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The Monoclonal Antibody CHO-131 Identifies a Subset of Cutaneous Lymphocyte-Associated Antigen T Cells Enriched in P-Selectin-Binding Cells¹

Zhenya Ni,* James J. Campbell,2†‡ Gloria Niehans,§ and Bruce Walcheck3*

T cells use the vascular adhesion molecules E- and P-selectin to enter inflamed skin. Previous studies have indicated the possibility for diversity in the synthesis of E- and P-selectin glycan ligands by activated T cells due to their different requirements for the O-glycan branching enzyme core 2 β1,6-N-acetylgalosaminyltransferase I and its independent regulation. It is known that T cell staining by the mAb HECA-452 (referred to as cutaneous lymphocyte-associated Ag (CLA) T cells) correlates with E-selectin binding, yet whether these cells uniformly bind P-selectin is less clear. The mAb CHO-131 and P-selectin binding require a glycan moiety consisting of a sialylated and fucosylated oligosaccharide properly positioned on a core-2 O-glycan. Interestingly, CHO-131 stains a subset of CLA⁺ T cells. A direct comparison of the selectin binding capacity of CHO-131⁺ and CHO-131⁻ CLA⁺ T cells revealed a significantly greater P-selectin, but not E-selectin, binding activity by the former subset. Based on the expression of homing and central and effector memory cell markers, CHO-131⁺ and CHO-131⁻ CLA⁺ T cells have an overlapping skin-tropic and memory phenotype. CHO-131⁺ T cells were considerably enriched in psoriatic skin, yet, unlike the peripheral blood of healthy individuals, HECA-452 and CHO-131 stained a similar proportion of T cells in the cutaneous lesions, indicating an accumulation advantage by CHO-131⁺ T cells. We conclude that the CHO-131⁺ CLA⁺ T cell subset is enriched in P-selectin binding cells. These findings should provide new insights into the regulation and function of skin homing T cells. The Journal of Immunology, 2006, 177: 4742–4748.

Similiar to Ag recognition, effector/memory T cells display memory in their lymphoid and extralymphoid tissue trafficking, with distinct subsets preferentially migrating into, for instance, intestinal mucosal sites or the skin for regional immunity. Adhesion molecules and chemokine recognition dictate this tissue selective accumulation. For instance, effector/memory lymphocytes that are defined by the expression of the adhesion molecule αβ-traffic to intestinal tissues, and carry memory for intestinal Ags, whereas skin homing T cells in the human are identified by expression of the cutaneous lymphocyte-associated Ag (CLA)⁴ (1). These T cells use the endothelial cell-expressed adhesion molecules E-selectin and P-selectin to initiate their accumulation along the microvasculature (2, 3).

Approximately one-fourth of peripheral T cells in healthy individuals express the CLA determinant. However, the vast majority of T cells in normal skin and at sites of cutaneous inflammation are CLA positive, whereas at sites of chronic inflammation in noncutaneous tissues these cells are typically rare (4–6). CLA expression is selectively up-regulated by T cells during the naive to effector transition in peripheral lymph nodes (7), and these lymphocytes consist of CD4 and CD8 T cells that express heterogeneous TCR specificities, and are composed of various subsets, including central and effector memory cells, Th1, Th2, and T regulatory cells (8–13). CLA T cells are important for flexible host immune responses in the skin and act on skin-invading pathogens and cutaneous neoplasms (14–16). However, CLA T cells have also been implicated in many skin diseases, including hypersensitivities, cutaneous graft-vs-host disease, and particular skin-infiltrating T cell malignancies (4, 5).

CLA T cells are detected most notably in the human by the mAb HECA-452 (17). As described above, this phenotypic marker has allowed for extensive study of their trafficking and effector capabilities. HECA-452 recognizes sialylated and fucosylated oligosaccharides including sialyl-LewisX (sLeX) (18), and its staining of T cells signifies α1,3-fucosyltransferase VII (FucT-VII) expression (19). It is well established that HECA-452 staining of T cells correlates with E-selectin binding (3, 8, 17). P-selectin glycan ligands involve sLeX as well; however, there are clear distinctions in the glycan specificities by E- and P-selectin when binding T cells. Activated T cells, for instance, require activity by the O-glycan branching enzyme core 2 β1,6-N-acetylgalosaminyltransferase I (C2GlcNAcT-I) for P-selectin but not E-selectin binding (3, 20). Of interest is that FucT-VII and C2GlcNAcT-I are independently regulated upon T cell activation (20–22). A caveat of the HECA-452 epitope is that its biosynthesis does not require the C2GlcNAcT-I enzyme, and thus its reactivity does not directly correlate with the expression of high-affinity P-selectin glycan ligands (23).

