The Cutaneous Lymphocyte Antigen Is a Skin Lymphocyte Homing Receptor for the Vascular Lectin Endothelial Cell–Leukocyte Adhesion Molecule 1

By Ellen L. Berg, Tadashi Yoshino, Lusijah S. Rott, Martyn K. Robinson, R. Aaron Warnock, Takashi K. Kishimoto, Louis J. Picker, and Eugene C. Butcher

From the Laboratory of Immunology and Vascular Biology, Department of Pathology and the Digestive Disease Center, Stanford University, Stanford, California 94305; and the Center for Molecular Biology in Medicine, Veterans Administration Medical Center, Palo Alto, California 94304

Summary

A skin-associated population of memory T lymphocytes, defined by expression of the cutaneous lymphocyte antigen (CLA), binds selectively and avidly to the vascular lectin endothelial cell–leukocyte adhesion molecule 1 (ELAM-1), an interaction that may be involved in targeting of CLA^+ T cells to cutaneous sites of chronic inflammation. Here we present evidence that CLA itself is the (or a) lymphocyte homing receptor for ELAM-1. Antigen isolated with anti-CLA monoclonal antibody HECA-452 from human tonsillar lysates avidly binds ELAM-1 transfected mouse cells. Anti-CLA antibody blocks T lymphocyte binding to ELAM-1 transfectants. HECA-452 and ELAM-1 binding to lymphocytes or to isolated tonsillar HECA-452 antigen is abrogated by neuraminidase treatment implying a prominent role for sialic acid in CLA structure and function. The dominant form of CLA on T cells is immunologically distinct from the major neutrophil ELAM-1 ligand, the sialyl Lewis x (sLe^x) antigen (NeuAcα2-3Galβ1-4[Fucα1-3]GlcNAc), which is absent, weakly expressed, or masked on T cells. However, neuraminidase treatment of CLA^+ T cells, but not of CLA^- T cells, reveals Lewis x (CD15) structures. In combination with the known requirement for terminal NeuAcα2-3Gal and fucose residues attached to *N*-acetylglucosamine for ELAM-1 and HECA-452 binding, this finding suggests that CLA may comprise an additionally sialylated or otherwise modified form of sLe^x. The identification of a lymphocyte homing receptor for skin may permit novel approaches to the diagnosis and therapy of cutaneous and inflammatory disorders.

Most T cells infiltrating cutaneous sites of inflammation express the cutaneous lymphocyte antigen (CLA)[1] defined by mAb HECA-452 (1). CLA^+ T cells represent a unique subset of previously activated ("memory") lymphocytes that constitute 10–25% of circulating $CD3^+$ PBLs, 5–10% of T cells in tonsils and peripheral lymph nodes, and ~80–90% of T cells in most cutaneous sites of chronic inflammation (1, 2). They are rare in most noncutaneous inflammatory sites. The selective localization of this skin-associated memory T cell population appears to reflect their ability to bind to endothelial cell–leukocyte adhesion molecule-1 (ELAM-1) an endothelial cell lectin of the selectin/LEC-CAM family that is preferentially expressed by venules in cutaneous sites of chronic inflammation (3). CLA^+ peripheral blood T cells bind avidly and almost quantitatively to ELAM-1 transfected COS cells whereas CLA^- T cells bind poorly in comparison. This selectivity of adhesion raised the possibility that CLA itself might be involved in or mediate T cell binding to ELAM-1. Here we present studies that confirm that CLA is a lymphocyte homing receptor for ELAM-1.

Materials and Methods

Binding of ELAM-1 cDNA Transfectants to Immunoisolated HECA-452 Antigen (Ag). Lymphocyte CLA is defined by the rat IgM mAb HECA-452 (1). Immunoisolated HECA-452 Ag or control glycoproteins (CD44) were prepared from tonsil extracts by mAb affinity chromatography, employing a two-step procedure, gener-

[1] *Abbreviations used in this paper:* CLA, cutaneous lymphocyte antigen; ELAM, endothelial cell–leukocyte adhesion molecule; HEV, high endothelial venules; HSA, human serum albumin; sLe^x, sialyl Lewis x; WGA, wheat germ agarose.

