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P-, E-, and L-Selectin Mediate Migration of Activated CD8\(^{+}\) T Lymphocytes into Inflamed Skin\(^1\)

Takako Hirata, Barbara C. Furie, and Bruce Furie\(^2\)

P- and E-selectin mediate CD4\(^{+}\) Th1 cell migration into the inflamed skin in a murine contact hypersensitivity model. In this model, not only CD4\(^{+}\) T cells but also CD8\(^{+}\) T cells infiltrate the inflamed skin, and the role of CD8\(^{+}\) type 1 cytotoxic T (Tc1) cells as effector cells has been demonstrated. Here we show that in mice deficient in both P- and E-selectin, the infiltration of CD8\(^{+}\) T cells in the inflamed skin is reduced, suggesting the role of these selectins in CD8\(^{+}\) T cell migration. We directly studied the role of selectins using in vitro-generated Tc1 cells. These cells are able to migrate into the inflamed skin of wild-type mice. This migration is partially mediated by P- and E-selectin, as shown by the reduced Tc1 cell migration into the inflamed skin of mice deficient in both P- and E-selectin or wild-type mice treated with the combination of anti-P-selectin and anti-E-selectin Abs. During P- and E-selectin-mediated migration of Tc1 cells, P-selectin glycoprotein ligand-1 appears to be the sole ligand for P-selectin and one of the ligands for E-selectin. P- and E-selectin-independent migration of Tc1 cells into the inflamed skin was predominantly mediated by L-selectin. These observations indicate that all three selectins can mediate Tc1 cell migration into the inflamed skin. The Journal of Immunology, 2002, 169: 4307–4313.

Eukocyte migration from blood to tissues is a multistep process that involves a variety of adhesion and signaling molecules. Selectins are a family of adhesion molecules that mediate the initial adhesive step, characterized by leukocyte capture and rolling on the endothelial surface under vascular shear flow. L-selectin is expressed on most leukocytes, while P- and E-selectin are expressed on activated vascular endothelium. Selectin-mediated cell-cell interaction is a prerequisite for subsequent firm attachment and transmigration of leukocytes (1–4). Selective migration is thus critically controlled by the expression of selectins and their ligands as well as other molecules mediating the adhesion cascade during migration. Naive T cells preferentially migrate into lymph nodes through interaction of L-selectin on T cells with peripheral node addressins (PNAd)\(^3\) on high endothelial venules (HEVs) (5). Following antigenic stimulation, some T cells down-regulate L-selectin, while the expression of other adhesion molecules is enhanced, directing the differentiated effector T cells to peripheral tissues. Differential expression of adhesion molecules also directs different sets of effector T cells to specific sites. For example, \(\alpha_4/\beta_2\) integrin is expressed on gut-homing T cells (6), whereas cutaneous lymphocyte Ag, which binds E-selectin, is preferentially expressed on T cells migrating into skin (7).

Depending on the cytokines present during Ag priming, CD4\(^{+}\) Th precursor cells differentiate toward Th1 or Th2 cells that produce distinct patterns of cytokines (8, 9). Recent studies have indicated that CD8\(^{+}\) T cells can also be differentiated into IFN-\(\gamma\)-producing CD8\(^{+}\) type 1 cytotoxic T (Tc1) cells or IL-4/IL-10-producing Tc2 cells (10, 11). Differentiated Th1 and Th2 cells not only produce different sets of cytokines, but also exhibit the preferential expression of adhesion molecules and chemokine receptors, which may result in distinct migration properties of these subsets (12, 13). Similarly, Tc1 and Tc2 cells may differentially express adhesion molecules and chemokine receptors (14). Differential ligand expression between type 1 and type 2 subsets was also reported for both CD4\(^{+}\) and CD8\(^{+}\) T cells (15, 16). It is speculated that these different migratory properties of various T cell subsets are important for the efficient regulation of the immune responses mediated by these effector cells.

