Glycosylation Might Provide Endothelial Zip Codes for Organ-Specific Leukocyte Traffic into Inflammatory Sites

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Inflammatory diseases are characterized by the leukocyte infiltration into tissues. L-selectin on lymphocytes and its endothelial glycosylated ligands are instrumental in the initiation of lymphocyte extravasation. Immunohistochemical stainings with monoclonal antibodies against functionally active glycan-decorated L-selectin ligands, ie, sialyl-Lewis x (sLex, 2F3, and HECA-452) or sulfated extended core 1 lactosamine (MECA-79), were performed on more than 400 specimen representatives for thyroiditis, myocarditis, psoriasis, vasculitis, ulcerative colitis, and their corresponding noninflamed tissues. The endothelial expression of sLex or sulfo sLex glycans in postcapillary venules was either absent or low in control tissues. The *de novo* induction of endothelial expression of sLex or sulfo sLex glycans was detected in all inflamed tissues. Furthermore, each organ carried its own modification of sLex or sulfo sLex glycans, ie, zip code. Our results suggest that these zip code glycans may provide means for organ selective leukocyte traffic that could be used in selective leukocyte traffic inhibition. (Am J Pathol 2002, 161:543–550)

Inflammatory diseases, such as acute kidney and heart allograft rejections or bronchial asthma, are characterized by a heavy infiltration of lymphocytes and monocytes, but not of granulocytes, leading finally to necrotic changes at the inflammatory site.^{1–4} The recruitment of lymphocytes across the microvascular postcapillary endothelium^{5–7} is a complex cascade, taking its origin from the initial tethering and rolling of leukocytes that are mediated by the members of the selectin family and their glycosylated ligands. These interactions are followed by a chemokine-mediated activation of integrins, leading to

a firm adhesion of rolling leukocytes and, subsequently, to transendothelial migration into inflammatory sites.^{8,9}

The tethering and rolling phases of lymphocyte extravasation have been proposed to be initiated by L-selectin. This molecule recognizes endothelial ligands, such as CD34, podocalyxin, MAdCAM-1, and sgp200, provided that these ligands are decorated with α 2,3-sialylated, α 1,3-fucosylated, and sulfated lactosamines.^{1,5–7,10–12} These sulfated sialyl Lewis x (sLex) glycans are necessary for the L-selectin-mediated binding of lymphocytes to endothelium and are constantly expressed at least on the CD34 glycans of lymph node high endothelium.¹³ Under normal conditions, the flat endothelium in parenchymal organs does not express properly glycosylated modifications of L-selectin ligands¹⁴ and no extravasation occurs. However, the induction of sLex- or sulfo sLex-decorated L-selectin ligands onto the postcapillary microvascular endothelium occurs both in rodents and humans undergoing heart and kidney allograft rejection and also in the early specimens taken from bronchial asthma patients.^{2–4,14} Moreover, the enzymatically synthesized multivalent sLex glycans can prevent the selectin-dependent lymphocyte adhesion to properly glycosylated endothelium *ex vivo*, pointing out a putative mean to inhibit the inflammatory reaction.^{14,15}

Here we expand these previous observations and analyze the endothelial sLex or sulfo sLex expression patterns in various inflammatory diseases. For this purpose, a large series of histological biopsies (>400) from patients with ulcerative colitis, psoriasis, thyroiditis, myocarditis, and vasculitis and their corresponding noninflamed tissues were collected. The endothelium of control noninflamed tissues did not express essentially any sLex or sulfo sLex epitopes whereas on the endothelium of inflamed organs they were prominently expressed. Most importantly, the up-regulated sLex or sulfo sLex expression patterns of inflamed tissues were clearly different between every organ analyzed, and when combined with

Supported in part by research grants from the Academy of Finland and the Technology Development Center, Helsinki; the Sigrid Juselius Foundation; grants from the Helsinki University Central Hospital Fund; and a grant from the Heart Association, Helsinki, Finland.