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§Abbreviations used in this paper: CLA, cutaneous lymphocyte-associated Ag; sLeX, sialyl-LewisX; FucT-VII, 1,3-fucosyltransferase VII; C2GlcNAcT, core 2 β1,6-N-acetylgalosaminyltransferase; PSGL-1, P-selectin glycoprotein ligand-1; C2-O-sLeX, core 2 O-glycan terminated with sialyl-LewisX.---
At this much, less is known about the role of P-selectin ligands on T cells in immune and disease events in the human. For instance, it has not been determined whether E- and P-selectin glycan ligands are expressed in conjunction by CLA+ T cells. P-selectin glycoprotein ligand-1 (PSGL-1; CD162) possess a sLe^\text{X}\text{^{(C2-O-sLe^X)}} (24). This structure has been directly demonstrated to confer high-affinity P-selectin binding (25). The mAb CHO-131 specifically recognizes C2-O-sLe^X, as demonstrated by using synthetic glycopeptides modeled after the N terminus of human PSGL-1 containing precise O-glycan structures (23). Moreover, cell reactivity by CHO-131 is dependent on the expression of, in part, C2GlcNAcT-I, and is sensitive to benzyl-GalNAc, an inhibitor of O-glycan modification (23, 26, 27). Interestingly, only a subset of CLA lymphocytes expresses the CHO-131 epitope (29). Here, we tested whether the CHO-131+ and CHO-131− CLA+ T cell subsets differentially bind to E- and P-selectin, and we examined their skin-tropic characteristics as well as their effector and central memory cell phenotypes.

Materials and Methods

Reagents

The anti-C2-O-sLe^X mAb CHO-131 (23, 26, 27) and the anti-αβ, mAb ACT-1 (28) have been described previously. Bioinhibition of CHO-131 was performed using NHS-SS-LC-biotin (Pierce), per the manufacturer’s instructions. HECA-452 conjugated to FITC or biotin, anti-CD45RA-PECy7, anti-CD27, anti-L-selectin, and anti-CCR4 were purchased from BD Pharmingen. The anti-human PSGL-1 mAb 215 was purchased from Santa Cruz Biotechnology. Anti-CCR9 and anti-CCR7 were purchased from R&D Systems. CD3-FITC, CD4-FITC, and CD4-FRPE were purchased from Ancell. Anti-CD3 polyclonal sera (rabbit) was purchased from DakoCytomation and used for immunohistochemistry. Mouse E-selectin/Fc was purchased from R&D Systems and used for flow cytometry. Human P-selectin/Fc and E-selectin/Fc were purchased from R&D Systems and used for the shear flow assay. Unconjugated mouse IgG isotypes and IgM, as well as mouse and rat IgM conjugated to FITC or biotin were purchased from CalTag. Cy2-, Cy3-, FITC-, or PE-conjugated F(ab')\text{2} goat anti-mouse IgM or IgG, Cy5-conjugated F(ab')\text{2} donkey anti-rabbit IgG, Cy3-conjugated F(ab')\text{2} donkey anti-mouse IgM, FITC-conjugated F(ab')\text{2} goat anti-human IgG, and allophycocyanin-, Cy3-, and PECy7-conjugated streptavidin were purchased from Jackson ImmunoResearch Laboratories. Normal mouse and normal rat serum were also purchased from Jackson ImmunoResearch. The cell nuclei stain YoYo-1 was purchased from Molecular Probes.

