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CD4⁺ T Cells Migrate into Inflamed Skin Only If They Express Ligands for E- and P-Selectin

Wolfgang Tietz, Yvonne Allemand, Eric Borges, Dorothea von Laer, Ruppert Hallmann, Dietmar Vestweber, and Alf Hamann

Previous data suggested a role of endothelial selectins in skin homing of lymphocytes. In the current study, we have analyzed the expression and functional role of E- and P-selectin ligands on CD4⁺ T cells induced in vivo upon skin sensitization, using soluble selectin-Ig chimera and blocking Abs. Only low numbers of CD4⁺ cells expressing significant levels of E- or P-selectin ligands were present in s.c. lymph nodes of untreated mice (0.5–1.5% and 2–4%, respectively). Induction of a delayed-type hypersensitivity reaction increased the percentage of E-selectin-binding CD4⁺ cells in the draining lymph nodes up to 6 to 9% and that of P-selectin-binding cells up to 14%. The majority of E- and P-selectin-binding cells displayed an activated phenotype as judged by the increase in IL-2R, CD71, or cell size. The populations of E- and P-selectin-binding cells were largely overlapping; all E-selectin-binding cells also bound to P-selectin, whereas only a subfraction of P-selectin-binding cells reacted with E-selectin. Both E- and P-selectin-binding CD4⁺ cells, isolated by FACS, efficiently migrated into inflamed, but not normal skin, whereas P- or E-selectin ligand-negative CD4⁺ T cells did not. Abs against one of the two endothelial selectins partially inhibited the entry of isolated, ligand-positive cells, whereas a combination of Abs against both selectins almost completely abrogated skin homing. These data indicate that the expression of functional ligands for E- and for P-selectin is essential for homing of CD4⁺ T cells into the inflamed skin. The Journal of Immunology, 1998, 161: 963–970.

The migration of leukocytes into tissues is dependent on several steps: tethering and rolling, activation, firm adhesion, and transendothelial migration (1, 2). During this process, selectins are the key mediators of initial transient binding to endothelium (3).

L-selectin is expressed on leukocytes; on naive T cells, it is crucial for recirculation through high endothelial venules (4), while murine CD4⁺ memory cells, which are L-selectinlow, enter lymph nodes only at low rates (5). In contrast, both E-selectin and P-selectin are expressed on endothelial cells and become up-regulated during inflammation. F-selectin was initially found on activated platelets; its expression on the surface of endothelial cells is induced within a few minutes by appropriate stimuli (6, 7).

A ligand for P-selectin, the P-selectin glycoprotein ligand-1 (PSGL-1), was first identified on human and murine myeloid cells (8–10). It has since been shown that subsets of T cells, notably activated or effector cells, can also bind to P-selectin and express functional PSGL-1 (3, 11–14). Both PSGL-1 and ESL-1 (E-selectin ligand-1), the major ligands for E-selectin on murine cells (15), are constitutively expressed on T cells, but gain a role as selectin ligands only after appropriate posttranslational modification, presumably glycosylation (14) (see Note added in proof).

A ligand for E-selectin on human lymphocytes was identified as an antigenic determinant selectively expressed by the majority of T cells in skin but by only a minor subset of memory/effector cells in the blood (16, 17). Accordingly, the epitope was called the cutaneous lymphocyte-associated antigen (CLA). Recently, it has been shown that the CLA epitope is an E-selectin-binding modification of the protein core of PSGL-1 (18). The striking association between expression and localization of the cells led to the assumption that E-selectin is involved in targeting the CLA⁺ subpopulation into skin. However, experimental proof for the role of E- as well as P-selectin in the recruitment of T cells into the (inflamed) skin was largely lacking until the generation of E- and P-selectin-deficient mice. Studies in P-selectin-deficient mice showed a significant reduction of CD4⁺ T cells accumulation into the skin in oxazolone-induced delayed-type contact hypersensitivity (DTH) (19). In contrast to the rather mild phenotypes observed in mice deficient in a single selectin gene (20), those they were doubly deficient showed a virtual absence of leukocyte rolling and low extravasation at sites of inflammation (21). Whether migration into the normal skin is also affected was not reported. These data supported the hypothesis that E-selectin and P-selectin are functionally redundant in the mouse (19–21).

