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

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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-acetylgalcosamine 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) 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-
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 agaroase (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, L1-2ELAM-1, or control vector cDNA, L1-2control-1, and NECA-452 Ag or CD44-coated glass slides prepared and blocked as described above. The proportion of L1-2 ELAM-1 cells bound 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 bind 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).

Results and Discussion

The lymphocyte CLA antigen is defined by mAb HEC 452 (1, 2). To determine whether HEC 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 (L1-2ELAM-1). As shown in Fig. 1, purified HECA-452 Ag mediates the binding of L1-2ELAM-1 cells, but not control transfected (L1-2control-1). Control proteins (e.g., H-CAM or CD44 isolated from human tonsil) are not adhesive for L1-2ELAM-1 cells (Fig. 2 a–d). Binding is inhibited by mAbs
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
jugated to human serum albumin binds ELAM-1 transfec-tants (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^a, the sialyl Lewis x (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^x-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^x), 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^x and sLe^x). As lymphocytes do not express significant levels of Le^x or sLe^x as indicated by minimal reactivity with anti-sLe^x or anti-Le^x 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
lymph node high endothelial venules (HEV) and is required homing lymphocytes mediates their interaction with peripheral or bsdectin). LECAM-1 expression on peripheral lymph node- peripheral lymph node homing receptor, LECAM-1 (LAM-1 cells. This situation is remarkably similar to that of the pe- in the skin ELAM-1 mediates accumulation of CLA + T phil recruitment, whereas in sites of chronic inflammation be conferred in part by expression of an α2-3 fucosyl trans- branched) carbohydrate structure. In either case, the presence 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 α 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 neutro- phil 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 per- imal 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. However, LECAM-1 is highly expressed on neutrophils and monocytes, which can bind in vitro to HEV but do not nor- mally migrate into lymph nodes in vivo (23). These observa- tions 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-medi- ated), but also requires secondary, integrin-mediated adhe- sion 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 pro- duced 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 Leα that is closely related to, albeit immunologically distinct from, the major neutrophil oligosaccharides presenting the sLeα 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. Im- portantly, CLA represents the first homing protein 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 marital and segregate immune responses in vivo.
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