The E-selectin Ligand-1 Is Selectively Activated in Chinese Hamster Ovary Cells by the $\alpha(1,3)$-Fucosyltransferases IV and VII*

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The entry of leukocytes into inflamed tissue is controlled by cell adhesion events between leukocytes and the endothelial cells of the blood vessel wall. The selectins initiate the first transient adhesive events in this process followed by leukocyte activation, firm adhesion to the apical endothelial cell surface, and finally transendothelial migration. Each of the three known selectins (L-, E-, and P-selectin) has been shown to mediate leukocyte rolling along the blood vessel wall and to be important for leukocyte extravasation in vivo (1–7).

E-selectin is specifically expressed by cytokine-activated vascular endothelial cells and mediates the binding of neutrophils, monocytes, eosinophils, basophils, and a small subset of CD4+ T-lymphocytes. It recognizes siaIyiated and fucosylated carbohydrate structures resembling the tetrasaccharide siaIyl Lewis x (sLex)$^1$ (NeuAcα2,3Galβ1,4(Fucα1,3)-GlcNAc) or its stereoisomer siaIyl Lewis a (8). Another closely related carbohydrate epitope is defined by the antibody HECA452 and is probably involved in the binding of certain T-cell subsets to E-selectin (9).

The expression of these carbohydrate epitopes is dependent on the activity of $\alpha(1,3)$-fucosyltransferases, which catalyze the last step in the synthesis pathway. Five human fucosyltransferases have been cloned in recent years (10–14). Four of them, namely FucTIII, -V, -VI, and -VII can use $\alpha(2,3)$-sialyl-$N$-acetyllactosamine to generate the sLex$^*$ moiety in vitro (15). Consequently, transfection of each of these fucosyltransferases into mammalian cells gives rise to sLex$^*$ structures on the cell surface. In contrast, transfection with FucTIV generates different results, depending on the type of mammalian cells used (16–20). In COS cells and in CHO cells derived from Pro5 parental strains no sLex$^*$ is generated, while in CHO cells derived from DHFR$^-$ parental strains FucTIV gives rise to sLex$^*$ on the cell surface (20). The generation of sLex$^*$ structures in different non-leukocyte, mammalian cell lines correlated with the binding of the transfected cells to E-selectin. This led to the early hypothesis that many different scaffold molecules could possibly serve as functionally relevant presenters of sLex$^*$ or sLex$^*$-like structures that bind to E-selectin (21).

Despite these findings and despite the lectin character of E-selectin, very few distinct glycoprotein ligands could directly be affinity-isolated with E-selectin probes analyzing various leukocyte cell lysates. The E-selectin ligand-1 (ESL-1) was isolated by this approach as the major 150-kDa glycoprotein ligand on mouse neutrophils (22, 23). This protein was found to be expressed on many different cell types, including CHO cells, but a glycoform of ESL-1 that was capable of binding to E-selectin was only found on myeloid cells. Modification of ESL-1 with sialic acid and fucose was found to be essential for ligand activity, and antibodies against ESL-1 could partially inhibit the binding of neutrophils to E-selectin in nonstatic (rotation) adhesion assays. The P-selectin glycoprotein ligand-1 (PSGL-1) which can be affinity-isolated with a P-selectin probe (24, 25) can also be affinity-isolated with an E-selectin probe from human (26) as well as from mouse neutrophils (27). Additional glycoprotein ligands that can be isolated with an E-selectin probe but have not yet been cloned are a 250-kDa glycoprotein (reduced 230 kDa) on mouse neutrophils (27) and a 250-kDa glycoprotein on bovine y6 T-cells (28). Thus, very few distinct glycoproteins can be affinity-isolated from E-selectin-binding leukocytes. This raises the question of which mechanism determines this surprising ligand selectivity. One possibility would be that only these few ligands acquire certain carbohydrate modifications that enable them to bind to E-fucosyltransferases III–VII, CHO, Chinese hamster ovary; ESL-1, E-selectin ligand-1; PSGL-1, P-selectin glycoprotein ligand-1; mAb, monoclonal antibody; PAGE, polyacrylamide gel electrophoresis; 3′SLN, sialyllactosamine; NAL, N-acetyllactosamine.