The relationship between expression of P-selectin and E-selectin ligands on T cells in vivo, their kinetics during an immune reaction, and their precise functional role have not yet been analyzed. We have recently shown that in vitro-generated Th1-type cells can express ligands for both P- and E-selectin and are recruited into the inflamed skin, but also into the inflamed synovium, by use of both selectins (13). In this study we analyze the development and characteristics of in vivo occurring CD4⁺ T cells, which express ligands for endothelial selectins. The data indicate that ligands for both P- and E-selectin become transiently expressed on a subset of CD4⁺ cells in murine lymph nodes after antigenic stimulation. These cells predominantly display an activated phenotype. FACS isolation and reinjection of these cells and the use of blocking Abs

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Note added in proof

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provide evidence that both selectins are involved in the entry of CD4⁺ cells into acutely inflamed sites in the skin.

Materials and Methods

Abs and selectin-IgG chimera

Anti-CD8 (53-6.72 (22)) and anti-Mac-1 (M1/70 (23)) were obtained from Becton Dickinson (San Jose, CA). The E-selectin-specific mAb UZ4 (24) and the P-selectin-specific mAb RB40.34 (25) were prepared as described. mAbs were purified by affinity chromatography as described (26) or precipitated by ammonium sulfate from serum-free supernatants. Phycoerythrin (PE)- or FITC-labeled anti-human IgG, anti-CD45RB, clone 23G2, anti-IFN-γ, clone R46A2, anti-IL-4, clone 1B11, anti-IL-2R, anti-CD71, and anti-α4β1 (L243) were obtained from Serotec (Düsseldorf, Germany). Biotin-conjugated anti-CD4 and PE-labeled goat anti-rat Ig (mouse absorbed) were obtained from Medac (Hamburg, Germany); rabbit anti-rat IgG was from Dako (Hamburg, Germany), and PerCP-conjugated streptavidin was from Becton Dickinson (Heidelberg, Germany). The E- and P-selectin-IgG fusion proteins were produced as described (27).

Animals and induction of skin inflammation

Female BALB/c mice, 8 to 16 wk of age, obtained from Harlan-Winkelmann (Borchen, Germany), were used. A cutaneous DTH reaction was induced by skin painting with 0.5% 2,4-dinitrofluorobenzene (DNFB) in acetone-oil (4:1) on day −21 and −20 and challenged with 0.5% DNFB on day −4 for isolation of P- and E-selectin-binding cells, if not otherwise stated, or on day −1 for homing experiments.

Cells

Lymphocytes from s.c. draining lymph nodes and other organs were prepared by teasing from skin-sensitized and rechallenged or control mice. Lymphocytes from peripheral blood or spleen were isolated by density centrifugation as described (5). Dead cells were removed by centrifugation on 17.0% isotonic Nycodenz (Nyegaard, Oslo, Norway), and the cells were washed twice with PBS. For the isolation of CD4⁺ T cells, they were incubated at 4°C with an Ab mixture containing anti-CD8 and anti-MAC-1. After incubation, cells were washed and depleted from non-T cells by two subsequent panning steps on petri dishes coated with rabbit anti-rat Ig (100 μg/ml, cross-reacting with mouse IgG) at 4°C. The purity of the CD4⁺ cells after panning was >95% as determined by FACS analysis.