Accepted for publication May 2, 2002.

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Table 1. Monoclonal Antibodies Used in this Study

mAb	Concentration	Epitope recognized
7C7	1.2 μ g/ml	Control for murine IgM antibodies
TIB-146	10 μ g/ml	Control for rat IgM antibodies
2F3	0.6 μ g/ml	Sialyl Lewis x, 6-, 6'-, 6, 6'-sulfo sialyl Lewis x
HECA-452	2 μ g/ml	Sialyl Lewis x
MECA-79	1 μ g/ml	Extended core 1 6-sulfated lactosamine
CD34	2 μ g/ml	CD34 glycoprotein

our previous data, it could be postulated that every inflamed organ expressed its own specific endothelial sLex or sulfo sLex zip code regulating lymphocyte traffic to a given organ. This thorough analysis of the endothelial sLex and sulfo sLex expression patterns in inflammatory diseases should warrant the more detailed analysis of the authentic glycosylations on the relevant endothelial glycoproteins acting as L-selectin ligands.¹³

Materials and Methods

Histological Specimens

Formalin-fixed, paraffin-embedded archived blocks of normal and diseased human tissues and corresponding slides were obtained from the Department of Pathology, Helsinki University Central Hospital. The selection of human samples from archives was based on the primary pathological reports; all specimens were also re-evaluated independently to be representative for a given type of inflammation or serve as relevant controls of normal tissues by three observers (JR, OT, and TP). The ethical committee of the Helsinki University Central Hospital had approved the study plan.

Altogether, nearly 1000 histological specimens were identified from the records of the Department of Pathology and screened through for further study. One hundred twenty-two histological specimens with lymphocytic thyroiditis, 130 with ulcerative colitis, 126 with psoriasis, 108 skin biopsies with vasculitis, and 57 with myocarditis were screened, and 50 cases from each disease were chosen for the study. As control specimens, 131 samples with goiter, 150 samples with noninflammatory colon, 98 samples of normal skin, and 20 samples with myocardial hypertrophy or myofibrosis were screened, and 30 cases from each organ were selected.

Immunohistochemistry

The glycans on selectin ligands were identified by immunohistochemistry with three different monoclonal antibodies (mAbs) each recognizing specific glycosylation modifications (Table 1). mAb HECA-452¹⁶ (rat IgM) and mAb MECA-79¹⁷ (rat IgM) were kindly provided by Dr. S. Jalkanen (University of Turku, Turku, Finland). mAb 2F3¹⁸ (IgM) was from Pharmingen (San Diego, CA). As a positive control for the detection of endothelial cells, anti-

human CD34, class II (mIgG1) (1:25; DAKO, Glostrup, Denmark) and as negative controls mAbs 7C7 (mouse IgM) and TIB-146 (rat IgM), both kindly provided by Dr. S. Jalkanen, were used. For immunohistochemical analysis, previously described protocols was used.^{3,4} In short, tissue specimens originally embedded in paraffin were cut into 5- μ m sections, deparaffinized, and rehydrated, followed by a microwave pretreatment and a serum blocking. Vector Vecstain IgG mouse and rat kits were used for immunoperoxidase stainings according to the manufacturer's instructions (Vector Laboratories, Inc., Burlingame, CA).

Every CD34⁺ vessel from a field of view using $\times 400$ magnification was calculated and because the size of this field was known the number of vessels was converted to vessels/mm². Ten to 20 high-power fields were analyzed and the mean $\pm$ SEM was calculated. The intensity of the staining was scored either positive or negative for each vessel. After determining the number of mAb 2F3-, HECA-452-, or MECA-79-reactive vessels this was divided by the number of CD34⁺ vessels from the same specimens yielding the percentage of sLex- or sulfo sLex-reactive vessels. Only the small capillaries/venules were included because the arteries or major vessels did not react with the antibodies used.