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1 The abbreviations used are: sLe$^*$, siaIyl Lewis x; FucTIII–FucTVII, fucosyltransferases III–VII; CHO, Chinese hamster ovary; ESL-1, E-selectin ligand-1; PSGL-1, P-selectin glycoprotein ligand-1; mAb, monoclonal antibody; PAGE, polyacrylamide gel electrophoresis; 3′SLN, sialyllactosamine; NAL, N-acetyllactosamine.

2 E. Borges and D. Vestweber, unpublished observations.
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E-selectin—An adhesion receptor found on neutrophils and monocytes that mediates the recruitment of leukocytes to sites of inflammation.

E-selectin-IgG—A fusion protein created by linking the extracellular domain of E-selectin to the Fc region of an antibody.

E-selectin ligands—Molecules that interact with E-selectin, facilitating leukocyte recruitment.

Cell culture—The process of growing cells in a controlled environment for research purposes.

Antibodies—Proteins produced by the immune system to identify and neutralize pathogens.

Western blot—A technique used to separate proteins and detect specific proteins or antigens in a sample.

Fucosylation—The addition of fucose to glycoproteins, a process that influences protein function and cell adhesion.

Results—The outcomes of experiments or observations.

Different Patterns of Glycoprotein Ligands for E-selectin in the CHO Mutant Cell Lines LEC11 and LEC12—A study examining the repertoire of glycoprotein ligands for E-selectin in different mutant cell lines.

Kidney—An organ responsible for filtering blood and producing urine.

Liver—A major organ in the digestive system responsible for processing nutrients and eliminating toxins.

Pancreas—An organ that produces hormones and digestive enzymes.

Fetal calf serum—A growth promoter used in cell culture that mimics the nutritional environment of the mother.

Dulbecco's modified Eagle's medium (DMEM)—A widely used cell culture medium.

Plasmid—A small, circular DNA molecule used to carry genes into cells for the purpose of genetic modification.

Affinity—The strength of the interaction between a ligand and its receptor.

Construct—A DNA fragment that encodes a specific protein or a functional domain.

Expression plasmid—A plasmid containing a gene of interest for expression in a host cell.

Flow cytometry—A technique for analyzing and sorting cells based on their physical and chemical properties.

Cytochemistry—The study of cell structure and function using various staining techniques.

Cell lysate—A sample containing cellular extracts used for protein analysis.

N-glycan—A carbohydrate group attached to proteins through the N-glycosylation process.

FucTIII—A fucosyltransferase that adds fucose to glycoproteins.

HepG2 cells—A human hepatoma cell line commonly used in cell culture studies.

CHO cells—Chinese hamster ovary cells, a model system for protein expression.

Mature—Refers to the final, functional form of a protein after post-translational modifications.

Transfected—Cells that have been modified by the introduction of foreign DNA or RNA.

Fucose—A monosaccharide commonly added to glycoproteins, influencing their biological activity.

CHO mutants—Cells that have been selectively bred for specific characteristics.

Hybridomas—Cell lines that produce monoclonal antibodies.

Immunoblot—A method used to identify specific proteins in a sample by their size and shape.

Macrophage—A type of white blood cell that plays a role in the immune response.

Sialyltransferase—An enzyme that adds sialic acid to glycoproteins.

Avidin—A protein that binds tightly to avidin-binding sites on other proteins, often used in bioassays.

PSGL-1—A protein that binds to E-selectin.

ESL-1—A protein that binds to E-selectin.

E-selectin-IgG—A fusion protein that retains the E-selectin binding site of the antibody.

E-selectin affinity matrix—A solid support with immobilized E-selectin used for affinity purification.

Western blot analysis—A method for detecting specific proteins by their size and shape.