Immunofluorescence staining and FACS sorting

For the phenotypical characterization of the CD4⁺ E- or P-selectin-binding cells, we used a FACScan or FACS caliber flow cytometer (Becton Dickenson). The cells were labeled with soluble E- or P-selectin chimera containing the PE or FITC fragment of human IgG at 0.4 to 10 μg/ml in HBSS with Ca²⁺/Mg²⁺ for 15 min, biotin-conjugated anti-CD4 mAb, and FITC-conjugated mAbs against various other cell surface markers. Biotin-selectin constructs were detected with PE- or FITC-labeled anti-human IgG (20 μg/ml), CD4 with PerCP-conjugated streptavidin. Straining was done strictly at 4°C. Cells were analyzed by FACS after fixation with 1% paraformaldehyde. For negative controls, a human CEA-Ig chimeric construct and isotype control mAbs were applied; the myeloid cell line 32DL63 served as a positive control for selectin-ligand expression.

For the sorting of E- or P-selectin-binding T cell subsets, isolated CD4⁺ T cells were labeled with E- or P-selectin as described above and subsequently sorted into positive and negative fractions on a FACS, M. F. I. cell sorter (Cytometry Bioinstruments, Ft. Collins, CO). The isolated populations were >95% pure with respect to CD4 and selectin ligand expression upon reanalysis. To remove the selectin-IgG chimera from the cells, the sorted fractions were treated for 20 min with 5 mM EDTA. Control experiments showed no effect of cell sorting or EDTA treatment on the migration behavior of total lymph node cells.

For FACS analysis of cytokine production, CD4⁺ T cells were isolated and stimulated with PMA (50 ng/ml) and ionomycin (500 ng/ml) for 3 h and, after addition of brefeldin A (10 μg/ml), for a further 2 h. The cells were labeled with selectin ligands, fixed, permeabilized, and stained with PE-conjugated anti-cytokine mAbs for either II-4 or IFN-γ production as described (28) and FITC-labeled anti-human IgG for selectin binding. Alternatively, CD4⁺ cells were triple labeled for selectin binding and intracellular II-4 and IFN-γ and the PMA/ionomycin stimulation. Control experiments had shown that the stimulation did not significantly alter the expression of the selectin ligands. As a negative control for intracellular cytokine staining, cells were either incubated with a PE-conjugated isotype controls, unlabeled anti-cytokine mAb, or labeled in the presence of an excess of soluble cytokines.

In vivo homing assays

The migration of radioactively labeled lymphocytes was analyzed as described (29, 30). In short, cells were labeled with 20 μCi/ml (750 kBq/ml) sodium ¹¹¹I (chormate for 1 h at 37°C. Blasts were selectively labeled by incorporation of [¹²⁵I]iododeoxyuridine (1 μCi (37 kBq)/ml) for 2 h. Dead cells were removed by centrifugation on a Nycodenz density cushion. Cells (0.5–2 × 10⁵) resuspended in 0.3 ml PBS were injected with or without added Ab (200 μg/ml Ab per mouse) into the tail vein. Mice were killed 1 or 24 h later, and the distribution of the radioactivity in skin pieces of 2.5 cm² in size, as well as in various other organs and in the remaining body, was measured. Blood values were computed for a 2-ml volume. Extended counting times and multiple background measurements were used allowing a statistical error below 10% for samples containing down to 0.1% of radioactivity recovered within the animals. The significance of differences between the means were analyzed with Student’s t test. A variety of isotype control mAbs, e.g., nonblocking anti-VCAM-1 IgM mAb (4E6) and and nonblocking anti-HEV IgG1 mAb (Meca 325) were used, among others, and tested in inflamed or noninflamed tissues with no significant differences compared with controls that did not receive Abs.

Results

The number of E- and P-selectin-binding T cells is low in resting lymph nodes, but increases transiently in draining lymph nodes upon cutaneous inflammation

The expression of ligands for the endothelial selectins was studied on freshly isolated CD4⁺ cells from s.c. lymph nodes by testing their ability to bind soluble E-selectin- or P-selectin-IgG chimera.