Contact hypersensitivity (CHS) is a T cell-mediated immune response induced by cutaneous application of a reactive hapten (17). During the sensitization phase, Langerhans cells migrate from epidermis to skin-draining lymph nodes, where they present hapten-MHC complexes to naive T cells. During the elicitation phase that develops after subsequent contact with the hapten, Ag-specific T cells migrate into the site of Ag challenge and release cytokines, thereby initiating the inflammatory response. CHS has long been considered a model of delayed-type hypersensitivity (DTH), which is mediated by CD4\(^{+}\) Th1 cells. Multiple studies suggest, however, that CHS responses are mediated by CD8\(^{+}\) T1 cells and down-regulated by CD4\(^{+}\) Th2 cells (18–20). The recent study using mice lacking either CD4\(^{+}\) or CD8\(^{+}\) T cells provided evidence that both CD4\(^{+}\) Th1 cells and CD8\(^{+}\) Tc1 cells function as effector cells in CHS responses (21). To exert effector functions, these cells need to migrate into the site of Ag challenge. The migration of in vitro-generated Th1 cells into the inflamed skin in a CHS model is mediated by P- and E-selectin (15). In addition, P-selectin glycoprotein ligand-1 (PSGL-1) on Th1 cells has been shown to function as a major P-selectin ligand and one of the E-selectin ligands (22, 23). However, the molecular mechanisms for CD8\(^{+}\) T cell trafficking into the inflamed skin have not been determined.

Here we investigated the migratory properties of CD8\(^{+}\) T cells into the inflamed skin in a CHS model using in vitro-generated Tc1 cells. Our results show that Tc1 cells are able to migrate into the
inflamed skin and that the migration is partially mediated by P- and E-selectin. During P- and E-selectin-mediated migration, PSGL-1 on Tc1 cells functions as a predominant ligand for P-selectin and also plays a role as an E-selectin ligand. In addition, L-selectin mediates P- and E-selectin-independent migration of Tc1 cells. These results indicate roles for three selectins in CD8+ T cell migration into the inflamed skin.

**Materials and Methods**

**Mice**

Mice homozygous for the PSGL-1-targeted mutation (PSGL-1<sup>−/−</sup> mice) (24) were backcrossed four times with C57BL/6j (B6). B6 mice, P-selectin-deficient mice on a B6 background, B6 × 129S3/SvImJ (129S) F<sub>1</sub> mice, and P- and E-selectin double-deficient mice on a B6/129S background were purchased from The Jackson Laboratory (Bar Harbor, ME). E-selectin-deficient mice on a B6 background were provided by Dr. A. Beaudet (Baylor College of Medicine, Houston, TX). All mice were used between 8–12 wk old. The mice were housed in the animal facility of Beth Israel Deaconess Medical Center. All studies and procedures were approved by the Animal Care and Use Committee of Beth Israel Deaconess Medical Center.

**Flow cytometry**

All mAbs used in flow cytometric analyses were purchased from BD PharMingen (San Diego, CA). They include anti-CD4-PD (GK1.5), anti-CD8α-FITC (53-6.7), anti-CD8α-PE (53-6.7), anti-CD162-PE (2PH1), anti-CD62L-PE (MEL-14), anti-CD49d-PE (9C10), anti-CD18-PE (CT7/16), anti-CD25-PE (PC61), anti-CD44-PE (IM7), and anti-CD45RB-PE (23G2). Cells were stained with mAbs for 30 min at 4°C, washed, and analyzed on a FACS Calibur (BD Biosciences, San Jose, CA) using CellQuest software (BD Biosciences). For assessment of selectin binding by flow cytometry, cells were incubated with selectin-IgM chimeras provided by Dr. J. Lowe (University of Michigan, Ann Arbor, MI) for 30 min at 4°C. Nonspecific binding was determined by the addition of 5 mM EDTA. Cells were then incubated with biotinylated goat anti-human IgM (American Qualex, San Clemente, CA) and stained with streptavidin-PE (BD Biosciences).