Immunoprecipitation—A technique for isolating specific proteins from a mixture.

Scanning laser confocal microscopy (SLM)—A microscope technique that uses laser light to produce detailed images of cellular structures.

Glycoprotein—A protein that contains carbohydrate moieties.

Mannose—A monosaccharide commonly added to glycoproteins, influencing their biological activity.

Asparagine—A common site for N-glycosylation in proteins.

ECL kit—A reagent kit used for chemiluminescent detection of proteins in Western blots.

Biosensor—An instrument that can detect the binding of molecules using a signal transduction system.

Glycolipid—A lipid that contains both a carbohydrate and a lipid moiety.

Hepadnavirus—A type of RNA virus that infects the liver and can cause hepatitis.

Fucosylated—Proteins that have been modified by the addition of fucose.

Fucosylation site—The specific location on a glycoprotein where fucose is added.

Fucosylation assay—A method for measuring the activity of fucosyltransferases in vitro.

ESL-1 affinity matrix—A solid support with immobilized ESL-1 used for affinity purification.

HPLC—High-performance liquid chromatography, a method for separating and analyzing complex mixtures.

HPLC analysis—A technique for analyzing the purity and composition of proteins.

Abundance—The relative concentration of a protein or molecule.

Table 1—A listing of the proteins isolated from LEC11 cells, showing their abundance, molecular weight, and other characteristics.
numbers of cells were analyzed. As expected, no proteins could be isolated from normal CHO cells (Fig. 1A). A similar broad range of glycoprotein ligands as in LEC11 cells was also found when we analyzed various colon carcinoma cell lines. In contrast, the result with LEC12 cells resembled that with mouse neutrophils, from which we have previously isolated ESL-1 as the major 150-kDa ligand that was isolated from LEC12 cells. When we analyzed various colon carcinoma cell lines, ESL-1 was found to be isolated from normal CHO cells (Fig. 1A). A similar broad range of glycoprotein ligands as in LEC11 cells was also found when we analyzed various colon carcinoma cell lines. In contrast, the result with LEC12 cells resembled that with mouse neutrophils, from which we have previously isolated ESL-1 as the major 150-kDa ligand that was isolated from LEC12 cells. When we analyzed various colon carcinoma cell lines, ESL-1 was found to be isolated from normal CHO cells (Fig. 1A). A similar broad range of glycoprotein ligands as in LEC11 cells was also found when we analyzed various colon carcinoma cell lines. In contrast, the result with LEC12 cells resembled that with mouse neutrophils, from which we have previously isolated ESL-1 as the major 150-kDa ligand that was isolated from LEC12 cells. When we analyzed various colon carcinoma cell lines, ESL-1 was found to be isolated from normal CHO cells (Fig. 1A).

Affinity Isolation Experiments with Fucosyltransferase-transfected CHO Cells—Since the two hamster fucosyltransferases in the two mutant cell lines have not yet been cloned and the molecular details of the mutations have not yet been fully revealed, we repeated the affinity isolation experiments as described above, using different CHO cell clones stably transfected with one of the five known human fucosyltransferases, numbered from III to VII. Cells were transfected with expression plasmids carrying the DNA sequence for the respective fucosyltransferase together with the neomycin resistance gene as selection marker. Clones resistant to G418 were analyzed in affinity isolation experiments with E-selectin-IgG as affinity probe. More than 80% of the G418-resistant clones were able to produce E-selectin-IgG precipitable glycoproteins. As shown in Fig. 2A, a large panel of glycoproteins was affinity-isolated with E-selectin-IgG from FucTIII transfected CHO cells very similar to the result with LEC11 cells (Fig. 1A). In contrast, a major ligand of 150 kDa was precipitated with E-selectin-IgG from FucTIV- and FucTVII-transfected cells, just as observed with LEC12 cells. This ligand, precipitated by E-selectin-IgG from FucTVII-transfected cells could be reprecipitated with affinity-purified antibodies against mouse ESL-1 (Fig. 2B). In addition to ESL-1, two minor higher molecular mass species of 220 and 280 kDa were found as well as a 120-kDa protein.