Mononuclear cell isolation

Peripheral blood was collected from normal donors in sodium heparin in accordance with an approved protocol by the Institutional Review Board: Human Subjects Committee at the University of Minnesota, or discarded in accordance with an approved protocol by the Institutional Review Board: Human Subjects Committee at the University of Minnesota Cancer Center Flow Cytometry Facility. Adhesion of the sorted CHO-131+ and CHO-131− CLA+ T cell subsets to human E-selectin or P-selectin was assayed in a parallel plate flow chamber (Glycotech), per the manufacturer’s instructions. P-selectin/Fc chimera or E-selectin/Fc was adsorbed to 35-mm petri dishes, and the plates were then blocked with 2.5% BSA. Optimal functional site densities for the chimeras were determined as described previously (29). CHO-131+ or CHO-131− CLA+ T cells were suspended in HBSS containing 2 mM CaCl_2 at a concentration of 5 × 10^6 cells/ml and perfused into the flow chamber. Their shear resistance and rolling velocities were determined as described previously (30–32). Image analyses were performed using the public domain NIH ImageJ program (developed at the National Institutes of Health and available on the Internet at [http://rsb.info.nih.gov/ij/](http://rsb.info.nih.gov/ij/)).

T cell staining for flow cytometric analyses

To assess P-selectin ligands on CHO-131+ and CHO-131− CLA+ T cells, enriched CD3+ T cells in PBS were incubated with 10 µg/ml human P-selectin/Fc at 4°C with gentle shaking for 30 min in the presence or absence of EDTA. Cells were then sequentially treated with FITC-conjugated F(ab')\text{2} goat anti-human IgG, CHO-131, PE-conjugated F(ab')\text{2} goat anti-mouse IgM, 10% normal mouse and rat serum, HECA-452-biotin, and streptavidin-allophycocyanin. To assess PSGL-1 expression levels on CHO-131+ and CHO-131− CLA+ T cells, enriched CD3+ T cells were sequentially stained with the anti-PSGL-1 mAb 215, PE-conjugated F(ab')\text{2} goat anti-mouse IgG, 10% normal mouse and rat serum, biotin-conjugated CHO-131, streptavidin-allophycocyanin, and HECA-452-FITC.

To assess αβ, CCR4, CCR7, CD27, or CCR9 levels on CHO-131+ CD4+ memory T cells, isolated mononuclear cells were first stained with unconjugated CHO-131 plus one of the IgG Abs—anti-αβ, CCR4, CCR7, CD27, or CCR9. Cells were next incubated with PE-conjugated F(ab')\text{2} goat anti-mouse IgM and Cy5-conjugated F(ab')\text{2} goat anti-mouse IgG. Cells were then blocked with 10% normal mouse and rat serum. Finally, the cells were stained with anti-CD45RA-PECy7 and anti-CCR7.

To assess CCR7, CD27, or L-selectin (CD62L) levels on CHO-131+ and CHO-131− CLA+ CHO-131− CLA+ T cell subsets were isolated mononuclear cells were first stained with unconjugated CHO-131 plus one of the IgG Abs—anti-αβ, CCR4, CCR7, CD27, or CCR9. Cells were next incubated with PE-conjugated F(ab')\text{2} goat anti-mouse IgM and Cy5-conjugated F(ab')\text{2} goat anti-mouse IgG. Cells were then blocked with 10% normal mouse and rat serum. Lastly, cells were stained with CD4-FRPE and HECA-452-biotin followed by streptavidin-PE-Cy7.

All cell staining steps were performed at 4°C with washing (PBS) between each step. Isotype-matched negative control mAbs were used to evaluate levels of background staining. Cells were fixed in 1% paraformaldehyde, and 5,000–50,000 cells were analyzed by flow cytometry on a FACSCalibur (BD Biosciences) or MoFlo cytometer (Cytometry) with CellQuest Pro (BD Biosciences) or Summit software (DakoCytomation), respectively.