Statistical Analysis

Fifty cases from inflamed specimens and 30 cases from specimens with normal histology entered the statistical analysis. The results were first analyzed by the Kruskal-Wallis one-way analysis of variance by ranks. This test is basically a comparison of medians of several unpaired groups and the null hypothesis is that the medians are equal; *P* values < 0.05 were considered as significant. The Kruskal-Wallis test indicated that we could use the nonparametric Mann-Whitney *U*-test for the multiple comparisons of medians in control and inflammation groups. The null hypothesis is that the medians are equal. In the Mann-Whitney *U*-test, differences were considered to be significant at *P* < 0.05 .

Results

The expression of an endothelial CD34 was analyzed in all specimens for two reasons. First, mAb CD34 was used as a marker to determine the number of vessels/mm², because this molecule has been shown to be expressed on all vascular endothelia, and second, because the CD34 glycoprotein is one of the prime protein ligands for L-selectin carrying sulfo sLex glycans. In control specimens representing noninflamed thyroid, heart, and colon, the number of CD34⁺ vessels was $86 \pm 3.0/\text{mm}^2$, $111 \pm 1.6/\text{mm}^2$, and $91 \pm 4.2/\text{mm}^2$, respectively (mean $\pm$ SEM) (Figure 1), whereas the vascularity in noninflamed skin biopsies was only $34 \pm 1.8/\text{mm}^2$. The numbers of anti-CD34⁺ vessels were modified only marginally in the inflammatory tissues when compared to their control noninflamed tissue samples ($107 \pm 2.0/\text{mm}^2$ in thyroiditis,

Figure 1. The number of postcapillary vessels/mm² in control and inflammatory tissue specimens. The expression of endothelial CD34 glycoprotein in thyroid gland, heart, skin, and colon specimens with noninflammatory histology (gray bars) and with the signs of inflammation (black bars).

99 ± 1.8/mm² in myocarditis, 87 ± 2.9/mm² in colitis, 47 ± 1.9/mm² in psoriasis, 49 ± 2.6/mm² in vasculitis).

High endothelial venules (HEVs) in lymph nodes express specific sulfo sLex-decorated versions of L-selectin ligands, such as CD34 glycoprotein, fulfilling the most if not all of the requirements for L-selectin recognition. However, the same endothelial protein backbone is not properly glycosylated in any other organ under normal conditions. Because there is not a single antibody available to recognize all known glycodecorations of L-selectin ligands, we needed to probe the differently decorated L-selectin ligands with a panel of mAbs. The reacting epitopes of antibodies used in this study are shown in Table 1. The original antigens in the immunization yielding mAbs 2F3 and HECA-452 sLex decorated glycolipids and stromal components of human lymph nodes, respectively.^{16,18} mAbs 2F3 and HECA-452 detect α2,3-sialylation and α1,3-fucosylation of lactosamine, but they do not distinguish between sulfated and nonsulfated epitopes. MECA-79 was originally defined to recognize an antigen-entitled peripheral lymph node addressin (PNAd).^{17,19} This is actually a family of glycoproteins, most of which are able to bind L-selectin. In humans only CD34 and podocalyxin have been identified at the molecular level among the glycoprotein species reacting with MECA-79.²⁰ Later the recognizing epitope of MECA-79 was shown to recognize an extended sulfated core 1 lactosamine structure. This antibody does not recognize α2,3-sialylation or α1,3-fucosylation, but is however able to block *in vitro* and *in vivo* lymphocyte homing to murine lymph nodes.^{17,19}

The sLex moiety and its sulfated forms are of importance in the extravasation process leading to leukocyte invasion into different human tissues. To assess whether distinct inflammatory diseases differ from each other in the relation to their endothelial glycosylation, the profiles of 50 specimens representing inflammation and 30 control specimens from five different noninflammatory conditions were studied by immunohistochemistry using two different sLex mAbs and one sulfation-dependent mAb with relevant controls.