ally as previously described (4). Tissue extracts were prepared in NP-40–containing lysis buffer and wheat germ agglutinin-binding materials were isolated by affinity chromatography on wheat-germ-agarose (WGA; Vector, Burlingame, CA) eluting with wash buffer containing 0.5 M *N*-acetylglucosamine. The WGA-binding material was passed through affinity columns of Hermes-1 (rat IgG2a anti–human CD44; reference 5), rat IgM mAb control and then HECA-452 (rat IgM anti-CLA; references 1 and 2) coupled to Sepharose 4B (Pharmacia, Uppsala, Sweden). Columns were washed and eluted with 50 mM β-octylglucoside-containing wash and acetic acid elution buffers. Fractions were collected and neutralized with 1 M Tris-HCl, pH 8.0. Peak fractions employed in these experiments contained 0.1–1 μg/ml protein. The purity and composition of the tonsillar HECA-452 Ag was as reported by Picker et al. (1). For the binding assay, samples of HECA-452 Ag or control glycoproteins isolated from tonsil extract were adsorbed onto glass wells of 8-chamber Lab-Tek slides (Nunc, Inc., Naperville, IL) by dilution in phosphate buffered saline, as previously described for the functional reconstitution of the peripheral lymph node addressin (4). After blocking in CM (5% normal bovine serum/10 mM HEPES, pH 7.0/DMEM; Applied Scientific, San Francisco, CA), mouse L1-2 cells transfected with ELAM-1 cDNA, L1-2^{ELAM-1}, or control vector cDNA, L1-2^{vector} (6), were applied to each well (1.5 × 10^6/0.15 ml CM). After a 25 min incubation at room temperature on a rotating shaker at 50 rpm, the tops of the wells were removed and the slides were washed and fixed in 1.5% glutaraldehyde in DMEM. Binding of ELAM-1 cDNA transfectants to HECA-452 antigen was similar whether isolated from whole tonsil lysates or from tonsil lymphocytes (separated on gradients of Ficoll-hypaque; L. Picker, personal observations). L1-2^{ELAM-1} cells did not bind control proteins, even when tested at concentrations 10–100-fold higher than HECA-452 antigen. In some experiments, glass slides coated with HECA-452 Ag or control protein (CD44) were incubated with anti-CLA (HECA-452; references 1 and 2) or control rat IgM (RA3-2C2; reference 7) and then washed before assay; alternatively, L1-2^{ELAM-1} cells were incubated with anti–ELAM-1 (CL2; reference 3), anti–human LECAM-1 (Dreg-56; reference 8), or anti–mouse T200 (30-G12, anti-CD45; reference 9) and after washing, were applied to HECA-452 Ag or CD44-coated slides. For other experiments, HECA-452 Ag and CD44-coated glass slides were treated with 5 mU/ml neuraminidase (*Vibrio cholera* neuraminidase; Calbiochem, San Diego, CA) in 50 mM sodium acetate, pH 5.6/100 mM NaCl/10 mM $CaCl_2$ with or without 50 mM sialyllactose (Sigma Chemical Co., St. Louis, MO) or buffer alone for 1 h at room temperature and afterwards were washed and blocked with CM before assay. For experiments testing the calcium dependence of adhesion, L1-2^{ELAM-1} cells were preincubated in HBSS (without calcium, except where indicated) or HBSS with 0.5 mM EGTA for 30 min on ice and then washed and resuspended in either HBSS with 0.5 mM EGTA or HBSS with 5 mM $CaCl_2$. The assay was performed in these buffers on HECA-452 Ag or CD44-coated glass slides prepared and blocked as described above. The proportion of L1-2^{ELAM-1} cells binding was a function of the amount of HECA-452 Ag added, and the extent of washing. Under typical conditions (selected to facilitate quantitation while conserving antigen), ~200 cells bound per high power field, representing 1–2% of input cells. Increasing the percent of cells bound had no effect on the results with antibody blocking or neuraminidase treatment, (n = 1, data not shown).

Lymphocyte–ELAM-1 Binding Assays. Normal PBMCs (isolated by gradient centrifugation on Ficoll-Hypaque 1077; Sigma Chemical Co.) were incubated in the presence of anti–LECAM-1 (Dreg-56; reference 8), anti-CLA (HECA-452; reference 1, 2), anti-CD45 (L3B12; reference 10), or control rat IgM (MECA-79; reference 11), at 50 μg/ml CM, 2 × 10^6 cells/ml, 3 ml per plate, on plates to which L1-2^{ELAM-1} cells were immobilized. The L1-2^{ELAM-1} plates were prepared by incubating 3 × 10^7 cells in 3 ml of CM in 60 mm plastic plates, previously coated overnight with 10 μg/ml poly-L-lysine (Sigma Chemical Co.) for 2 h at room temperature. After 30-min rotation at 50 rpm, unbound PBMC were removed by two washes in HBSS and adherent cells removed by incubation with HBSS containing 1 mM EDTA and 1 mM EGTA for 20 min. PBMC, which bound the ELAM-1 transfectants, were counted and the number of $CD3^+CLA^+$ cells bound in each experiment was calculated by staining an aliquot of the cells and analyzing them by FACS® analysis. The percent of CLA^+ T cells in the PBMC preparations employed ranged from 7–23%. The percent of CLA^+ T cells which bound ELAM-1 transfectants ranged from 11–74% in these experiments. Previous studies have shown that essentially all CLA^+ T cells can be depleted from PBMC by serial incubations on ELAM-1 transfected COS cells (3).