**Cell culture**

For generation of Tc1 and Tc2 cells, splenic CD8<sup>+</sup> T cells were isolated by negative selection using the Mouse T Cell CD8 Subset Column Kit (R&D Systems, Minneapolis, MN). Purified CD8<sup>+</sup> T cells were cultured on 24-well tissue culture plates coated with 20 μg/ml anti-CD3ε (145-2C11; R&D Systems) and 10 μg/ml anti-CD28 (37.51; BD PharMingen) for 2 days in the presence of 4 nM of IL-2 and IL-4 (R&D Systems) and either 2 ng/ml IL-12 (R&D Systems) or 4 μg/ml anti-IL-4 (11B11; BD PharMingen) to promote Tc1 differentiation or 50 ng/ml IL-4 (Pierce Endogen, Rockford, IL) to promote Tc2 differentiation. After 2 days, the cells were transferred to uncoated plates and cultured for an additional 4 days.

**Analysis of cytokine production**

After 5 days of culture, Tc1 or Tc2 cells were harvested, washed, and restimulated with plate-coated anti-CD3ε and anti-CD28 for 24 h at 1 × 10<sup>6</sup> cells in 1 ml medium containing 4 ng/ml IL-2. Concentrations of IFN-γ and IL-4 in the culture supernatants were determined by ELISA using Quantikine M kits (R&D Systems).

**Cell adhesion assays**

Ninety-six-well plates (Flow Laboratories, McLean, VA) were coated with 10 μg/ml selectin-IgG chimeric proteins for 2 h at 37°C and blocked with 1% BSA overnight at 4°C as described previously (23). The cells were labeled with 2',7'-bis-(2-carboxethyl)-5-(and-6)-carboxyfluorescein (BCECF; Molecular Probes, Eugene, OR) for 30 min at 37°C, washed, and resuspended in DMEM with 10% FCS. The labeled cells were added to each well (5 × 10<sup>5</sup>/well) and incubated for 20 min at 4°C under rotation (60 rpm). The plates were washed three times with HBSS with CaCl<sub>2</sub> and MgCl<sub>2</sub>, and bound cells were quantitated by measuring the fluorescence using a Cytofluor 2300 (Millipore, Bedford, MA). Each assay was conducted in triplicate and repeated three to six times.

**Induction of CHS**

Mice were sensitized by the application of 100 μl 2% (w/v) oxazolone (Sigma-Aldrich, St. Louis, MO) in acetone/olive oil (4/1; Sigma-Aldrich) solution on the shaved abdominal skin on day 0. Some mice were painted with acetone/olive oil alone. Mice were challenged on day 6 by applying 20 μl 0.5% (w/v) oxazolone in the same vehicle on the left ear (10 μl/side). The right ear was painted with the vehicle only. Ear swelling responses were quantitated using ear thickness measurements taken with a dial thickness gauge (Mitutoyo, Kanagawa, Japan).

**Immunohistochemistry**

Ear specimens were taken 24 h after challenge. Ears were shaved from mice, embedded in OCT compound (Miles, Elkhart, IN), and frozen. Cryostat sections were fixed in acetone and stained for CD8 using rat anti-CD8 (Caltag Laboratories, Burlingame, CA), biotinylated rabbit anti-rat IgG (Vector Laboratories, Burlingame, CA), and ABC-alkaline phosphatase reagent (Vector Laboratories). Sections were also stained with MECA-79 (BD PharMingen), biotinylated anti-rat IgM, and avidin-biotin peroxidase complex-alkaline phosphatase reagent. Color was developed using Vector Red alkaline phosphatase substrate solution. Slides were counterstained with methylene green. The number of CD8<sup>+</sup> T cell infiltrates was counted on 10 separate 0.1-mm<sup>2</sup> regions/section. The sections were examined in a blinded fashion.

**Isolation of skin-infiltrating cells**

Skin-infiltrating cells were isolated via enzymatic digestion as previously described (25). Briefly, ears taken 24 h after challenge were separated into vential and dorsal sheets and incubated in HBSS containing 1% FCS and 1 mg/ml collagenase/dispace (Sigma) for 2 h at 37°C. The sheets were minced and disrupted on a 46-μm pore size stainless steel mesh (Belco, Vineland, NJ) in cold HBSS. The resulting cell suspension was filtered sequentially through a 46-μm pore size stainless steel mesh (Belco) and a 35-μm pore size nylor mesh filter (BD Falcon, Bedford, MA) and then washed twice in HBSS.

