The Journal of Immunology

Homing in on L-Selectin

Steven D. Rosen

J Immunol 2006; 177:3-4; ;
doi: 10.4049/jimmunol.177.1.3
http://www.jimmunol.org/content/177/1/3

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Homing in on L-Selectin

Steven D. Rosen[1]

By 1983, when the article by Gallatin, Weissman, and Butcher (1) was published in *Nature*, it was clear that blood-borne lymphocytes migrate selectively ("home") to secondary lymphoid organs. It was known that their ability to enter lymphoid organs and their preferences for particular lymphoid organs depend on their state of development and their history of Ag stimulation. A seminal study by Gowans and Knight (2) in 1964 had determined that the route of lymphocyte entry into lymph nodes is through specialized postcapillary venules located in the cortex "which are remarkable for the height of their endothelium." These vessels are now commonly referred to as high endothelial venules (HEV).[2] Gowans and Knight noted that the walls of HEV contain infiltrated lymphocytes (but generally not other leukocytes) and reached the remarkably prescient conclusion that there must be a "special affinity" between the endothelium and lymphocytes.

It became possible to investigate the molecular basis for this interaction through the development of an in vitro adhesion assay by Stamper and Woodruff (3) in 1976. Their basic observation was that when lymphocytes were overlaid onto a cryostat-cut section of rat lymph node and the sections were rotated, lymphocytes bound selectively to profiles of HEV. Additional studies by Woodruff's group and by Butcher, Weissman, and coworkers (who perfected the assay in the murine system) established that the in vitro adhesive assay reflected the in vivo interaction between lymphocytes and HEV with high fidelity (4).

Taking advantage of this remarkable assay and using the newly emerging mAb technology, Gallatin et al. (1) set about identifying the "lymph node homing receptor," which was postulated to be responsible for the selective adhesion of lymphocytes to lymph node HEV and ultimately for their migration into lymph nodes. These efforts led to the first biochemical identification of the receptor now known as L-selectin.

They started with a mouse B cell lymphoma (38C-13), which bound selectively in the Stamper-Woodruff assay to lymph node HEV but not to HEV in Peyer's patches, a gut-associated lymphoid organ. They immunized rats with this cell type and generated hybridomas. They screened for hybridoma supernatants that stained the immunogen cell type as well as normal mouse lymphocytes but not HEV nonbinding cells. This yielded the MEL-14 mAb. In critical functional tests, MEL-14 blocked the in vitro attachment of both 38C-13 cells and normal lymphocytes to lymph HEV in the in vitro assay and also inhibited short-term homing of fluorescently labeled lymphocytes to lymph nodes in vivo. Immunoprecipitation of surface-iodinated lymphocytes with MEL-14 revealed a single diffuse band of 80–92 kDa on SDS-PAGE with no evidence of another subunit. Formal proof for an actual receptor function for the MEL-14 Ag came later with the demonstration that the isolated native molecule could interact with HEV of lymph node and block subsequent in vitro lymphocyte attachment, thus establishing that the Ag could bridge the lymphocyte to HEV (5).

Realizing that the lymphocyte-HEV interaction is one example of specific heterotypic cell-cell adhesion events, the article concluded with the speculation that "MEL-14 defined structures may be part of a larger family of evolutionarily related molecules mediating many cell-cell recognition events . . . ." This view was dramatically validated with the subsequent discovery that the MEL-14 Ag belongs to a family of lectin-like adhesion receptors (i.e., the selectins) that are involved in a plethora of adhesion events within the blood vascular compartment. Thus, at the same time that the MEL-14 Ag was being elucidated, our group was showing that lymphocyte binding to lymph node HEV involves a lectin-like receptor on the lymphocyte that recognizes carbohydrate-based determinants on HEV (6, 7). Subsequent biochemical studies showed the MEL-14 Ag is identical to the lectin-like receptor (8). Confirmation of the lectin nature of the MEL-14 Ag was achieved with its molecular cloning in 1989 and the demonstration that it possesses a C-type lectin domain at its extracellular terminus (9, 10). Accordingly, the MEL-14 Ag was renamed as L-selectin. Fulfilling the prediction of "a family of evolutionarily related molecules," E-selectin (ELAM-1) and P-selectin (GMP-140) were cloned at the same time (11, 12) and were shown subsequently to function as lectins in leukocyte-endothelial interactions (E and P) and platelet-leukocyte interactions (P). Following the cloning of L-selectin, definitive proof of its critical participation in lymph node homing came with the analysis of a knockout mouse (13).

The concept of a simple heterotypic receptor-ligand model guided the experiments of Gallatin et al. (1), which culminated in the identification of L-selectin. However, work over the next several years established that leukocyte trafficking in general (including lymphocyte homing) occurs through a cascade of adhesion and signaling events involving the sequential (and sometime overlapping) action of selectins, chemokine signaling, and integrins. In the case of the lymphocyte homing to lymph nodes, L-selectin functions in the first step by mediating the tethering and rolling of lymphocytes along HEV (14). Specificity is determined not by a single receptor-ligand pairing but via

Department of Anatomy and Program in Immunology, University of California, San Francisco CA, 94143

[1] Address correspondence and reprint requests to Dr. Steven D. Rosen, Department of Anatomy and Program in Immunology, University of California, San Francisco CA, 94143-0452. E-mail address: steven.rosen@ucsf.edu

[2] Abbreviation used in this paper: HEV, high endothelial venule.

0022-1767/06/$02.00

a succession of adhesion and signaling steps, each involving a distinct receptor-ligand pairing (15, 16).