Fluorescence Activated Cell Sorting and Analysis. For two-color FACS® analysis, cells were incubated with FITC-conjugated HECA-452 for 30 min, washed in 2.5% normal bovine serum/PBS, then incubated with biotin-conjugated anti-CD3 (AMAC, Westbrook, ME) for 30 min. After washing, cells were then incubated in PE-conjugated streptavidin (Chromaprobe, Mountain View, CA), and again washed before analysis. For three-color FACS® analysis, cells were incubated with anti-CD15 (anti-Le^x; AMAC), CSLEX-1 (anti-sLe^x; reference 12), or control mouse IgM (MOPC 104E; Sigma Chemical Co.) for 20 min, then washed and incubated with PE-conjugated goat anti–mouse Ig (Tago Inc., Burlingame, CA) in PBS containing 10% normal rat serum, for 20 min. After washing again, cells were incubated in 10% normal mouse serum in PBS, then FITC-conjugated HECA-452 and biotin-conjugated anti-CD3 (AMAC). After a final wash, cells were incubated in streptavidin-PE/allophycocyanin (Southern Biotechnology Associates, Birmingham, AL) for 20 min, washed and then fixed with 1% paraformaldehyde/PBS before analysis. FACS® analyses were performed on a FACScan® (Becton Dickinson and Co., Mountain View, CA). For experiments with separated CLA^+ and CLA^- T cells, HECA-452^+ (CLA^+) or HECA-452^- (CLA^-) lymphocytes were obtained by staining Ficoll-Hypaque-separated PBMC with FITC-conjugated HECA-452 (for 20 min at 1 μg/10^6 cells), washing and sorting positive and negative lymphocytes using a FACStar® (Becton Dickinson and Co.). >95% of the lymphocytes in the sorted population were HECA-452^+. Neuraminidase treatment was performed by incubating cells at 10^7/ml in PBS containing 40 mU/ml *Vibrio cholera* neuraminidase (CalBiochem, San Diego, CA) and 2 mM $CaCl_2$ for 40 min at 37°C before staining. Similar results were obtained using *Arthrobacer ureafaciens* neuraminidase or treating cells at 4°C.

Results and Discussion

The lymphocyte CLA antigen is defined by mAb HECA-452 (1, 2). To determine whether HECA-452 might define functional ELAM-1 natural glycoprotein ligand(s), we used affinity isolated tonsillar HECA-452 antigen (HECA-452 Ag) as an adhesion substrate for ELAM-1 transfected mouse L1-2 cells (L1-2^{ELAM-1}). As shown in Fig. 1, purified HECA-452 Ag mediates the binding of L1-2^{ELAM-1} cells, but not control transfectants (L1-2^{vector}). Control proteins (e.g., H-CAM or CD44 isolated from human tonsil) are not adhesive for L1-2^{ELAM-1} cells (Fig. 2 *a–d*). Binding is inhibited by mAbs

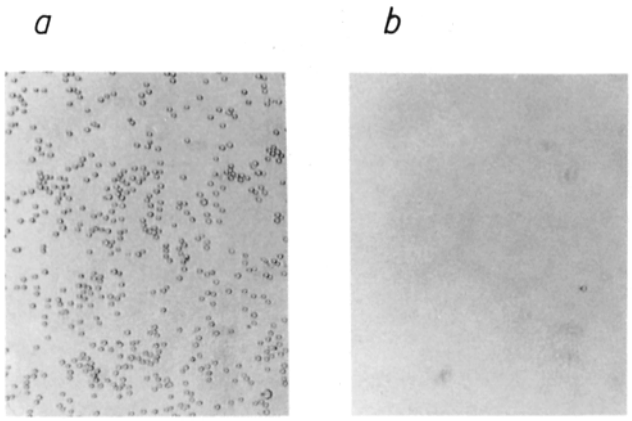

Figure 1. Immunoisolated HECA-452 Ag is a ligand for ELAM-1 transfected cells. (*a*) L1-2^{ELAM-1} cells and (*b*) L1-2^{vector} cells binding to HECA-452 Ag immunoisolated from human tonsil lysates and coated onto glass slides was as described in Materials and Methods.