**In vivo migration assays**

Tc1 and Tc2 cells were harvested after 6 days of culture, and dead cells were removed by centrifugation on Histopaque 1083 (Sigma). The cells (1 × 10<sup>7</sup>/ml) were radiolabeled with 100 μCi/ml sodium <sup>32</sup>Chromate (NEN Life Science Products, Boston, MA) for 1 h at 37°C in RPMI 1640 with 10% FCS. Cells were washed twice and resuspended in PBS. Cells (4 × 10<sup>4</sup>) in 0.4 ml PBS were injected into the tail vein of a mouse that had been sensitized 7 days before and challenged on the left ear 24 h previously as described above. In some experiments anti-P-selectin (RB40.34; BD PharMingen), anti-E-selectin (9A9; provided by Dr. B. Wolitzky, Coela- canth, East Windsor, NJ), anti-L-selectin (MEL-14; BD PharMingen), anti-CD49d R1-2 (BD PharMingen) or PS/2 (Sero tec, Oxford, U.K.), or isotype control (200 μg/mouse) was injected together with the cells. Mice were sacrificed 3 h after injection, the ears were removed, and the radioactivity in the skin was measured using a Packard gamma scintillation counter (Downers Grove, IL).

**Statistical analysis**

For statistical comparison of two samples, two-tailed Student’s t test was used.

**Results**

Mice deficient in both P- and E-selectin exhibit reduced CD8<sup>+</sup> T cell infiltration in inflamed skin in a CHS model

CD4<sup>+</sup> T cell migration into the inflamed skin is dependent on P- and E-selectin (15). To examine whether P- and E-selectin mediate the migration of CD8<sup>+</sup> T cells into the inflamed skin, we examined cell infiltration into the inflamed skin using an oxazolone-induced CHS model in mice lacking both P- and E-selectin (P/E<sup>−/−</sup> mice). These mice are reported to show impaired CHS response characterized by decreased ear swelling and leukocyte accumulation (26). P/E<sup>−/−</sup> mice and their control wild-type mice were sensitized with 2 mg oxazolone on the shaved abdominal skin, and 6 days later they were challenged on the left ear with 100 μg oxazolone. Some mice were painted with acetone/olive oil vehicle alone at the time of sensitization to serve as unsensitized controls. Right ears were painted with vehicle alone to serve as unchallenged control ears. Similar to previous results (26), the ear swelling response in P/E<sup>−/−</sup> mice measured 24 h after challenge was decreased by 51% compared with that in wild-type mice (P/E<sup>−/−</sup>, 12.3 ± 6.2 × 10<sup>−2</sup>).
mm (n = 8); wild type, 25.0 ± 3.1 × 10^{-2} mm (n = 8)). Immunohistochemical analyses of the skin sections from the ears 24 h after challenge showed that CD8^+ T cell infiltrate in P/E^-/- mice was reduced by 79% compared with that in wild-type mice (Fig. 1). These results suggest that P- and E-selectin play an important role in the migration of CD8^+ T cells into inflamed skin in a CHS model.

**Tc1 cells migrate into inflamed skin**

To directly determine whether CD8^+ T cells can migrate into the site of Ag challenge, the migratory behavior of in vitro-stimulated CD8^+ T cells was studied. Enriched splenic CD8^+ T cells were stimulated with anti-CD3ε and anti-CD28 either in the presence of IL-12, anti-IL-4, and IL-2 to promote Tc1 differentiation or in the presence of IL-4 and IL-2 for Tc2 differentiation. The cytokine profiles of differentiated Tc1 and Tc2 cells were confirmed (data not shown). Tc1 and Tc2 cells were labeled with ^51Cr and injected into the mice previously sensitized with oxazolone and challenged on the left ear. The right ear was painted with the vehicle and served as noninflamed control skin. Three hours after injection, the radioactive cells that accumulated in the ear were measured. As shown in Fig. 2A, Tc1 cells migrated into the inflamed ear at a concentration 3 times higher than Tc2 cells. No differences were observed between Tc1 and Tc2 cells in the migration into the control ear. These results are similar to those of CD4^+ T cells that showed that Th1 cells entered the inflamed skin in greater numbers than Th2 cells (15). The migration of Tc1 cells into the inflamed skin, however, was 39% lower than that of Th1 cells (Fig. 2B).