Thyroid Gland

In the thyroid gland (Figure 2; A to F), the anti-sLex antibody mAb 2F3 did not react with the endothelium in control goiter specimens with noninflammatory histology. As noninflamed controls, both the diffuse and multinodular types of thyroid hyperplasias but not adenomas were included. In thyroiditis specimens, the percentage of mAb 2F3-reactive vessels was significantly increased to 26.3 ± 2.4% (mean ± SEM) (Figure 3A) when compared to control specimens ($P < 0.001$, Mann-Whitney *U*-test). It should be noted that only capillaries and venules expressed sulfo sLex-decorated L-selectin ligands whereas arteries or arterioles did not.

The second anti-sLex antibody used, mAb HECA-452, reacted positively only in 2.1 ± 0.9% of vessels in goiter specimens with noninflammatory histology. Again, a strong induction in the endothelial mAb HECA-452 reactivity was found in thyroiditis specimens (32.8 ± 2.4%) and this increase in the expression level of mAb HECA-452 positively reactive endothelia was significant when compared to controls ($P < 0.001$, Mann-Whitney *U*-test) (Figure 2, A to F, and Figure 3A).

To analyze the sulfated variants of functionally active L-selectin ligands on the endothelium, the antibody recognizing the extended core 1 polylactosamine structure, mAb MECA-79, was used. Although only 0.6 ± 0.3% of the vessels reacted positively with mAb MECA-79 in specimens with noninflamed histology, a strong endothelial reactivity was observed in thyroiditis specimens (37.5 ± 2.3%) ($P < 0.001$, Mann-Whitney *U*-test).

Heart

In myocarditis, a similar analysis of the endothelial expression of various glycosylation variants was performed (Figure 2; G to L). Although specimens with noninflamed myocardium remained essentially negative with all of the three antibodies used (Figure 3B), myocarditis lesions showed some slight, but yet significant endothelial up-regulated expressions of sLex epitopes, detected with mAb HECA-452, rising from 0.0 ± 0.0% to 2.5 ± 0.9% (mean ± SEM, $P = 0.017$).

Skin

The control noninflamed skin specimens expressed a small proportion of positively stained sulfo sLex vessels (Figure 2, mol/L-R), especially when detected with mAb HECA-452 (9.2 ± 1.3%, mean ± SEM) (Figure 3C). In the psoriasis specimens with active skin lesions, the endothelial reactivity with mAb HECA-452 (24.6 ± 1.3%) was significantly elevated ($P < 0.001$, Mann-Whitney *U*-test). Concomitantly, the endothelial reactivity with mAb 2F3 remained totally negative.

In the skin biopsy specimens with vasculitis, the mAb 2F3 and MECA-79 reactivities remained essentially at a background level, and the staining with mAb HECA-452 was practically not altered either (9.2 ± 1.3% to 11.8 ± 1.2%).

Colon

In the colon, the endothelial sulfo sLex expression was already present in control specimens with noninflamed histology (Figure 2, S-X). However, the endothelial expression levels analyzed with all three mAbs were even more enhanced in colitis specimens. The percentage of mAb 2F3-expressing vessels increased from $2.6 \pm 0.5\%$ to $12.8 \pm 1.4\%$, the mAb HECA-452-reactive vessels from $7.5 \pm 1.4\%$ to $15.8 \pm 1.7\%$, and mAb MECA-79 from $1.0 \pm 0.4\%$ to $11.0 \pm 1.1\%$ (mean $\pm$ SEM) (Figure 3D). All these elevations in the expression of endothelial sulfo sLex variants were statistically significant between the noninflamed controls and the inflammatory specimens ($P < 0.001$ for each pair, Mann-Whitney *U*-test).