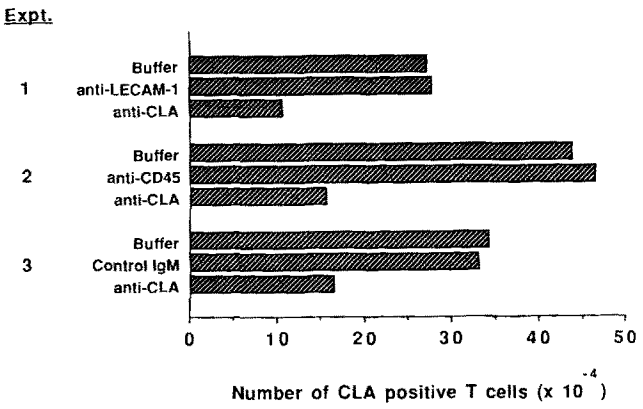

Figure 3. Binding of T lymphocytes to immobilized L1-2^{ELAM-1} cells is blocked by anti-CLA. Normal human PBMCs (6×10^6) were incubated on plates containing immobilized L1-2^{ELAM-1} cells in the presence of the indicated antibodies. After washing, the bound PBMC were removed, counted and analyzed by FACS®, as described in Materials and Methods. For each experiment, replicate plates were analyzed and the number of CLA$^+$ T cells binding is indicated. Similar results were obtained when total CD3$^+$ T cells were counted (data not shown).

HECA-452 (anti-CLA) (Fig. 2 *a*) and anti–ELAM-1 (Fig. 2 *b*) and is abrogated by treatment of the antigen with neuraminidase (Fig. 2 *c*). Adherence of the ELAM-1 transfectants is also divalent cation-dependent (Fig. 2 *d*). These results demonstrate that HECA-452 recognizes ELAM-1 binding species in the tonsillar lysate. To confirm the participation of CLA (lymphocyte HECA-452 Ag) in the specific binding

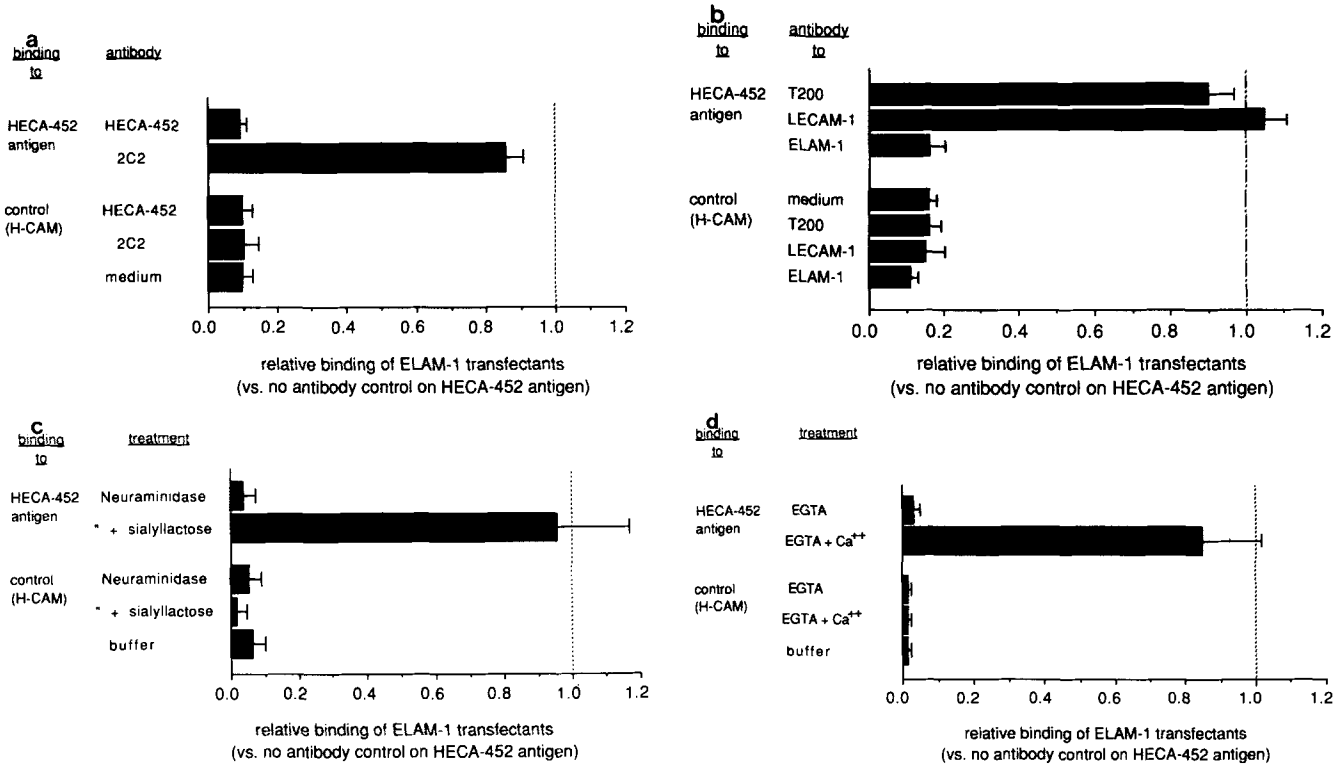

Figure 2. The HECA-452 Ag/ELAM-1 interaction is blocked by anti–ELAM-1 and anti-CLA mAbs, is calcium dependent and neuraminidase sensitive. Binding of L1-2^{ELAM-1} cells to HECA-452 Ag immobilized on glass slides, under the various conditions indicated, was performed as described in Materials and Methods. Data shown are from representative experiments in which the number of cells in three to six 100× fields for each data point were counted and standard deviations obtained. The data were normalized by dividing the number of cells bound by the number of cells binding to HECA-452 Ag in the absence of any pretreatment.