**Tc1 cells bind to P- and E-selectin**

To study which molecules are responsible for the differential migration of Tc1 and Tc2 cells, we first examined adhesion molecule expression on Tc1 and Tc2 cells. The expression of L-selectin (CD62L) and α4 integrin (CD49d) is higher on Tc1 cells than Tc2 cells, while PSGL-1 (CD162), β2 integrin (CD18), and CD44 are expressed at similar levels on both subsets (Fig. 3A). Most skin-infiltrating CD8^+ T cells also appeared to express PSGL-1, L-selectin, α4 integrin, β2 integrin, and CD44 (Fig. 3B). This adhesion molecule expression profile is similar to that observed with in vitro-differentiated Tc1 cells. Therefore, at least several adhesion molecules are similarly expressed on CD8^+ T cells infiltrating the skin in vivo and in vitro-differentiated CD8^+ T cells.

Since CD8^+ T cell infiltrate into the inflamed skin was reduced in P/E^-/- mice, P- and E-selectins are implicated in CD8^+ T cell migration into inflamed skin. We therefore tested Tc1 and Tc2 cells for their ability to bind to selectins using selectin-IgG chimeric proteins. The in vitro adhesion assays were performed under nonstatic conditions. Tc1 cells bound to both P-selectin-IgG and E-selectin-IgG, while less Tc2 cells bound to either P-selectin-IgG or E-selectin-IgG (Fig. 3C). The adhesion profiles of Tc1 and Tc2 cells are similar to those of Th1 and Th2 cells, respectively (Fig. 3C). No binding to L-selectin-IgG by Tc1 cells was detectable (data not shown). Selectin binding of Tc1 and Tc2 cells was also tested by flow cytometry. Fifty-eight percent of Tc1 cells bound to P-selectin-IgM, and 81% bound to E-selectin-IgM, while 10 and 12% of Tc2 cells bound to P-selectin-IgM and E-selectin-IgM, respectively (Fig. 3D).

PSGL-1 on Th1 cells has been shown to be a ligand for P-selectin and in part for E-selectin (23). We therefore tested whether PSGL-1 is on Tc1 cells functions as a ligand for P- and E-selectin. In contrast to the binding of wild-type Tc1 cells to P-selectin-IgG, the binding of PSGL-1^-/- Tc1 cells to P-selectin was almost completely absent (Fig. 3E). The binding of PSGL-1^-/- Tc1 cells to E-selectin was reduced by 70% compared with that of wild-type Tc1 cells (Fig. 3E). Therefore, PSGL-1 on Tc1 cells functions as a major ligand for P-selectin and also plays a role in Tc1 cell binding to E-selectin in vitro. The results are similar to those with Th1 cells (23), suggesting that PSGL-1 on Th1 and Tc1 cells has similar selectin-binding characteristics.

**P- and E-selectin partially mediate Tc1 cell migration into the inflamed skin**

To examine whether P- and E-selectin ligand expression observed in vitro is relevant during the migration of Tc1 cells into the inflamed skin, the cells were injected into P/E^-/- mice previously

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**FIGURE 1.** CD8^+ T cell infiltration in a CHS response. A, Anti-CD8-stained frozen ear sections from the challenged ears from sensitized wild-type (left panel) or P/E^-/- (right panel) mice. Bar = 100 μm. B, The number of infiltrating CD8^+ T cells was quantitated on frozen sections stained with anti-CD8. Data are the mean ± SEM from six mice of each genotype. *p < 0.01 compared with wild type.