The multistep paradigm rationalizes the broader involvement of L-selectin in many instances of leukocyte trafficking. Thus, for example, L-selectin works collaboratively with the integrin $\alpha_4\beta_7$ in the homing of naive lymphocytes to Peyer's patches (17). The initial failure to detect L-selectin's contribution in the Stamper-Woodruff assay (1) is likely attributable to the relatively weak or low abundance of ligands on Peyer's patch HEV in comparison to those on lymph node HEV. L-selectin also has important functions on other classes of leukocytes in instances of acute and chronic inflammation. In addition to mediating endothelial interactions, L-selectin functions in leukocyte-leukocyte interactions (18). This so-called "secondary tethering" serves to amplify the overall number of leukocytes that are recruited to the tissue sites. Moreover, the L-selectin mediated interaction of leukocytes with "accidental" ligands on cancer cells, CNS myelin, and other sites may have profound pathophysiological consequences (19). The many roles of L-selectin and its ligands continue to occupy researchers in many disciplines.

Gallatin et al. (1) anticipated that by solving the problem of lymph node homing, general principles and mechanisms might emerge. Indeed, this article not only led to the molecular characterization of a novel adhesion molecule but also set the stage for our current understanding of how leukocytes traffic to tissues sites throughout the body. No doubt this article richly deserves the "Pillars of Immunology" accolade.

References

1. Gallatin, W. M., I. L. Weissman, and E. C. Butcher. 1983. A cell-surface molecule involved in organ-specific homing of lymphocytes. *Nature* 304: 30–34.
2. Gowans, J. L., and E. J. Knight. 1964. The route of recirculation of lymphocytes in the rat. *Proc. R. Soc. Lond. Ser. B.* 159: 257–282.
3. Stamper, H., and J. Woodruff. 1976. Lymphocyte homing into lymph nodes; in vitro demonstration of the selective affinity of recirculating lymphocytes for high-endothelial venules. *J. Exp. Med.* 144: 828–833.
4. Butcher, E. 1986. The regulation of lymphocyte traffic. *Cur. Top. Microbiol. Immunol.* 128: 85–122.
5. Geoffroy, J. S., and S. D. Rosen. 1989. Demonstration that a lectin-like receptor (gp90MEL) directly mediates adhesion of lymphocytes to high endothelial venules of lymph nodes. *J. Cell Biol.* 109: 2463–2469.
6. Rosen, S. D., M. S. Singer, T. A. Yednock, and L. M. Stoolman. 1985. Involvement of sialic acid on endothelial cells in organ-specific lymphocyte recirculation. *Science* 228: 1005–1007.
7. Stoolman, L. M., and S. D. Rosen. 1983. Possible role for cell surface carbohydrate-binding molecules in lymphocyte recirculation. *J. Cell Biol.* 96: 722–729.
8. Yednock, T. A., E. C. Butcher, L. M. Stoolman, and S. D. Rosen. 1987. Receptors involved in lymphocyte homing: relationship between a carbohydrate-binding receptor and the MEL-14 antigen. *J. Cell Biol.* 104: 725–731.
9. Lasky, L. A., M. S. Singer, T. A. Yednock, D. Dowbenko, C. Fennie, H. Rodriguez, T. Nguyen, S. Stachel, and S. D. Rosen. 1989. Cloning of a lymphocyte homing receptor reveals a lectin domain. *Cell* 56: 1045–1055.
10. Siegelman, M. H., M. Van De Rijn, and I. L. Weissman. 1989. Mouse lymph node homing receptor cDNA clone encodes a glycoprotein revealing tandem interaction domains. *Science* 243: 1165–1172.
11. Bevilacqua, M. P., S. Spengeling, M. A. Gimbrone, Jr., and B. Seed. 1989. Endothelial leukocyte adhesion molecule 1: an inducible receptor for neutrophils related to complement regulatory proteins and lectins. *Science* 243: 1160–1165.
12. Johnston, G. I., R. G. Cook, and R. P. McEver. 1989. Cloning of GMP-140, a granule membrane protein of platelets and endothelium: sequence similarity to proteins involved in cell adhesion and inflammation. *Cell* 56: 1033–1044.
13. Arbonés, M. L., D. C. Ord, K. Ley, H. Ratech, C. Maynard-Curry, G. Otten, D. C. Capon, and T. F. Tedder. 1994. Lymphocyte homing and leukocyte rolling and migration are impaired in L-selectin-deficient mice. *Immunity* 1: 247–260.
14. Warnock, R. A., S. Askari, E. C. Butcher, and U. H. von Andrian. 1998. Molecular mechanisms of lymphocyte homing to peripheral lymph nodes. *J. Exp. Med.* 187: 205–216.
15. Butcher, E. C. 1991. Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. *Cell* 67: 1033–1036.
16. Springer, T. A. 1994. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* 76: 301–314.
17. Bargatze, R. F., M. A. Jutila, and E. B. Butcher. 1995. Distinct roles of L-selectin and integrins $\alpha_4\beta_7$ and LFA-1 in lymphocyte interactions with Peyer's Patch HEV in situ: the multi-step model confirmed and refined. *Immunity* 3: 99–108.
18. Bargatze, R. F., S. Kurk, E. C. Butcher, and M. A. Jutila. 1994. Neutrophils roll on adherent neutrophils bound to cytokine-induced endothelial cells via L-selectin on the rolling cells. *J. Exp. Med.* 180: 1785–1792.
19. Rosen, S. D. 2004. Ligands for L-selectin: homing, inflammation, and beyond. *Ann. Rev. Immunol.* 22: 129–156.