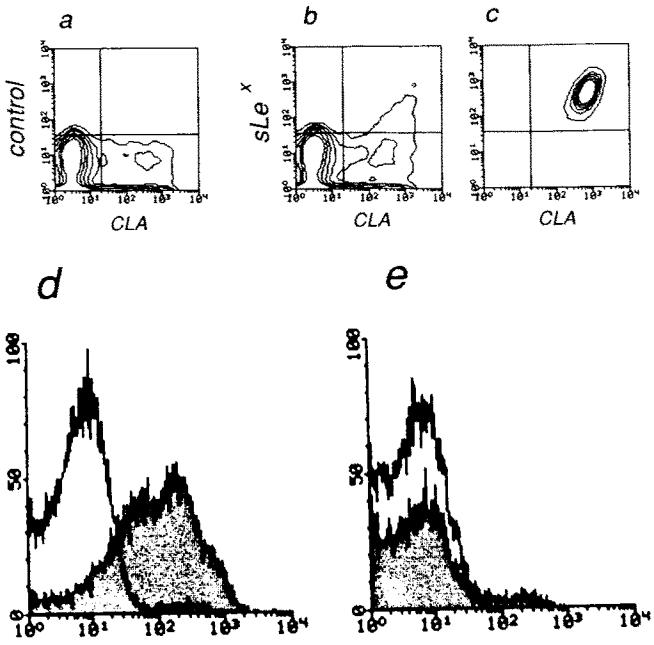

Figure 4. Comparison of the CSLEX1 (anti-sLe^x) and HECA-452 (anti-CLA) reactivity of T cells and neutrophils. *a* and *b* are two-color FACS® contour plots of CD3$^+$ T lymphocytes, and *c* is a similar plot of neutrophils from the same individual. This representative example shows a small subpopulation of sLe^{x+} T cells, all of which are CLA$^+$ (*b*). Neutrophils stain positively with anti-CLA (HECA-452) and with anti-sLe^x (CSLEX1), and have three–five-fold greater means of sLe^x expression than the sLe^{x+} T cells (*c*). Sorted CLA$^+$ T cells (*d*), but not CLA$^-$ T cells (*e*) express CD15 (Le^x) after treatment with neuraminidase. Grey shaded plots represent neuraminidase-treated cells, unshaded plots represent cells stained before treatment. All lymphocyte plots display only CD3$^+$ T cells, selected by light scatter gating and by staining with anti-CD3 in a third color.

of peripheral blood T cells to ELAM-1, antibody inhibition studies were also performed. The anti-CLA mAb HECA-452, but not control mAbs to LECAM-1, CD45, or isotype matched control mAbs, inhibited T cell binding to ELAM-1 transfectants (Fig. 3).

The dominant neutrophil ligand for ELAM-1 has recently been demonstrated to contain the sialyl Lewis x (sLe^x) antigen or NeuAcα2-3Galβ1-4(Fucα1-3)GlcNAc (13–15). SLe^x is an abundant terminal oligosaccharide of neutrophil N-linked glycans in which it occurs linked β1-3 to galactose (16). MAb CSLEX1 against sLe^x blocks neutrophil binding to ELAM-1 (13, 15); oligosaccharide derivatives of sLe^x-β1-3Gal inhibit neutrophil binding to ELAM-1 (15); and sLe^x-β1-3Gal conjugated to human serum albumin binds ELAM-1 transfectants (6). The lymphocyte ELAM-1 ligand (i.e., CLA) however, is believed to be distinct from this neutrophil ligand (17, 18).