In untreated mice, the number of E-selectin-binding CD4⁺ T cells was very low, amounting to only 0.5 to 1.5% of total CD4⁺ cells in the cutaneous lymph nodes. The percentage of P-selectin-binding T cells was slightly higher, in the range of 2 to 4% of total CD4⁺ T cells (Figs. 1, a and b, and 3d). Similar numbers of these cells were found in the spleen (not shown).

Previous studies had shown that ligands for endothelial selectins are associated with the memory-activated phenotype (31, 32) and become expressed in vitro upon activation and differentiation into Th1 effector cells (13). We therefore analyzed the development of CD4⁺ cells expressing selectin ligands upon induction of an immune response in the skin. Mice were treated with DNFB on day 1 and 2 and challenged on day 15. Cells from cutaneous lymph nodes were removed both before and at different time points after treatment to analyze the kinetics of the E- and P-selectin-binding CD4⁺ T cells. After induction of the DTH response, the number of both E-selectin ligand- and P-selectin ligand-expressing cells was strongly increased. The level of expression of selectin ligands was generally found to be very heterogeneous within all populations. In this study, only T cells with an intermediate to high level of ligand expression were considered positive (Fig. 1, a–d).

Analysis of cells sorted from draining lymph nodes in skin-sensitized animals showed that all isolated E-selectin-binding CD4⁺ T cells also expressed P-selectin ligands, whereas 70% of the P-selectin-binding CD4⁺ T cells also bound E-selectin (Fig. 1, e and f).

Figure 2 shows the kinetics of E-selectin ligand expression of two separate experiments and P-selectin ligand expression. The percentage of the E-selectin-binding T cells increased to 4% on day 4 after the second priming; the percentage of the P-selectin-binding CD4⁺ T cells increased to 10%. After challenge, the percentage of E-selectin-binding CD4⁺ T cells increased to 6 to 9% and that of the P-selectin-binding CD4⁺ T cells to 14% (Fig. 1, c and d). Similar ranges were found after challenge in repeated experiments. At late time points after antigenic stimulation, the levels

Appendix

Table 1. Kinetics of E- and P-selectin ligand expression of CD4⁺ T cells on the skin after different times of antigenic stimulation

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>E-selectin (%)</th>
<th>P-selectin (%)</th>
</tr>
</thead>
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<tr>
<td>0</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>3</td>
<td>2.0</td>
<td>2.2</td>
</tr>
<tr>
<td>7</td>
<td>4.0</td>
<td>4.2</td>
</tr>
<tr>
<td>14</td>
<td>6.0</td>
<td>6.2</td>
</tr>
</tbody>
</table>

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Flow cytometry analysis of total CD4+ T cells from pooled cutaneous (axillary, brachial, and inguinal) lymph nodes of 3 to 10 untreated (a and b) and DNFB-treated mice at day 4 after rechallenge (c–f) for expression of E-selectin ligands (left) or P-selectin ligands (right). The cells were stained with E-selectin-Ig and P-selectin-Ig chimerae (solid lines) or a control chimeric protein (dotted line). The broken vertical lines indicate the position of gates used to distinguish negative (or low) vs positive (intermediate to high) populations; the numbers indicate the percentage of positive cells. e, Staining of isolated P-selectin binding cells (P-L+) for E-selectin ligands; f, staining of isolated E-selectin binding cells (E-L+) for P-selectin ligands. Representative example of more than three experiments.

FIGURE 1. Flow cytometry analysis of total CD4+ T cells from pooled cutaneous (axillary, brachial, and inguinal) lymph nodes at day 4 after rechallenge. The mice were treated with DNFB on day 1 and 2 and rechallenged on day 15. Cells from pooled cutaneous lymph nodes (two animals) were removed before and at different time points after treatment to analyze the expression of the E- and P-selectin ligands on CD4+ T cells with FACS. The development of the percentage of the selectin ligand-positive CD4+ T cells of the total CD4+ T cells within 36 days is shown. Data are from two independent experiments.