Affinity isolation analysis of FucTV- and FucTVII-transfected CHO cell clones revealed comparable results, although the selectivity with which the 150-kDa ligand was precipitated by E-selectin-IgG was not as distinctive as that observed with FucTIV- and FucTVII-transfected cells (Fig. 2A). The affinity isolation results depicted in Fig. 2 for each fucosyltransferase transfection are representative in each case for four or five independent clones.

The expression levels of ESL-1 in the various CHO mutants and CHO transfecants were examined by immunoprecipitations with anti-ESL-1 antibodies analyzing equal amounts of metabolically labeled cells. As shown in Fig. 3, the amounts of precipitated ESL-1 were similar for all tested clones.

Expression Levels of Fucosyltransferase Activity Are Not Responsible for the Generation of Different Patterns of Glycoprotein Ligands—Since the expression of FucTIII led to the synthesis of numerous E-selectin binding glycoproteins while FucTIV and FucTVII generated almost exclusively ESL-1 as a ligand for E-selectin, we examined whether these differences would be caused by different expression levels of the enzymes. Detection of the enzymes in immunoblots using specific rabbit antisera (obtained from John Lowe) was unsuccessful for each of the stably transfected CHO clones. This was most likely due to insufficient sensitivity, since the enzymes were only detectable when they were transiently overexpressed in COS cells (data not shown). As an alternative method, we analyzed the expression levels of the fucosyltransferases in vitro fucosyltransferase enzyme assays using detergent extracts of the transfected cells. Extract aliquots of identical protein content were analyzed, revealing comparable levels of enzyme activity for each enzyme (Fig. 4). In agreement with published data (13, 39), FucTIII containing cell lysates could utilize NAL as well as 3’S-LN as acceptor substrate, while FucTIV samples showed a clear preference for the nonsialylated neutral acceptor NAL (the precursor of Le*) and FucTVII preferentially fucosylated...
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**Fig. 2. Affinity isolation of E-selectin-binding proteins from fucosyltransferase-transfected CHO cells with E-selectin-IgG.** A, equal numbers of CHO cells transfected with FucTIII (CHO-III), FucTVII (CHO-VII), FucTV (CHO-V), and FucTIV (CHO-IV) were labeled as in Fig. 1, and detergent extracts were incubated with protein A-Sepharose loaded either with human IgG (IgG) or with E-selectin-IgG (E-Sel-IgG). Beads were washed, and specifically bound proteins were eluted with EDTA, electrophoresed on a 6% polyacrylamide gel under reducing conditions, and detected by fluorography. Samples of the FucTIV-transfected cells were electrophoresed on a separate gel. For each transfection, four to five independent clones were analyzed, giving similar results as the one which is shown. B, FucTVII-transfected CHO cells were subjected to affinity isolation with human IgG (IgG) or E-selectin-IgG (E-Sel.-IgG), and EDTA-eluted proteins from the E-selectin-IgG matrix were either directly electrophoresed or subjected to reprecipitations either with IgG of a rabbit nonimmune serum (re.control) or with affinity-purified IgG of the rabbit anti-E-Sel-1 serum 65 (re.α-Sel-1). Specifically bound proteins were eluted with SDS-PAGE loading buffer, electrophoresed, and visualized as in A. Molecular mass markers (in kDa) are indicated on the left.

**Fig. 3.** All analyzed CHO mutants and CHO transfectants express similar levels of ESL-1. The cells that were analyzed in Figs. 1 and 2 were metabolically labeled as described, and identical cell numbers were subjected to immunoprecipitations with affinity-purified antibodies from the rabbit anti-ESL-1 antiseraum 65. Precipitated proteins were electrophoresed on a 6% polyacrylamide gel and visualized by fluorography. Molecular mass markers (in kDa) are indicated on the left.