Previous studies with mAb FH6 against sLex-i [NeuAcα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4GlcNAcβ1-3-R], thought to represent a prominent or dominant form of neutrophil sLe^x] have suggested that sLe^x is not detectably expressed by CD3$^+$ T cells (19). Here we used mAb CSLEX1 (12) to assess the expression of sLe^x on T cells. CSLEX1 recognizes neutrophil sLe^x-containing oligosaccharides (as described above); binds glycolipids and glycoconjugates of the structure sLe^x-β1-3Gal-R (6, 12), and also recognizes neuraminidase-sensitive carbohydrate determinants of human α1-acid glycoprotein (T. Yoshino, personal observations) in which the fucosylated N-linked glycans contain single lactosamine units which are linked β(1–4) to mannose (13, 20). Thus, the minimal recognition structure of CSLEX1 appears to be the sLe^x tetrasaccharide itself. Sensitive flow cytometric analysis with peripheral blood cells from 5 different individuals revealed a small if consistent T cell subset staining with CSLEX1. The mean levels of CSLEX1 reactivity of these weakly positive T cells, however, were three- to five-fold less than on neutrophils from the same individuals (Fig. 4). Furthermore, although all of the CSLEX1$^+$ T cells (representing ~5% of CD3$^+$ T cells) were CLA high, they constituted only a fraction (15–30%, n = 6) of the CLA$^+$ T cell population (Fig. 4 *b*), and both the CSLEX1 positive and negative fractions bind almost quantitatively to ELAM-1 transfectants (3, and data not shown). These results confirm that the predominant ELAM-1 binding structure comprising CLA on T cells is immunologically distinct from the major neutrophil ligand.

Recent studies of the reactivity of mAb HECA-452 with defined oligosaccharides reveal a strong correlation between HECA-452 recognition and ELAM-1 binding (6). MAb HECA-452 recognizes not only CLA but also neutrophil ELAM-1 ligands, including sLe^x. The HECA-452 antigen isolated from myeloid cell lines HL60 or U937 cells, as from tonsils, binds ELAM-1 transfectants, and this binding is inhibited by HECA-452 (data not shown). Furthermore, HECA-452 binds sLe^x-β1-3Gal conjugated to human serum albumin (HSA) (6). Interestingly, HECA-452 also recognizes the isomer of sLe^x, the sialyl Lewis a antigen (sLe^a), NeuAcα2-3Galβ1-3(Fucα1-4)GlcNAc, which is a strong ELAM-1 ligand as well (6). Both HECA-452 and ELAM-1 transfectants bound sLe^a-β1-3Gal-conjugated HSA at least as avidly as sLe^x-β1-3Gal-conjugates. Based on computer modeling, these results suggested that ELAM-1 and HECA-452 must react with a structurally common face of these related carbohydrate structures, in which terminal fucose and sialic acid residues are presented similarly. Consistent with this, ELAM-1 and HECA-452 bound poorly to lacto-*N*-fucopentaose I, lacto-*N*-fucopentaose II (Le^a), lacto-*N*-fucopentaose III (Le^x), sialyllacto-*N*-tetraose a and c, implying that the terminal neuraminic acid and fucose residues are essential for both antibody and lectin recognition (6). In contrast, CSLEX1 does not bind sLe^a, implying that its reactivity depends on the orientation of the GlcNAc (which differs in sLe^a and sLe^x). As lymphocytes do not express significant levels of Le^a or sLe^a as indicated by minimal reactivity with anti-sLe^a or anti-Le^a mAbs, these findings suggested that, although antigenically distinct from neutrophil oligosaccharides bearing sLe^x, CLA might be closely related to them.

Consistent with this hypothesis, treatment of sorted CLA$^+$ T cells with neuraminidase unmasks high levels of Le^x (CD15) (Fig. 4 *d*), whereas sorted CLA$^-$ T cells remain

largely CD15$^-$ (Fig. 4 *e*). Treated lymphocytes failed to stain with anti-Le^a mAbs. Thus, although CLA$^+$ T cells bear little immunologically detectable sLe^x, they appear to express a sialylated Lewis x structure. Given the structural constraints predicted based on ELAM-1 and HECA-452 binding to sLe^a and sLe^x, CLA could comprise disialyl Le^x, bearing an additional neuraminic acid linked α2-6 to the core GlcNAc; this additional neuraminic acid would not interfere with the predicted ELAM-1 binding face of sLe^x (6) but would be expected to prevent recognition by anti-sLe^x mAb CSLEX1. Neuraminic acid substitutions have also been reported in α2-6 and α2-8 linkage to galactose, but such modifications would be predicted to severely alter the presentation of the essential α2-3-linked neuraminic acid in relation to the fucose residue (J. Magnani, personal communication). Alternatively, CLA may bear modified sugar residues preventing recognition by anti-sLe^x antibodies, or may comprise a more complex (or branched) carbohydrate structure. In either case, the presence of cryptic CD15 on CLA$^+$ lymphocytes but not other T cells suggests that their unique ELAM-1 binding ability may be conferred in part by expression of an α(1-3) fucosyl transferase, perhaps identical to the myeloid enzyme (21). This fucosyl transferase could operate in conjunction with other lymphocyte selective glycosyl transferases (sialyl transferases) or other carbohydrate modifying enzymes to generate CLA.