These stimulated lymph nodes, a significant fraction of the total CD4+ cells is in an activated stage. Among P- and E-selectin-binding cells, the percentage of activated cells is much higher: roughly 75% of the E-selectin-binding CD4+ T cells were found to carry the IL-2R and expressed the transferrin receptor (CD71, not shown). The percentage of P-selectin-binding CD4+ T cells that express activation markers was found to be slightly lower, with between 50 and 70% carrying the above markers. Similar ranges were found in repeated experiments. Half of both populations also expressed the early activation marker CD69 (data not shown). It has not yet been possible to determine whether these activation markers are expressed, for the most part, on the same subfraction of cells or whether they delineate only partially overlapping subsets.

Less stringent was the association of selectin-binding and markers delineating a memory phenotype: L-selectin-negative as well as CD45RBlow cells were moderately enriched within the selectin-binding subsets. Interestingly, the subsets of cells expressing selectin ligands also clearly contained a resting cell population that was small, CD45RBlow and L-selectinhig (Fig. 3c).

In the human, the expression of CLA, a ligand for E-selectin, has been shown to be inversely correlated with expression of the mucosal homing receptor α4β7. We did not observe this correlation in the mouse (Fig. 3). Selectin-binding cells were also observed in other organs such as Peyer’s patches, spleen, or peripheral blood (Fig. 3h), and also in contralateral lymph nodes (not shown), albeit in lower numbers (corresponding to the lower numbers of activated cells found in these compartments after stimulation via a skin site). High numbers of selectin-binding cells were also found in the lamina propria and other noncutaneous sites in a model of intestinal inflammation (33).

E- and P-selectin-binding T cells isolated from untreated mice (Fig. 3d) or from mice several weeks after a sensitization cycle (Fig. 3e) displayed a similar, largely activated phenotype. Also, in this setting, no evidence was found that the selectin-binding cells were enriched in the subset of memory cells.

In conclusion, CD4+ cells expressing ligands for endothelial selectins are found in increased numbers only in stimulated mice. This cell population is heterogeneous with respect to activation markers, with a bias for activated cell stages. In the smaller subset...
expressing ligands for E-selectin, the activation is more pronounced.

**A large percentage of selectin-binding CD4\(^+\) cells are cytokine-producing effector cells**

Previously, we have shown that in vitro-generated Th1 cells, but not Th2 cells, express ligands especially for P-selectin (13, 14). It was therefore of interest to determine whether the expression of selectin ligands on cells differentiated in vivo correlates with an effector cell stage characterized by the production of distinct cytokines. Figure 4 shows that slightly more than half of the IFN-γ-producing Th1 cells are found in the P-selectin binding fraction, and high producers are enriched in this fraction (mean fluorescence in the IFN-γ channel: 1.8 \(\times\) that of the P-selectin-negative cells). In the DTH model used, very few IL-4-producing Th2 cells are found in the draining lymph nodes; among these, however, both selectin-binding and non-binding cells were found. Ligands for E-selectin are expressed only on a smaller percentage of cytokine-producing Th1 cells. In conclusion, approximately half of the cytokine producers express ligands for P-selectin, while less of them express ligands for E-selectin; thus, activated effector cells are enriched in the selectin-binding fraction of CD4\(^+\) cells. The close association between cytokine phenotype and expression of selectin ligands is, however, not found in this model of in vivo activation of T cells.

**Only selectin-binding CD4\(^+\) T cells are able to enter inflamed skin**

The use of soluble selectin-Ig chimeras that bind reversibly, in a Ca\(^{2+}\)-dependent manner, to their ligands allowed us to isolate CD4\(^+\) subsets expressing the functional ligands in a native state, to analyze their migratory properties in vivo.