**Fig. 4.** α(1,3)-fucosyltransferase activity of CHO transfectants. α(1,3)-fucosyltransferase enzyme assays were performed with detergent extracts of CHO cells transfected with FucTIII (CHO-III), FucTIV (CHO-IV), or FucTVII (CHO-VII). NAL or 3'-SLN was used as acceptor substrates. In each case, measurements of transfected clones (open bars) were compared with negative control measurements performed with pcDNA3 mock-transfected CHO cells (closed bars). The transfected clones were identical with the ones depicted in Fig. 2. For each transfection, three independent clones were analyzed giving similar results as the one depicted here. Data shown correspond to fucosyltransferase activities (expressed as pmol/min/mg) measured with samples containing acceptor substrate minus activities measured without acceptor substrate and represent the mean and standard deviation from three replicate assays.

In vitro expressed different levels of enzyme activity. As determined by enzyme assays, FucTIV-transfected CHO clone 9 expressed 6 times higher levels of FucTIV than clone 7. However, as illustrated in Fig. 5, there was no difference in the glycoprotein pattern that could be isolated from both clones with E-selectin-IgG. In both clones, ESL-1 was the major isolated ligand. The only observed difference was the increased overall level of isolated material in clone 9 versus clone 7.

**ESL-1 Is a Major Carrier for HECA452 Epitopes in FucTIV- or FucTVII-expressing CHO Cells—** We wanted to examine whether the surprising selectivity with which ESL-1 was generated as an E-selectin ligand in FucTIV and FucTVII expressing CHO cells was indeed based on the selective generation of certain carbohydrate modifications on this protein. The monoclonal antibody HECA452 was described as recognizing carbohydrate epitopes that can be recognized by E-selectin and that are related to but not identical with sLex (8, 33–36). All FucT-III-transfected CHO clones were positive for HECA452 as well as for the anti-sLex antibody CSLEX-1, as was analyzed by flow cytometry (Fig. 6). Probing immunoprecipitates of ESL-1 from CHO cells transfected with FucTIII, FucTIV, or FucTVII in immunoblots with the HECA452 antibody revealed that ESL-1 was positive for the HECA452 carbohydrate epitope in each of the acidic acceptor 3'-SLN (the precursor of sLeα). Based on these measurements, the FucTIII-transfected cells did not express higher specific activities for the transfer of fucose to NAL or to 3'-SLN than the FucTIV or FucTVII transfected, respectively. The results depicted in Fig. 4 were obtained with the same clones that were analyzed by affinity isolation with E-selectin-IgG (Fig. 2). Thus, differences in the expression level of the various fucosyltransferases, as far as they can be determined in in vitro enzyme assays, are unlikely to be the reason for the different patterns of E-selectin-binding glycoproteins in the various transfectants.

This was also verified by analyzing different CHO clones that were transfected with the same fucosyltransferase but expressed different levels of enzyme activity. As determined by in vitro enzyme assays, FucTIV-transfected CHO clone 9 expressed 6 times higher levels of FucTIV than clone 7. However, as illustrated in Fig. 5, there was no difference in the glycoprotein pattern that could be isolated from both clones with E-selectin-IgG. In both clones, ESL-1 was the major isolated ligand. The only observed difference was the increased overall level of isolated material in clone 9 versus clone 7.
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the transfectants, while ESL-1 in mock-transfected CHO cells was negative (Fig. 7). Immunoblot analysis with CSLEX-1 did not generate conclusive results, since signals were almost undetectable even in cell extracts of the sLe^a-expressing human monocytic cell line HL60 (not shown).

When total cell extracts of the same CHO clones were analyzed in immunoblots, FucTIII transfectants were found to express a large panel of glycoproteins that were positive for HECA452 (Fig. 8). In contrast, in FucTIV- and FucTVII-expressing CHO cells, a 150-kDa glycoprotein was detected as the major carrier for HECA452-reactive epitopes (Fig. 8).