Putative Endothelial Zip Codes

In addition to the *de novo*-induced or -enhanced endothelial sLex or sulfo sLex expression shown in this study, we propose that each organ also possessed its own sLex- or sulfo sLex-specific zip code. A zip code could be determined by combining the percentages of reactive vessels for each of the three mAbs used, as shown in Table 2. Thus the glycosylation zip code for endothelium in thyroid gland would be 00-02-00 during noninflamed conditions and 26-33-38 during thyroiditis. Similarly the sulfo sLex-specific zip code for heart endothelium is normally 00-00-00, 00-003-00 during mild myocarditis and 38-25-45 during severe allograft rejections (Table 2).

Discussion

The present study with more than 400 histological specimens from inflammatory diseases and their relevant noninflamed control tissues showed that control specimens did not essentially express sulfoglycans when detected by a panel of mAbs 2F3, HECA-452, and MECA-79. In contrast, the relevant tissue specimens obtained from the inflammatory sites from patients with thyroiditis, myocarditis, psoriasis, vasculitis, or ulcerative colitis showed significant increases of the endothelial expressions of the glycan decorations being detectable with one, two, or all three mAbs 2F3, HECA-452, and MECA-79. Because this triplet is a representative of functionally active L-selectin

ligands, we suggest that the *de novo*-induced glycans expressed on endothelial glycoproteins could be of importance and involved both in the lymphocyte traffic and in the generation of lymphocytic infiltration, the two common hallmarks for all of these inflammatory conditions.

These observations are well in line with previously published data and extend further the earlier results with both rodent and human inflammatory lesions, such as allograft rejection.^{2,4,14,19,21} In rat kidney and heart allograft studies, the venous endothelium expressed *de novo* sLex glycans at the onset of rejection. This phenomenon did presumably promote the lymphocyte extravasation into tissue parenchyma as shown with anti-sLex antibodies, the L-selectin-IgG fusion protein, as well as with the *ex vivo* Stamper-Woodruff lymphocyte-binding assay.^{14,15,22} In addition, after synthesizing multivalent sLex poly(lactosamine) glycans with different organ specificities, we were able to show that these extended sialyl diLex glycans were able to inhibit lymphocyte binding to heart endothelium with 10-fold lower IC_{50} values when compared to the lymphocyte binding to lymph node endothelium in the same animals.²³ Thus, the manipulation of the regulation of sLex ligand expression could provide means to a site-specific immunosuppression, by inhibiting lymphocyte traffic only to a given organ, but not interfering the common normal host immunity in lymph nodes.

For this study, we selected five different inflammatory diseases and compared the endothelial expression of sLex- and sulfo sLex-decorated L-selectin ligands in inflamed tissues to their noninflamed counterparts. In lymphocytic thyroiditis, there is a marked and intense infiltrate of lymphocytes and plasma cells in thyroid gland that finally and virtually ends up in replacing the actual thyroid parenchyma and, thus, the histology of a late-stage disease may resemble the histology of lymphatic tissue. Myocarditis is defined as an inflammatory involvement of the heart muscle characterized by a leukocyte infiltrate and the necrosis or degeneration of myocytes. In psoriasis, the most typical lesions are well-demarcated pink plaques, which have a characteristic marked epidermal thickening and a superficial lymphocytic infiltrate, but the most diagnostic sign is the great number of neutrophils forming small aggregates. The term vasculitis is applied to any blood vessel inflammation. The classic