**FIGURE 2.** Migration of Tc1 cells into the inflamed skin. ^51Cr-labeled Tc1 or Tc2 cells (A) or Tc1 or Th1 cells (B) were injected into the tail vein of wild-type mice that had been sensitized 6 days previously with oxazolone and challenged 24 h previously on the left ear. The mice were sacrificed 3 h after injection, and radioactivity in the control and challenged ears was assayed. Values are the mean ± SEM from four mice. Results represent one of three similar experiments.
sensitized and challenged with oxazolone. The migration of Tc1 cells into inflamed skin of P/E/H11002 mice was reduced by 59% compared with the migration into inflamed skin of wild-type mice (Fig. 4A). The migration of Tc1 cells was also reduced when the cells were injected into wild-type mice with the combination of anti-P- and anti-E-selectin Abs (Fig. 4B). These results indicate that P- and E-selectins are involved in the migration of Tc1 cells into inflamed skin. However, we observed a considerably greater accumulation of Tc1 cells in the inflamed skin of P/E/H11002 mice or wild-type mice treated with anti-P- and anti-E-selectin Abs compared with Th1 cells (Fig. 4), suggesting the existence of a P- and E-selectin-independent mechanism of Tc1 cell migration.

PSGL-1 on Tc1 cells is the sole ligand for P-selectin and one of the ligands for E-selectin in vivo

The in vitro adhesion assays showed that PSGL-1 on Tc1 cells is a ligand for P-selectin and also functions as a ligand for E-selectin. To study the role of PSGL-1 in Tc1 cell migration in vivo, the migrations of wild-type and PSGL-1−/− Tc1 cells to the inflamed skin of wild-type mice were compared. A slightly reduced migration of PSGL-1−/− Tc1 cells to the inflamed skin of wild-type mice on both B6 and B6/129S backgrounds was observed, although the reduction was not statistically significant (Fig. 5).

Based on the in vitro observation that PSGL-1 is a ligand for P- and E-selectin, PSGL-1 is most likely to be functioning during P- and E-selectin-mediated migration of Tc1 cells. To prove this, wild-type and PSGL-1−/− Tc1 cells were injected into sensitized and challenged E-selectin-deficient (E/H11002) mice, the migration of PSGL-1−/− Tc1 cells was reduced to the level of P- and E-selectin-independent migration (Fig. 5B). Thus, it appears that PSGL-1 on Tc1 cells is the major ligand for P-selectin in vivo.

FIGURE 3. Characterization of Tc1 and Tc2 cells generated in vitro and CD8+ T cells infiltrating in skin in vivo. A, Flow cytometric analysis of Tc1 and Tc2 cells. Tc1 and Tc2 cells were harvested after 6 days of culture, dead cells were removed, and the remaining cells were stained with the indicated mAbs (solid lines) or isotype-matched control IgGs (dotted lines) and analyzed by flow cytometry. B, Flow cytometric analysis of skin-infiltrating cells. Skin-infiltrating cells were isolated from challenged ears, stained with anti-CD8-FITC plus the indicated PE-labeled mAbs (solid lines) or isotype controls (dotted lines), and analyzed by flow cytometry. CD8+ for lymphocytes were gated for analysis. C, Selectin-binding activities of type 1 and type 2 cells. In vitro-generated type 1 and type 2 cells were labeled with BCECF and added to the wells of 96-well plates coated with human IgG (Ig), P-selectin-IgG chimeric protein (P-Ig), or E-selectin-IgG chimeric protein (E-Ig). The plates were incubated for 20 min at 4°C under rotating conditions, unbound cells were removed by washing, and the fluorescence per well was determined using a Cytofluor 2300. Percent adhesion equals 100 x bound cells/total cells added. D, Flow cytometric analysis of selectin binding of Tc1 and Tc2 cells. In vitro-generated Tc1 and Tc2 cells were stained with human IgM, P-selectin-IgM, or E-selectin-IgM in the presence of CaCl2 and MgCl2 (solid lines) or EDTA (dotted lines) and analyzed by flow cytometry. E, Adhesion of wild-type or PSGL-1−/− Tc1 and Tc2 cells to selectin-IgG chimeras. Values are the mean ± SEM from triplicate wells. Results represent one of three similar experiments.
In contrast, when PSGL-1−/− Tc1 cells were injected into P-selectin-deficient (P−/−) mice, the migration was not reduced to the level of P- and E-selectin-independent migration (Fig. 5B). Therefore, it is likely that there are E-selectin ligands other than PSGL-1, although PSGL-1 functions as one of the E-selectin ligands.