CD4\(^+\) T cells were labeled with the E- or P-selectin-Ig construct and a secondary Ab, then sorted in positive and negative fractions on a FACS. The resulting populations of the E- or P-selectin-binding fraction were 95% positive (Fig. 5, c and d).
The negative fraction contained 1% or less of selectin-binding cells (Fig. 5, a and b). After removal of the selectin-Ab complexes by washing in EDTA, cells were labeled with sodium $[^{51} \text{Cr}]$chromate and $[^{125} \text{I}]$iododeoxyuridine and injected i.v. Control experiments showed that neither cell sorting nor EDTA treatment had an effect on the migration behavior of lymph node cells (data not shown).

As shown in Figure 6a, the E-selectin-binding CD4$^{+}$ T cells entered the inflamed skin to a much higher extent within the first hour after i.v. injection, compared with the total fraction of negative cells or to $[^{125} \text{I}]$iododeoxyuridine-labeled blasts present in the negative fraction. CD4$^{+}$ cells of memory phenotype, not enriched for selectin-binding subsets, also lack a significant capacity to enter the inflamed skin (34). Similar results were obtained with P-selectin-binding CD4$^{+}$ T cells (Fig. 7a). Twenty-four hours

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CD4 strongly reduced and a high localization within the lung was observed. These trafficking preferences contrast to those of resting CD4 accumulation in the liver was observed in several experiments.

1 The exclusive capacity of CD4 to migrate into inflamed skin strongly suggests, but does not prove, a crucial role of these epitopes for homing into the inflamed skin. The capacity of isolated E- or P-selectin-binding CD4 T cells to migrate into sites of cutaneous inflammation was tested, therefore, in the presence of mAbs blocking either E-selectin or P-selectin.

Figure 8 shows that the migration of E-selectin- and P-selectin-binding CD4 T cells depends on both selectins. E-selectin- or P-selectin-binding CD4 T cells show a partial reduction in migration into the inflamed skin by mAb against either E- or P-selectin. An additive effect was observed when both Abs were combined, resulting in almost complete inhibition.

Significant effects of the Abs on the migration into other compartments such as lymph nodes, blood, intestine, spleen, lung, and liver were not observed. Control experiments with various isotype control mAbs recognizing normal or inflamed endothelium had no effect on the migration (not shown).

These results clearly show that the two selectins together play a crucial role in migration into inflamed skin, but their function largely overlaps under the conditions tested.

Discussion

In this study, we have analyzed the occurrence and role of ligands for the endothelial selectins on murine CD4 T cells. By using soluble receptor reagents, the minor T cell populations carrying epitopes binding to either P- or E-selectin could be characterized and isolated for functional experiments. In untreated mice, the percentage of E- or P-selectin-binding CD4 T cells in cutaneous lymph nodes was found to be very low. It increased in the draining lymph nodes after induction of a DTH, especially after rechallenge, but with time, it decreased again, suggesting that the occurrence of such cells is dependent on an active immune reaction.

Indeed, the majority of E- and P-selectin-binding CD4 T cells was found to express activation markers such as the IL-2R or CD71, although a distinct fraction of small, phenotypically naive/resting cells could also be distinguished among them. L-selectin and CD45RB, which are low on activated as well as on memory T cells (34), were less strongly associated with the selectin-binding cell subset. A slightly increased cell size, especially of the E-selectin binding cells, as indicated by the scatter values, would argue against these cells being true resting memory cells. This phenotype is compatible with the previous characterization of human T cells carrying the E-selectin-binding epitope CLA as cells in the activated-to-memory transition (16, 32), but suggests that, in mice, selectin ligands are expressed on activated rather than on memory cells. This conclusion is also supported by the merely transient occurrence of selectin-binding cell subsets after antigenic challenge.