Figure 2. The histological distribution of sulfo sLex-decorated L-selectin ligands in both noninflamed and inflamed tissues of various organs. Thyroid gland: **A**, mAb 7C7, a mouse IgM control mAb, showed no endothelial reactivity in noninflamed thyroid gland; **B**, mAb TIB-146, a rat IgM control mAb, showed no endothelial reactivity in thyroiditis; **C**, anti-CD34 antibody showing a strong positive reaction with the endothelium of all capillaries and venules within a specimen with histological signs of thyroiditis; **D**, anti-sLex mAb, mAb 2F3, showing endothelial reactivity in thyroiditis; **E**, mAb HECA-452 stained strongly capillaries in thyroiditis; **F**, a vascular endothelial reactivity with mAb MECA-79 in several vessels in the inflammatory focus of thyroiditis. Heart: **G**, mAb 7C7, a mouse IgM control mAb, showed no endothelial reactivity in a heart specimen with noninflamed histology; **H**, mAb TIB-146, a rat IgM control mAb, showed no endothelial reactivity in myocarditis; **I**, anti-CD34 antibody showing a strong positive reaction with the endothelium of all capillaries and venules within a specimen with noninflamed histology; **J**, anti-sLex mAb, mAb 2F3, showing no endothelial reactivity in myocarditis; **K**, mAb HECA-452 stained strongly a small proportion of capillaries during myocarditis; **L**, mAb MECA-79 showed no endothelial reactivity in the mild myocarditis. Skin: **M**, mAb TIB-146, a rat IgM control mAb, showed no endothelial reactivity in a skin specimen with noninflamed histology; **N**, mAb 7C7, a mouse IgM control mAb, showed no endothelial reactivity in psoriasis; **O**, anti-CD34 antibody showing a strong positive reaction with the endothelium of capillaries and venules within psoriasis; **P**, anti-sLex mAb, mAb 2F3, showing no endothelial reactivity in a psoriatic lesion with local; **Q**, mAb HECA-452 stained strongly capillaries within a skin specimen with histological signs of psoriasis; **R**, no endothelial reactivity with mAb MECA-79 shown in the inflammatory focus of vasculitis. Colon: **S**, mAb TIB-146, a rat IgM control mAb, showed no endothelial reactivity in a colon specimen with noninflamed histology; **T**, mAb 7C7, a mouse IgM control mAb, showed no endothelial reactivity in a ulcerative colitis specimen; **U**, anti-CD34 antibody showing a strong positive reactivity with the endothelium of capillaries and venules within a colon specimen with noninflamed histology; **V**, anti-sLex mAb, mAb 2F3, showing a strong endothelial reactivity in a colitis specimen; **W**, mAb HECA-452 stained strongly a proportion of capillaries during colitis; **X**, a vascular endothelial reactivity with mAb MECA-79 shown in several vessels in the inflammatory focus of colitis. Original magnifications: $\times 200$ (**A**, **C**, **F**, **G**, **H**, **K**, **L**, **N**, **P**, **T**, **W**, **X**); $\times 100$ (**B**); $\times 400$ (**D**, **E**, **I**, **J**, **M**, **O**, **Q**, **R**, **S**, **U**, **V**).

Figure 3. The percentages of vessels expressing sulfo sLex epitopes analyzed with two anti-sLex antibodies, mAbs 2F3 and HECA-452, and an anti-sulfated extended core 1 lactosamine antibody, mAb MECA-79 in various organs. In the Mann-Whitney *U*-test, differences between the expression of sulfo sLex decorations in control and inflamed specimens were considered significant at $P < 0.05$ and marked with an **asterisk**. **A:** Thyroiditis. **B:** Myocarditis. **C:** Psoriasis and vasculitis. **D:** Colitis.

Table 2. The Organ-Specific Endothelial Zip Codes

	2F3-HECA-MECA		Origin of biopsies
	Normal	Inflamed	
Skin	02-09-06	00-25-15 01-12-02	Psoriasis Vasculitis
Thyroid gland	00-02-00	26-33-38	Thyroiditis
Heart	00-00-00	00-03-00 38-25-45	Myocarditis Fulminant heart allograft rejection
Colon	03-08-01	13-16-11	Ulcerative colitis
Lungs	00-00-00	05-05-06	Bronchial asthma

First two digits represent the percentages of mAb 2F3-reactive vessels, second two digits for mAb HECA-452-reactive vessels, and third two digits for mAb MECA-79-reactive vessels. Data pooled from this study as well as from our earlier works.^{3,4,19}

histological feature is an aggregation of neutrophils within and about venules, which can in severe cases lead to a lymphocyte infiltration of venule walls. Ulcerative colitis is a common recurrent disease expressing both active inflammatory ulceration areas and healing areas, both showing a microscopic evidence of inflammation. Histologically, the active phase is characterized by crypt abscesses and ulcerations extending down to the muscular layer and surrounded by a prominent mucosal infiltrate of inflammatory cells.²⁴