Immunofluorescence histology

The files of the Minneapolis Veterans Affairs Medical Center Pathology and Laboratory Service were searched, and stored paraffin blocks were retrieved for skin biopsies that carried a diagnosis of psoriasis and for normal skin. Procedures were performed in accordance with protocols approved by the Institutional Review Board: Human Subjects Committee at the University of Minnesota and Minneapolis Department of Veterans Affairs Medical Center. Formaldehyde-fixed and paraffin-embedded blocks of punch biopsies of psoriatic lesions from three different patients or normal skin (two patients) were sectioned onto poly-L-lysine-coated slides and were identified only by a randomly assigned case number. Approximately 10 serial sections were evaluated per biopsy. The first and last sections were stained by H&E to verify that the psoriasis sections were within the lesion. Tissue section dewaxing, rehydration, and Ag retrieval (hot, 0.01 M citrate buffer (pH 6.0)) were performed by standard procedures. Sections were stained with CD3-FITC and either CHO-131+ or HECA-452-biotin plus streptavidin-allophycocyanin. Other Ab staining procedures involved HECA-452-FITC, but the mean fluorescence intensity conferred by this Ab was >4-fold lower than by HECA-452-biotin plus streptavidin-allophycocyanin in our hands, which affected cell staining and sorting efficiency. All staining steps were performed at 4°C and cells were washed with PBS between steps. Nonspecific Ab labeling was determined using the appropriate isotype negative control Abs. Cell sorting was performed on the FACS Diva instrument (BD Biosciences) at the University of Minnesota Cancer Center Flow Cytometry Facility.

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incubated with protein block (DakoCytomation) for 30 min. The sections were then treated with anti-CD3 rabbit polyclonal sera, Cy3-conjugated F(ab’), donkey anti-rabbit IgG, and CHO-131 or HECA-452-biotin, followed by Cy3-conjugated F(ab’), donkey anti-mouse IgM or strepavidin-Cy3, respectively. Cell nuclei were counterstained using YoYo-1 (Molecular Probes). The slides were mounted in the mounting medium Slow-Fade Light (Vector Laboratories). All staining steps were performed at 4°C and slides were washed with TBS between steps. Isotype-matched negative control mAbs were used to evaluate levels of background staining. Analysis of fluorescence was performed on a Bio-Rad 1024 confocal laser-scanning microscope, as described previously (32).

**Results**

**P-selectin binding activity by CHO-131+ and CHO-131− CLA+ T cells**

Leukocytes stained with CHO-131 or HECA-452 have been previously used in adhesion assays involving E- and P-selectin. For instance, HECA-452 has been previously used to isolate CLA+ T cells for use in functional assays (2, 33), and CHO-131 has been shown not to disrupt leukocyte tethering to P-selectin (23). We also found that CHO-131 and HECA-452, whether alone or when complexed with second-stage reagents, did not significantly affect HL-60 cell tethering to P-selectin under shear flow conditions. However, HECA-452 when used at highly saturating concentrations of >25 μg/ml resulted in some HL-60 cell aggregation that diminished P-selectin tethering (data not shown). For the functional assays described below, T cells were stained with CHO-131 and HECA-452 at concentrations well below that which caused cell aggregation.

The P-selectin binding activity of the CHO-131+ and CHO-131− CLA+ T cell subsets (Fig. 1A) was initially examined using an in vitro shear flow assay that allows for cellular analyses under hydrodynamic conditions simulating the microvasculature (29, 30, 32). CHO-131+ and CHO-131− CLA+CD3+ T cells were first isolated from the peripheral blood of normal individuals by sorting, and then the shear resistance of each subset was assessed over
a range of shear stresses (1.5–4 dyn/cm²). During the initial incremental increases in shear stress, the number of CHO-131 CLA⁺ T cells tethering to P-selectin increased on average and then marginally declined under higher shear stress levels (Fig. 1B). In marked contrast, the number of tethered CHO-131 CLA⁺ T cells abruptly declined as shear stress increased (Fig. 1B). This disparity in adhesion behavior by the CHO-131⁺ and CHO-131⁻ CLA⁺ T cell subsets was the result of at least two distinct events. First, the tethered CHO-131 CLA⁺ T cells were more resistant to detachment. Second, free-floating CHO-131 CLA⁺ T cells demonstrated an increased attachment rate, in particular, under lower shear stress conditions (Fig. 1C). The mean rolling velocity of CHO-131 CLA⁺ T cells compared with CHO-131 CLA⁺ T cells on P-selectin was also reduced (Fig. 1D).