P- and E-selectin-independent migration of Tc1 cells into inflamed skin is mediated predominantly by L-selectin

We sought to identify the molecules mediating P- and E-selectin-independent migration of Tc1 cells into inflamed skin. Based on the observation that α4 integrin and L-selectin are expressed at high levels on Tc1 cells (Fig. 3A), we examined P- and E-selectin-independent Tc1 cell migration in the presence of function-blocking Abs against α4 integrin or L-selectin. α4 integrin has been suggested to mediate lymphocyte rolling (27). However, neither mAb PS/2 nor R1-2 against α4 integrin had any effect on Tc1 cell migration into the inflamed skin of P/E−/− mice (Fig. 6A), indicating that α4 integrin is not involved in the P- and E-selectin-independent process of Tc1 cell migration into inflamed skin. In contrast, the anti-L-selectin mAb MEL-14 inhibited the migration by 44% compared with its isotype control (Fig. 6B). The inhibitory effect of MEL-14 was not observed when the migration was examined in wild-type mice, most likely due to the compensation by P- and E-selectin (data not shown). These data suggest that L-selectin is involved in the migration of Tc1 cells into inflamed skin. Taken together, all three selectins can mediate the migration of Tc1 cells into the inflamed skin in this model.

Discussion

In the present study, we investigated the role of selectins in mediating the migration of activated CD8+ T cells into the inflamed skin. We demonstrated that in vitro-generated Tc1 cells are able to migrate into the inflamed skin in a CHS model. Our data show that Tc1 cell migration into the inflamed skin is partly mediated by P- and E-selectin. During P- and E-selectin-mediated migration of Tc1 cells, PSGL-1 functions as a predominant ligand for P-selectin and also plays a role as an E-selectin ligand. In addition, we show that L-selectin plays a role during P- and E-selectin-independent migration of Tc1 cells into the inflamed skin.

As an experimental model we used an oxazolone-induced CHS response, which is a widely used model of a T cell-mediated response. Although a large number of studies have focused on the respective roles of CD4+ and CD8+ T cells in CHS, conflicting results have been reported. CHS is thought to represent a model of DTH, which is mediated by CD4+ Th1 cells. However, recent studies demonstrated that IFN-γ-producing CD8+ Tc1 cells play an effector role in this model. Although the exact mechanisms of whether CD4+ Th1 or CD8+ Tc1 cells predominate as effector cells and their differential roles have not been well clarified, in a
study of human nickel allergy, CD8 Tc1 cells have been implicated in the initiation of tissue damage, while CD4 Th1 cells are implicated in a later phase, based on results showing disparate cytotoxic activity of CD4 and CD8 T cell subsets against keratinocytes (28). As both CD4 Th1 and CD8 Tc1 cells have been shown to function as effector cells in CHS responses to dinitrofluorobenzene and oxazolone, these cells may have similar differential roles. In an oxazolone-induced CHS model, we showed that in response to dinitrofluorobenzene and oxazolone, these cells may have similar differential roles. In an oxazolone-induced CHS model, we showed that in vitro-generated Tc1 cells were able to migrate into the inflamed skin, but not into the control skin. Our data also showed that Tc1 cells migrated more efficiently into the inflamed skin than Tc2 cells, just as Th1 cells migrated more efficiently than Th2 cells (15). The differential migration between type 1 and type 2 cells into inflamed skin may be explained by their differential expression of ligands for P- and E-selectin: type 1 cells express more functional ligands for P- and E-selectin than type 2 cells. It is still possible that the expression of other adhesion molecules and chemokine receptors affects the differential migration between Tc1 and Tc2 cells. Indeed, different chemokine receptor expression patterns are observed between Tc1 and Tc2 cells (14).