In order to examine whether this 150-kDa protein would be reactive with anti-ESL-1 antibodies, we depleted cell extracts of the FucTIII and FucTVII transfectants for the ESL-1 glycoprotein by two rounds of incubations with protein A-Sepharose beads bearing anti-ESL-1 antibodies. Mock depletions were performed with nonimmune IgG. Depleted cell extracts were analyzed in immunoblots with HECA452 antibody. As shown in Fig. 9, the 150-kDa glycoprotein reactive for HECA452 was specifically removed by anti-ESL-1 antibodies. In FucTIII transfectants, only this 150-kDa protein and no other HECA452-reactive glycoprotein was removed by anti-ESL-1 antibodies (Fig. 9). We conclude that it is ESL-1 that is selectively decorated with HECA452 epitopes in FucTVII-transfected CHO cells.

DISCUSSION

In this study we show that different \( \alpha(1,3) \)-fucosyltransferases, when expressed in CHO cells, generate different repertoires of E-selectin-binding glycoprotein ligands. While FucTIII generates a large panel of glycoprotein ligands, among which the hamster equivalent of mouse ESL-1 is just one, FucTIV and FucTVII almost exclusively modify ESL-1 in a way that allows it to bind to E-selectin. The same selectivity was seen for the generation of the carbohydrate epitope HECA452. These data demonstrate that FucTIV and FucTVII, known to be expressed in myeloid cells, are indeed very selective in choosing acceptor glycoproteins that they can transform into E-selectin ligands.

Our analysis of FucTIII-expressing CHO cells shows that simply expressing this enzyme is sufficient to transform numerous glycoproteins into E-selectin “ligands,” which bind with sufficient strength to allow affinity isolation. This suggests that \( \alpha(1,3) \)-fucosylation of carbohydrate side chains that are found on many different proteins is sufficient to generate high affinity recognition epitopes for E-selectin. Although possible, it is unlikely that all of these proteins share additional structural elements that are necessary for E-selectin binding. This would argue for the hypothesis that high affinity binding does not necessarily require additional structural elements besides certain carbohydrate components. This is in agreement with the work of Patel et al. (40), who showed that certain tetraantennary carbohydrate compounds that contained a di-sLex structure on one branch could be affinity-isolated by an E-selectin affinity matrix out of a mixture of all carbohydrate side chains that had been released by hydrazinolysis from plasma membrane glycoproteins of human myeloid cells. These results indicate that carbohydrate moieties can bind with high affinity to E-selectin in the absence of any other structural element.

Expression of each of the five fucosyltransferases in CHO cells gave rise to the expression of CSLEX-1- and HECA452-reactive epitopes on the cell surface. The FucTIV was originally characterized as a fucosyltransferase that generates E-selectin ligand activity in transfected cells and was therefore named ELFT for E-selectin ligand fucosyltransferase (16). However, it was found later that FucTIV-transfected COS and CHO cells did not bind to E-selectin and did not display CSLEX-1-reactive epitopes on the cell surface. In contrast, FucTIV facilitated the isolation of carbohydrate epitopes that were not able to metabolize sialylated type II acceptor carbohydrate structures (17, 18). Although possible, these contradictory results were then partly explained when Goelz et al. (20) found that FucTIV can generate CSLEX-1-reactive epitopes only in DHFR \(^+\) CHO DUKX B1 cells and not in CHO cell clones derived from Pro\(^-\) parental strains. Since we have used CHO

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**Fig. 5. Different levels of in vitro fucosyltransferase activity do not alter the substrate specificity of transfected fucosyltransferase.** Detergent extracts of two FucTIV-transfected CHO cell clones (CHO-IV, clone 7 and clone 9) were analyzed and compared for their in vitro fucosyltransferase activity (FT-activity) as well as for their content of E-selectin IgG-precipitable glycoproteins. A value of 100% was assigned to the fucosyltransferase activity that was measured for clone 9, using the acceptor NAL. E-selectin-binding proteins isolated from identical numbers of cells were electrophoresed on a 6% polyacrylamide gel and visualized by fluorometry. Note that the relative enzyme activities of clones 7 and 9 correlated with the amount of precipitable proteins from the respective cell lines but did not influence the pattern of E-selectin-binding glycoproteins.