P-selectin binding by CHO-131⁺ and CHO-131⁻ CLA⁺CD3⁺ T cells was further examined by using soluble P-selectin/Fc in flow cytometric assays. We found that P-selectin/Fc reactivity with CHO-131⁺ CLA⁺ T cells was much greater than with CHO-131⁻ CLA⁺ T cells, which was sensitive to EDTA (Fig. 2A). Hence, consistent among the various adhesive measures assessed above was that CHO-131⁺ CLA⁺ T cells demonstrated a greater P-selectin binding activity than CHO-131⁻ CLA⁺ T cells. Despite this, we observed that PSGL-1, the primary protein scaffold of the P-selectin glycan ligand on human T cells (34, 35), was not differentially expressed by the CHO-131⁺ and CHO-131⁻ CLA⁻CD3⁺ T cell subsets (Fig. 2B).

E-selectin binding activity by CHO-131⁺ and CHO-131⁻ CLA⁺ T cells

These T cell subsets were sorted and their adhesive behavior on E-selectin assessed by the shear flow assay, as was done for P-selectin. Interestingly, we observed equivalent binding activities by CHO-131⁺ and CHO-131⁻ CLA⁻CD3⁺ T cells on adsorbed E-selectin/Fc. For instance, in Fig. 3, it is shown that the shear resistance of CHO-131⁺ and CHO-131⁻ CLA⁺ T cells was similar on E-selectin/Fc at shear stresses ranging from 1.5 to 4 dyn/cm². Even under more stringent conditions (i.e., E-selectin/Fc site density decreased up to 60%), the shear resistance of the CHO-131⁺ and CHO-131⁻ CLA⁺ T cell subsets was observed not to be significantly different (data not shown). Hence, in contrast to our studies involving P-selectin, the CHO-131⁺ and CHO-131⁻ T cell subsets did not demonstrate different E-selectin binding activities under shear flow conditions.

Enrichment of CHO-131⁺ T cells in psoriatic skin lesions

The majority of T cells in psoriasis skin lesions express CLA (36), and the dermal venules express P-selectin (37). CHO-131 reactivity was also evident in psoriatic lesions, which primarily colocalized with CD3 staining (Fig. 4A). Interestingly, HECA-452 and CHO-131 were reactive with similar proportions of CD3⁺ T cells (68.7 ± 9.0 and 65.0 ± 5.6%, respectively (mean ± SD; n = 150 CD3⁺ T cells examined for CHO-131 or HECA-452 reactivity from three separate patients)) (Fig. 4B). In the peripheral blood of healthy donors, a much smaller proportion of CD3⁺ T cells are CHO-131 positive (4.93 ± 0.97% SD (n = 11 separate donors)), indicating that the representation of CHO-131⁺ T cells in psoriatic lesions was greatly enriched. Because HECA-452 and CHO-131 were reactive with a similar proportion of CD3⁺ T cells in the inflamed skin, the accumulated CLA⁺ T cells would thus appear to be primarily CHO-131⁺, whereas in the peripheral blood of healthy donors only 46 ± 14% SD (n = 11 separate donors) of CLA⁺ T cells were CHO-131⁺. Therefore, CHO-131⁺ CLA⁺ T cells may have an advantage over CHO-131⁻ CLA⁺ T cells in their ability to traffic to at least psoriatic lesions. It is possible that CHO-131⁺ T cell accumulation in psoriatic lesions results from the induction of C2-O-sLeX expression by re-stimulated T cells, which remains to be determined. However, it has been shown that CLA⁺ T cell accumulation occurs in conjunction with evolving skin lesions rather than as an acquisition of this phenotype following T cell migration into skin (36).