Previously, we have shown that in vitro-generated Th1 cells, but not Th2 cells, express the PSGL-1 protein in a modified form, rendering it a functional P-selectin ligand (13, 14). These data suggested that differentiation into distinct effector cell stages is linked with an up-regulation of the synthesis of functional selectin ligands. In the DTH model elicited by DNP, almost exclusively, Th1 cells are induced in vivo. In this model, roughly half of the IFN-γ-producing Th1 cells express P-selectin ligands, and this subset comprised especially the high producers. However, the small number of IL-4-producing Th2 cells found in the draining lymph nodes also showed selectin binding on part of them. These data indicate that selectin-binding epitopes are up-regulated on cytokine producers. It appears, however, that CD4 + effector cells generated in the skin inflammation model used here do not exhibit the close correlation between selectin binding and cytokine subset found for in vitro-generated Th1/Th2 cells.
The CD4+ subset binding E-selectin was smaller than that binding P-selectin in all compartments studied. Double staining with E- vs P-selectin-Ig, to analyze the relationship between cells expressing the respective ligands, was not possible due to technical reasons. An analysis of sorted (either P- or E-selectin binding) cells indicated that the E-selectin-binding fraction is a subtraction of cells binding P-selectin. E-selectin-binding cells display a higher degree of activation compared with the total P-selectin-binding fraction. In addition, those cells that carry ligands for E-selectin were found to express especially high levels of P-selectin ligands. This was also found for cultured CD4+ effector cells (unpublished data; see also Note added in proof).

This study shows that both the E-selectin-binding CD4+ T cells and the P-selectin-binding CD4+ T cells efficiently migrate into the inflamed skin, compared with a poor homing into skin displayed by CD4+ cells lacking ligands for E- and P-selectin by ligand-negative CD4+ blasts and by the total memory cell fraction of peripheral lymph nodes from untreated mice.1 The expression of ligands for endothelial selectins suggests, therefore, a crucial role of these epitopes for homing into the inflamed skin.

The migration of selectin-binding cells into other, noninflamed tissues was found to be very similar to that of activated or memory CD4+ T cells, in which migration is characterized by a localization in nonlymphoid and a reduced recirculation through lymphoid tissues (5, 35); a specific influence of selectin ligands for trafficking through these organs could be identified neither with sorted cells nor in Ab inhibition experiments. Additional data indicate that the expression of selectin ligands enables CD4+ cells to migrate not only into the inflamed skin, but also into other inflamed tissues (Ref. 13; Y.A. and A.H., unpublished observations).

The present results do not support the view that expression of ligands for endothelial selectins predisposes T cells toward homing into normal, noninflamed skin.

The crucial role of ligands for E- and P-selectin for migration into the inflamed skin is confirmed by Ab inhibition experiments. Blocking of either E- or P-selectin reduced the migration of the E-selectin-binding CD4+ T cells into the inflamed skin by 50% and reduced that of P-selectin-binding CD4+ T cells somewhat less. Inhibition of both endothelial selectins together blocked completely the migration into the inflamed skin, indicating that E- and P-selectin can largely substitute for each other in recruiting cells into the inflamed skin. Similar findings were observed with in vitro-generated Th1-type cells (13, 14). Thus, the overlapping function of E- and P-selectin identified by studies on neutrophils (19, 20) also applies in its role in T cell recruitment into inflamed tissue.

In conclusion, E- and P-selectin-binding CD4+ T cells are a heterogeneous population of, predominantly, activated effector T cells with a high capacity to migrate into inflamed, but not normal skin. The two endothelial selectins together and their ligands are obligatory for a recruitment of the CD4+ cells into the inflamed site, and their function cannot be replaced by integrins or L-selectin.

Note added in proof. A recent study shows that fucosyltransferase-VII is a key element in regulating the synthesis of P- as well as E-selectin ligands in lymphocytes and demonstrates that the level of this enzyme determines whether P-selectin ligands are expressed alone or in conjunction with E-selectin ligands (Knibbs, R. N., R. A. Craig, P. Mäly, P. L. Smith, F. M. Wolber, N. E. Faulkner, J. B. Lowe, and L. M. Stoolman. 1998. α-(1,3)Fucosyltransferase-VII-dependent synthesis of P-selectin ligands on cultured T lymphoblasts. J. Immunol. In press.)

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References