The expression of functional selectin ligands during Th cell differentiation in vitro appears to be regulated by the cytokine milieu; the Th1 cytokine IL-12 up-regulates functional selectin ligands through α(1,3)-fucosyltransferase VII (Fuc-T VII), while the Th2 cytokine IL-4 down-regulates Fuc-T VII (29, 30). As IL-12 directs the differentiation of either CD4+ or CD8+ T cells into effector populations that express functional selectin ligands, it is likely that the same mechanism through Fuc-T VII regulates selectin ligand expression on CD8+ T cells. Indeed, Fuc-T VII is required for selectin ligand expression on Tc1 cells (31). PSGL-1 on Th1 cells is the glycoprotein core that is post-translationally modified by Fuc-T VII and other enzymes to function as a major P-selectin ligand and one of the E-selectin ligands. Our in vitro adhesion assays show that PSGL-1 on Tc1 cells is the predominant ligand for P-selectin and also plays a role as one of the ligands for E-selectin. The in vivo migration assays support this observation. Thus, the role of PSGL-1 on Tc1 cells is very similar to that on Th1 cells. In vivo, the expression of functional selectin ligands is also dependent on the local environment, and selectin ligand-expressing lymphocytes are found particularly in cutaneous inflammatory sites (32, 33).

In contrast to Th1 cells that depend almost completely on P- and E-selectin to migrate into the inflamed skin, absence of P- and E-selectin caused only partial inhibition of Tc1 cell migration. We show that the P- and E-selectin-independent migration of Tc1 cells was predominantly mediated by L-selectin. This may be explained by the presence of a cell population within Tc1 cells that does not have selectin-binding activities and yet has the capacity to migrate into inflamed skin. Alternatively, the cooperative function of P- and E-selectin ligands with L-selectin on Tc1 cells is required for efficient migration. Such cooperative functioning of all three selectins has been demonstrated for neutrophil rolling on cytokine-activated endothelium (34).

Although L-selectin is primarily a lymphocyte homing receptor that mediates the tethering and rolling of lymphocytes on HEVs within lymph nodes, its role in lymphocyte recruitment into inflamed tissues has also been suggested. MEL-14 can block lymphocyte migration into a DTH-like site produced by sensitization and challenge to Bordetella pertussis vaccine (35). L-selectin has also been shown to be involved in lymphocyte migration to cutaneous site of inflammation in an allogenic skin graft model (36) and to inflamed lacrimal glands (37). The Tc1 cells used in our studies express L-selectin at high levels. Although naive T cells down-regulate L-selectin following activation, there is heterogeneity of effector and memory cells, and both L-selectin high and low populations have been reported.

The involvement of L-selectin in Tc1 cell migration further suggests that L-selectin ligands may be expressed on skin vessels at sites of inflammation. HEV ligands for L-selectin, collectively called PNAd, are stained with mAb MECA-79. In certain chronic skin diseases, postcapillary venules of the skin are reactive with MECA-79, suggesting that L-selectin ligands can be expressed not only on peripheral node HEVs, but also on skin endothelial cells (38, 39). Although the skin vessels in this oxazolone-induced CHS model were negative for MECA-79 (data not shown), the existence of MECA-79-unreactive L-selectin ligands has also been suggested (40).

α4 integrin has been shown to play a role in lymphocyte rolling and migration. Both selectin ligands and α4 integrin participate in T lymphoblast recruitment during the pulmonary immune response (41). Although in vitro-generated Tc1 cells express α4 integrin at high levels, anti-α4 integrin mAb administration had no effect during P- and E-selectin-independent migration, suggesting that α4 integrin is not involved in Tc1 cell migration in this model. In this regard, it is of note that in vitro-differentiated human Th2 cells, but not Th1 cells, are capable of interacting with VCAM-1, although both subsets express α4 integrin (42).

CD8+ cytotoxic cells are major effector cells of the immune defense system against viruses and tumors. The migration of Tc1 effector cells to infected sites during viral infection does not require P- and E-selectin (43). Tissue specificity of selectin ligands has been shown for Th1 cells (33). Thus, it is likely that selectins may be important for Tc1 cell migration into the inflamed skin, but may not be required for migration to other tissues. It is speculated.
that different sets of molecules mediate Tc1 cell migration to different sites of inflammation.

In conclusion, our data show that all three selectins can mediate the migration of Tc1 cells into the inflamed skin, with P- and E-selectin playing the dominant roles, and L-selectin playing a role in the absence of P- and E-selectin. As CD8+ T cells are found in lesions of a variety of human skin diseases, such as psoriasis, alergic contact dermatitis, atopic dermatitis, and cutaneous graft-vs-host disease, and play a critical role in the development and course of these diseases (44), selectins are possible targets for therapeutic modulation of these conditions.

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