**Fig. 6. Flow cytometry of fucosyltransferase transfectants with mAbs CSLEX-1 and HECA452.** CHO cells transfected with FucTIII (CHO-III), FucTIV (CHO-IV), or FucTVII (CHO-VII) were analyzed by flow cytometry with the mAbs CSLEX-1 and HECA452, as indicated. Dark shaded plots represent pDNA3 mock-transfected CHO cells, unshaded plots represent fucosyltransferase transfectants. First, antibodies were detected with fluorescein isothiocyanate-labeled anti-mouse IgM or fluorescein isothiocyanate-labeled anti-rat IgM antibodies.
markers (in kDa) are indicated on the left.

The 150-kDa major HECA452-reactive antigen in FucT-VII transfectants can be removed from cell extracts with anti-ESL-1 antibodies. Detergent extracts of CHO cells, transfected with FucTIII (CHO-III), FucTIV (CHO-IV), or FucT VII (CHO-VII), using affinity-purified antibodies from the anti-ESL-1 rabbit antiserum 65, electrophoresed on 6% polyacrylamide gels, transferred onto nitrocellulose filters, and analyzed in immunoblots with mAb HECA452 or with affinity-purified antibodies against ESL-1 (α-ESL-1) from antiserum 89060, as indicated. The additional band at 120 kDa, which reacts with anti-ESL-1 antibodies, most likely is a breakdown product of ESL-1. Molecular mass markers (in kDa) are indicated on the left.

What determines the selectivity with which FucTIV or FucT VII generate the HECA452 carbohydrate epitope on ESL-1 but not on most other glycoproteins although many of them are able to acquire this epitope upon expression of FucTIII? Two possibilities exist. The first is that FucTIV and FucTVII could fucosylate many different cellular proteins but only ESL-1 could carry acceptor structures that allow the generation of the HECA452 epitope. Indeed, FucTIV and FucT VII only accept type II substrates where they fucosylate GlcNAc in the α(1→3)-position, while FucTIII accepts type II and type I substrates, i.e. can add fucose in position 3 as well as in position 4. The selective generation of HECA452 epitopes could thus be based on the selective appearance of type II acceptor-like carbohydrate modifications on ESL-1. However, such a selective expression pattern of type II acceptor structures on only very few proteins has not been described. Alternatively, it could be FucT IV and FucT VII that selectively modify ESL-1 and very few other glycoproteins. This selective interaction could be based on structural elements on ESL-1 that are favorable for the interaction with FucTIV or FucT VII, or it could be based on the specific distribution of fucosyltransferases in subdomains of the Golgi membranes. FucTIII might be more broadly distributed, while FucTIV and FucT VII could be preferentially located in subdomains of the Golgi membranes that are passed by only a few glycoproteins and preferentially by ESL-1. Thus the specificity of fucosylation would be based on a “targeting” mechanism. Such a hypothesis is testable now.

Like ESL-1, PSGL-1 can be selectively precipitated with E-selectin-Ig from detergent extracts of myeloid cells (24, 27). In analogy to our results for ESL-1 it might be possible that this ligand is also selectively glycosylated. Indeed, Wilkins et
al. (41) have shown recently that PSGL-1 from HL60 cells carries two species of fucosylated O-glycans that were not found on the sialomucin CD43 purified from the same cells.

In summary, our data show that the remarkable selectivity with which E-selectin binds to ESL-1 from mouse neutrophils can be mimicked in CHO cells transfected with fucosyltransferases from myeloid cells. While our data do not rule out that ESL-1, besides specific carbohydrate modifications, might also carry other structural elements that are directly involved in the recognition by E-selectin, they suggest that ESL-1 is a selective target for the generation of carbohydrate structures that are necessary for the binding to E-selectin.

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