Our findings are consistent with the proposal that ELAM-1 on venules in sites of acute inflammation supports neutrophil recruitment, whereas in sites of chronic inflammation in the skin ELAM-1 mediates accumulation of CLA$^+$ T cells. This situation is remarkably similar to that of the peripheral lymph node homing receptor, LECAM-1 (LAM-1 or L-selectin). LECAM-1 expression on peripheral lymph node-homing lymphocytes mediates their interaction with peripheral lymph node high endothelial venules (HEV) and is required for trafficking through peripheral lymph nodes in vivo (22). However, LECAM-1 is highly expressed on neutrophils and monocytes, which can bind in vitro to HEV but do not normally migrate into lymph nodes in vivo (23). These observations indicate that selectin-based primary adhesion is not sufficient for extravasation and that additional levels of control must exist (3, 23). Indeed we have proposed previously that leukocyte extravasation involves an initial, specific but reversible, adhesion to endothelium (homing receptor-mediated), but also requires secondary, integrin-mediated adhesion step triggered by leukocyte activation (23–26). Thus, both neutrophils and CLA$^+$ T cells may interact with ELAM-1$^+$ venules, whether in sites of acute inflammation or in chronically inflamed skin, but their subsequent firm attachment and extravasation may be regulated by locally produced leukocyte-specific activation/chemotactic factors.

In conclusion, skin-associated memory T cells express the cutaneous lymphocyte antigen, CLA, a carbohydrate ligand for ELAM-1 which appears to function as a skin lymphocyte homing receptor. CLA comprises a sialylated carbohydrate structure, probably a sialylated form of Le^x that is closely related to, albeit immunologically distinct from, the major neutrophil oligosaccharides presenting the sLe^x ligand for ELAM-1. Interestingly, the carbohydrate epitope defining CLA decorates at least one glycoprotein species with molecular mass of 200 kD (1). The significance of this protein component to T cell binding to ELAM-1 remains to be determined. Importantly, CLA represents the first homing receptor identified that is selective for an extralymphoid tissue rather than an organized lymphoid organ. Its expression by a unique subset of memory T lymphocytes supports the concept that memory T cells participate in tissue-selective homing pathways that martial and segregate immune responses in vivo.

The authors thank R. Bargatze, L. McEvoy, and P. Altevogt for generous contributions; and J. Magnani for comments and suggestions.

This work was supported by grants from the National Institutes of Health and the Veteran's Administration. E. L. Berg is a Special Fellow of the Leukemia Society. E. C. Butcher is an Established Investigator of the American Heart Association.

Address correspondence to Ellen L. Berg, Stanford University School of Medicine, Laboratory of Experimental Chemistry, Department of Pathology, L-235, 300 Pasteur Drive, Stanford, CA 94305-5324. The present address of M. K. Robinson is Cell Tech, Ltd., 216 Bath Road, Slough, SL1 4EN Berkshire, England; the present address of T. K. Kishimoto is the Department of Immunology, Boehringer-Ingelheim, 90 East Ridge Road, Ridgefield, CT 06877; and the present address of L.J. Picker is the Department of Pathology, University of Texas Southwestern Medical School, 5323 Harry Hines Boulevard, Dallas, TX 75235-9072.

Received for publication 15 July 1991 and in revised form 19 August 1991.

References

1. Picker, L.J., S.A. Michie, L.S. Rott, and E.C. Butcher. 1990. A unique phenotype of skin-associated lymphocytes in humans. *Am. J. Pathol.* 136:1053.
2. Picker, L.J., L.W.M.M. Terstappen, L. Rott, P.R. Streeter, H.

