centrifuged at 400 g for 15 min at 4°C. Mononuclear leukocytes, representing lymphocytes and monocytes, were collected at the plasma-gradient interface and washed in 0.1 M phosphate-buffered saline (PBS; Sigma, St Louis, MO) at 4°C. Neutrophils were collected as a band below the interface, contaminating erythrocytes were removed by hypotonic lysis, and the cells resuspended in PBS. Purity of the isolated leukocyte populations were examined by light microscopy of smears stained with Wright-Giemsa.

**Transfection of murine L1-2 to express human L-selectin**

L1-2 cells are pre-B cells, derived from a murine lymphoma, that do not express the murine analogue of L-selectin (Gallatin et al., 1983). These cells were transfected with either the pMRB101 vector alone, or a vector incorporating the cDNA for human L-selectin, as previously described (Berg et al., 1991a). Transfected cells were stained with fluorescein-(FITC-) conjugated DREG 200 Fab, a murine monoclonal anti-human L-selectin antibody, and cells expressing the highest level of L-selectin were recovered by fluorescence activated cell sorting (FACS; Becton Dickinson, Mountain View, CA) (Berg et al., 1991b). Sorted cells were then grown and used for experiments within 1 week of sorting. Prior to labelling of L-selectin, cells were assessed for viability in terms of Trypan Blue exclusion. Viability was found to be >90% in all cases.

Human mononuclear cells at a density of 10⁶/100 μl were stained with FITC-labelled DREG 200 Fab antibody (10 μg ml⁻¹) or isotypic control murine IgG (10 μg ml⁻¹) for 30 min at 40°C. The fluorescence intensities of these cell preparations were measured by flow cytometry to confirm the specificity of staining with the DREG 200 antibody, and to establish the gate setting for sorting of L-selectin-positive lymphocytes. Cell preparations stained with the specific antibody were then sorted (at 4°C) and the lymphocytes (identified by forward and orthogonal light scattering) with the highest expression of L-selectin (upper 20%) collected. Neutrophils and L1-2 cells were also stained with specific or control antibodies under the conditions described and examined by flow cytometry to confirm staining specificity.

**Immunogold staining of leukocytes for L-selectin**

The human lymphocytes sorted for high expression of L-selectin, murine L1-2 cells transfected to express human L-selectin, and isolated human neutrophils were labelled with immunogold at 4°C by incubation with goat anti-mouse IgG conjugated to 12 nm colloidal gold (Jackson ImmunoResearch, Westgrove, PA) diluted 1:20 in PBS-0.2% bovine serum albumin (Sigma, St Louis, MO) (Erlandsen et al., 1993).

**Analysis of immunogold-labelled cells by FESEM**

Immunogold-labelled cells were attached to glass specimen carriers pretreated with 0.1% poly-L-lysine (Sigma) and further processed as previously described (Erlandsen et al., 1993). Briefly, after attachment at 4°C for 5–10 min to minimize cell activation, the cells were fixed overnight at room temperature with 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, containing 7.5% sucrose, and postfixed for 15–30 min in the same buffer containing 1% osmium tetroxide (Fischer Scientific, Fair Lawn, NJ). Fixation was followed by dehydration in an ascending alcohol series, critical point drying with CO₂, and sputter coating with platinum. The cells were examined at accelerating voltages of 3.0–4.0 keV in a Hitachi S-900 field emission SEM by high-resolution backscatter electron imaging using a modified YAG detector; images were recorded on Polaroid type 55 film.

**Results**

**Expression of L-selectin on human lymphocytes**

A contour plot deriving from flow cytometric analysis of FITC-DREG 200 Fab-stained lymphocytes from a representative cell donor is shown in Fig. 1A. Note that there is a significant range in fluorescence intensity among L-selectin-positive lymphocytes. Quadrant 4 in this figure identifies the lymphocytes with the highest expression of L-selectin that were collected by sorting for FESEM analysis.

The surface morphology of the human lymphocytes sorted for high expression of L-selectin can be seen at low magnification in Fig. 1B. These cells were covered with numerous microvilli and ruffles projecting from the cell body. This morphology was also observed for unsorted lymphocytes, demonstrating that sorting did not affect cell surface morphology or select a morphologically unique subset of lymphocytes (not shown). Sorting also did not interfere with the detection or distribution of immunogold staining for L-selectin.

Figure 1C illustrates the typical distribution of immunogold-stained L-selectin on the sorted human...