Expression of skin tropic and memory markers by CHO-131⁺ T cells

The studies below focus on CD4⁺ T cells because the expression of tissue-selective trafficking phenotypes and memory phenotypes by CLA⁺ T cells has mostly involved this T cell compartment. Similar to CD3⁺ T cells, CHO-131 stained only a portion of
CHO-131+ T CELLS BIND P-SELECTIN

Discussion

C2-O-sLeX is a physiological glycan ligand for P-selectin (24, 25). CHO-131 directly detects this glycan structure and its reactivity requires the functional activity of the glycosyltransferases C2GlcNAcT-I, α2,3-sialyltransferase, and α1,3-fucosyltransferase (23, 26, 27). Of interest is that CHO-131 is reactive with only a subset of CLA+ T cells (23, 26, 27). The major focus of this report was the characterization of the P-selectin binding activity by CHO-131+ and CHO-131− CLA+ T cells to further delineate subsets of CLA+ T cells and mechanisms responsible for T cell trafficking to psoriatic skin lesions. The CHO-131− memory CD4+ T cell population was highly enriched for cells expressing the skin-associated chemokine receptor CCR9, and for the most part lacked cells expressing the gut-associated chemokine receptor CCR9 and the homing receptor α4β7, when compared with CHO-131− memory CD4+. A direct comparison of CD62L, CCR7, and CCR9 expression by CHO-131+ and CHO-131− CLA+ T cells revealed that these subsets did not vary greatly in the proportion of cells expressing these markers (Fig. 5C). Hence, CHO-131 reactivity with CLA+ T cells appears not to correspond with a particular memory cell subdivision, based on the markers assessed here.

CHO-131+ T cells accumulate within psoriatic lesions. A, Approximately 10 serial sections were cut per biopsy and the first and last sections were stained by H&E to verify that the sections were within the lesion (data not shown). Ab staining involved anti-CD3 and CHO-131 or HECA-452, as described in Materials and Methods. Isotype-matched negative control mAbs were used to evaluate levels of background staining (data not shown). Arrowheads indicate some of the CHO-131 and anti-CD3 dual-stained cells (magnification, ×200). YoYo-1, CD3, and CHO-131 staining were colorized blue, green, and red, respectively. Data from a representative patient are shown. B, Three patients with psoriasis were examined and a total of 50 CD3+ T cells per patient were assessed for either CHO-131 or HECA-452 reactivity. Each bar indicates a separate patient.

Figure 4. CHO-131+ T cells accumulate within psoriatic lesions. A, Peripheral blood lymphocytes were gated on CD4+ T cells and dual analyzed for their expression of the CHO-131 epitope and E-selectin/Fc reactivity (left dot plot) or the CHO-131 epitope and HECA-452 reactivity (right dot plot), as described in Materials and Methods. For all dot plots, the indicated Ab or chimera reactivities on the x- and y-axes represent log 10 fluorescence. B, PBLs, gated on CD4+CD45RA− memory T cells, were dual analyzed for their expression of the CHO-131 epitope and α4β7, CCR9, and CCR4 (solid lines), as described in Materials and Methods. Nonspecific Ab labeling was determined using the appropriate isotype negative control mAbs (dotted lines). C, PBLs, gated on CHO-131+ or CHO-131− CLA+CD4+ T cells, were analyzed for their expression of CD62L, CCR7, or CD27 (solid lines), as described in Materials and Methods. Nonspecific Ab labeling was determined using the appropriate isotype negative control mAbs (dashed lines). Cell staining levels were examined by flow cytometry. For all histograms, the indicated Ab reactivities on the y-axes represent log 10 fluorescence. Data from a representative healthy donor are shown.
the skin. Our findings demonstrate that the P-selectin binding activity of CHO-131 CLA- T cells was significantly greater than CHO-131- CLA- T cells by all adhesive measures assessed. Thus, CLA- T cells appear not to uniformly express P-selectin glycan ligands, as they do E-selectin glycan ligands. This contrasts with neutrophils, which are homogeneous in their expression of E- and P-selectin ligands and in their staining by HECA-452 and CHO-131 (23).