Stein, and E.C. Butcher. 1990. Differential expression of homing-associated adhesion molecules by T cell subsets in man. *J. Immunol.* 145:3247.
3. Picker, L.J., T.K. Kishimoto, C.W. Smith, R.A. Warnock, and E.C. Butcher. 1991. ELAM-1 is an adhesion molecule for skin-homing T cells. *Nature (Lond.).* 349:796.
4. Berg, E.L., M.K. Robinson, R.A. Warnock, and E.C. Butcher. 1991. The human peripheral lymph node vascular addressin is a ligand for LECAM-1, the peripheral lymph node homing receptor. *J. Cell Biol.* 114:343.
5. Jalkanen, S.T., R.F. Bargatze, L.R. Herron, and E.C. Butcher. 1986. A lymphoid cell surface glycoprotein involved in endothelial cell recognition and lymphocyte homing in man. *Eur. J. Immunol.* 16:1195.
6. Berg, E.L., M.K. Robinson, O. Mansson, E.C. Butcher, and J.L. Magnani. 1991. A carbohydrate domain common to both Sialyl Le^a and Sialyl Le^x is recognized by the endothelial cell leukocyte adhesion molecule, ELAM-1. *J. Biol. Chem.* 266:14869.
7. Coffman, R.L., and I.L. Weissman. 1981. A monoclonal antibody that recognizes B cells and B cell precursors in mice. *J. Exp. Med.* 153:269.
8. Kishimoto, T.K., M.A. Jutila, and E.C. Butcher. 1990. Identification of a human peripheral lymph node homing receptor: a rapidly down-regulated molecule. *Proc. Natl. Acad. Sci. USA.* 87:2244.
9. Ledbetter, J.A., and L.A. Herzenberg. 1979. Xenogeneic monoclonal antibodies to mouse lymphoid differentiation antigens. *Immunol. Rev.* 47:63.
10. Wood, G.S., M. Link, R.A. Warnke, J. Dilley, and R. Levy. 1984. Pan-leukocyte monoclonal antibody L3B12. Characterization and application to research and diagnostic problems. *Am. J. Clin. Pathol.* 81:176.
11. Streeter, P.R., B.T. Rouse, and E.C. Butcher. 1988. Immunohistologic and functional characterization of a vascular addressin involved in lymphocyte homing into peripheral lymph nodes. *J. Cell Biol.* 107:1853.
12. Fukushima, K., M. Hirota, P.I. Terasaki, A. Wakisaka, H. Togashi, D. Chia, N. Suyama, Y. Fukushi, E. Nudelman, and S.-I. Hakomori. 1984. Characterization of sialosylated $Lewis^x$ as a new tumor-associated antigen. *Cancer Res.* 44:5279.
13. Walz, G., A. Aruffo, W. Kolanus, M. Bevilacqua, and B. Seed. 1990. Recognition by ELAM-1 of the sialyl-Le^x determinant on myeloid and tumor cells. *Science (Wash. DC).* 250:1132.
14. Lowe, J.B., L.M. Stoolman, R.P. Nair, R.D. Larsen, T.L. Berhend, and R.M. Marks. 1990. ELAM-1-dependent cell adhesion to vascular endothelium determined by a transfected human fucosyltransferase cDNA. *Cell.* 63:475.
15. Phillips, M.L., E. Nudelman, F.C.A. Gaeta, M. Perez, A.K. Singhal, S.-I. Hakomori, and J.C. Paulson. 1990. ELAM-1 mediates cell adhesion by recognition of a carbohydrate ligand, sialyl-Le^x. *Science (Wash. DC).* 250:1130.
16. Fukuda, M., E. Spooncer, J.E. Oates, A. Dell, and J.C. Klock. 1984. Structure of sialylated fucosyl lactosaminoglycan isolated from human granulocytes. *J. Biol. Chem.* 259:10925.
17. Springer, T.A., and L.A. Lasky. 1991. Sicky sugars for selectins. *Nature (Lond.).* 349:196.
18. Mackay, C.R. 1991. Skin-seeking memory T cells. *Nature (Lond.).* 349:737.
19. Ohmori, K., T. Yoneda, G. Ishihara, K. Shigeta, K. Hirashima, M. Kanai, S. Itai, T. Sasaoki, S. Arii, H. Arita, and R. Kannagi. 1989. Sialyl SSEA-1 antigen as a carbohydrate marker of human natural killer cells and immature lymphoid cells. *Blood.* 74:255.
20. Fournet, B., J. Montreuil, G. Strecker, L. Dorland, J. Haverkamp, J. Vliegenthart, J. Binet, and K. Schmid. 1978. Determination of the primary structures of 16 asialo-carbohydrate units derived from human plasma α1-acid glycoprotein by 360 MHz ^1H NMR spectroscopy and permethylation analysis. *Biochemistry.* 17:5206.
21. Goelz, S.E., C. Hession, D. Goff, B. Griffiths, R. Tizard, B. Newman, G. Chi-Rosso, and R. Lobb. 1990. ELFT: a gene that directs the expression of an ELAM-1 ligand. *Cell.* 63:1349.
22. Gallatin, W.M., I.L. Weissman, and E.C. Butcher. 1983. A cell surface molecule involved in organ-specific homing of lymphocytes. *Nature (Lond.).* 304:30.
23. Lewinsohn, D.M., R.F. Bargatze, and E.C. Butcher. 1987. Leukocyte-endothelial cell recognition: evidence of a common molecular mechanism shared by neutrophils, lymphocytes, and other leukocytes. *J. Immunol.* 138:4313.
24. Kishimoto, T.K., M.A. Jutila, E.L. Berg, and E.C. Butcher. 1989. Neutrophil Mac-1 and MEL-14 adhesion proteins inversely regulated by chemotactic factors. *Science (Wash. DC).* 245:1238.
25. Jutila, M.A., L. Rott, E.L. Berg, and E.C. Butcher. 1990. Function and regulation of the neutrophil MEL-14 antigen in vivo: comparison with LFA-1 and Mac-1. *J. Immunol.* 143:3318.
26. von Andrian, U.H., J.D. Chambers, L.M. McEvoy, R.F. Bargatze, K.E. Arfors, and E.C. Butcher. 1991. *Proc. Natl. Acad. Sci. USA.* In press.