Recently, a novel sulfotransferase (HEC-GlcNAc6ST or LSST) responsible for synthesizing the 6 sulfation of GlcNAc has been cloned.^{25,26} The gene deletion of this high endothelial venule-specific enzyme results in a significantly hampered lymphocyte traffic to lymph nodes *in vivo*.²⁷ Because such sulfations could be of a crucial importance for the recruitment of lymphocytes into the site of inflammation, the presence and the role of such enzymes warrant study in future. The endothelial expressions of MECA-79 epitopes, sulfated extended core 1 lactosamine structures,²⁸ have been shown earlier to be induced during inflammation at the sites of acute transplant rejection, bronchial asthma, as well as inflamed muscle or skin lesions.^{3,29,30} Here we expand these observations and show that endothelial mAb MECA-79 reactivity was present in many sites of inflammations, such as thyroiditis, psoriasis, ulcerative colitis, asthma, and organ transplant rejections.

In addition to the *de novo*-induced or -enhanced endothelial sLex or sulfo sLex expression shown in this study, we propose that each organ could also possess its own sLex- or sulfo sLex-specific zip code. When expressed as a percentage of reactive vessels for each of the three mAbs used, we could designate these zip codes as shown in Table 2. These induced expression levels of glycans that fulfill the criteria of L-selectin ligands suggest that these glycans could be crucial in the initiation of leukocyte extravasation into sites of inflammations. To test this hypothesis either an indirect Stamper-Woodruff type of *ex vivo* lymphocyte-binding assay could be performed in each organ, but this would require the use of fresh and not formalin-fixed tissues. Another more direct approach could be to isolate the endothelial glycoproteins, such as CD34, known to act as L-selectin ligands and directly analyze with mass spectrometry the

O-glycans on both normal and inflamed tissues and testing their ability to support L-selectin-dependent leukocyte rolling. We have already begun the latter approach and characterized the glycans on CD34 of tonsillar high endothelial venules.¹³

Furthermore, we were able to observe differences even between the inflammations of the same organ, such as between psoriasis and vasculitis in the skin. Nonetheless, whether this observation was caused by the severity of the inflammatory incidence or by the difference in the putative pathogenesis of lymphocytic infiltration remains to be defined. However, our observations about inducible organ- and inflammation-specific zip codes could be understood as an extension to the previously reported organ-specific peptides and their ligands.^{31–34} Both the glycan and peptide specificities of various organs could serve as the means of targeted interventions for re-circulating cells, whether or not these cells are leukocytes or malignant tumor cells during hematogenous metastasis.

The down-regulation of the endothelial expression of sulfo sLex-bearing glycoforms is taking place concomitantly with the inflammation resolving.² As previously shown, of six or more human α 1,3-fucosyltransferases, FucTVII is the one capable of synthesizing sLex glycans at the level of endothelial expression and, thus, linked to the induction of heart allograft rejections.^{23,35,36} The studies with L-selectin-deficient mice as well as with FucTVII-null or FucTIV and FucTVII double-null animals have demonstrated and supported the importance of L-selectin in the extravasation of lymphocytes into lymph nodes and inflamed tissues.^{37–39}

Taken together, we show here that the *de novo* induction of endothelial sLex or sulfo sLex glycans is a common event in all organs analyzed here and, thus, suggests that these glycans play a crucial role in the early-state induction of tissue inflammation. In addition, because each organ was shown to carry its own zip code, ie, a specific modification of sLex or sulfo sLex glycans, it suggests that this phenomenon could putatively provide a mean for the organ-selective inhibition of leukocyte trafficking.

Acknowledgments

We thank Eriika Wasenius for excellent technical assistance and Maaret Helintö for expertise in statistics.

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