Two different functional assays were used to assess P-selectin binding by CHO-131- and CHO-131- CLA- T cells. CHO-131- CLA- T cells were found to be considerably more reactive with soluble P-selectin/Fc than CHO-131- CLA- T cells, as determined by flow cytometry. However, unlike most adhesion molecules, the selectins require shear force for their proper function (42). A caveat of using soluble selectins to determine ligand expression is that their reactivity does not always correspond with cell adhesion behaviors occurring under the conditions of shear flow (20). In shear flow assays, we observed that CHO-131- CLA- T cells tethered to P-selectin significantly better than CHO-131- CLA- T cells, as revealed by various adhesion parameters, including shear resistance, accumulation efficiency, and rolling velocities. In contrast to P-selectin binding, essentially all CLA- T cells bind soluble E-selectin/Fc (8), and the CHO-131- and CHO-131- CLA- T cell subsets did not differentially bind adsorbed E-selectin in a shear flow assay. However, we cannot rule out that in vivo CHO-131- T cells may preferentially accumulate on E-selectin expressing endothelium in the inflamed skin. The apparent equivalent E-selectin binding activity by CHO-131- and CHO-131- CLA- T cells in our in vitro assays is consistent with core 2 O-glycan modifications not being essential for T cell binding to E-selectin (20). There are also differences in E- and P-selectin glycoprotein scaffolds on T cells, in which PSGL-1 is the primary ligand for P-selectin (34, 35), whereas E-selectin is less selective (43–46).

CHO-131- and CHO-131- CLA- T cells expressed equivalent levels of surface PSGL-1, indicating that its expression did not contribute to the differential P-selectin binding by the T cell subsets. CHO-131- T cells represent a portion of the brightest CLA expressers (Figs. 1A and 5A; Ref. 23), and we cannot rule out a role for their higher levels of sLeX expression contributing to P-selectin binding. Yet this seems unlikely because we did not observe preferential binding of CHO-131- CLA- T cells on E-selectin. Moreover, Yago et al. (47) have reported that sLeX properly positioned on a core 2 O-glycan vs sLeX expression levels are critical for PSGL-1 binding by P-selectin. In addition to C2-O-sLeX, tyrosine O-sulfation of PSGL-1 is also critical for P-selectin binding (48, 49), and this may occur at different levels by the CHO-131- and CHO-131- CLA- T cell subsets. Two tyrosylprotein sulfotransferases have been identified in mammalian cells (50–52). However, unlike particular glycosyltransferases involved in the synthesis of selectin glycan ligands, tyrosylprotein sulfotransferase expression appears not to be differentially regulated (53).

Recent studies reveal a strong likelihood for diversity in the synthesis of E- and P-selectin glycan ligands by activated T cells due to their different glycosyltransferase requirements and that the regulation of these enzymes appears to occur by discrete cytokine-induced signaling pathways (20–22). For instance, C2GlcNAcT-I has been shown to be required for the synthesis of P-selectin but not E-selectin glycan ligands (20); thus quantitative differences in C2GlcNAcT-I expression levels may regulate the synthesis of E- and P-selectin glycan ligands by T cells. Yet this may be more complex, because inhibitory mechanisms also regulate O-glycan modification. The enzyme α2,3-sialyltransferase-I is an inhibitor of core 2 O-glycan synthesis, and, interestingly, its expression is differentially regulated by polarized T cells (54). Quantitative differences in the expression levels of other critical glycosyltransferases may also be important in regulating the synthesis of E- and P-selectin glycan ligands, including α2,3-sialyltransferase-IV and FucT-VII. Although FucT-VII is required for the synthesis of E- and P-selectin glycan ligands, lower levels of this enzyme have been shown to be necessary for optimal P-selectin glycan ligand biosynthesis (55).

In conclusion, our findings demonstrate a subdivision in CLA- T cells based on the expression of a high-affinity P-selectin glycan ligand. At this time, it is unclear whether the HECA-452/CHO-131 epitope disparity by T cells reflects overlapping or distinct regulatory or effector activities. Based on the expression of CCR7, CD27, and CD62L, peripheral blood CHO-131- and CHO-131- CLA- T cells appear to have an overlapping memory phenotype. CLA- T cells consist of other subdivisions as well, such as Th1, Th2, and T regulatory cells (8, 9, 12, 56), that may be enriched in CHO-131 expressers. In addition, Harrington et al. (57) have reported that T cells undergo a transient up-regulation of CD2GlucNAcT-I gene expression and core 2-brached O-glycan synthesis upon their stimulation. It will be interesting to determine whether the apparent preferential accumulation of CHO-131- T cells in psoriatic lesions is the result of recent T cell activation or due to a particular regulatory function.

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Disclosures

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