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In vitro experimental studies of sialyl Lewis x and sialyl Lewis a on endothelial and carcinoma cells: crucial glycans on selectin ligands

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Extravasation from the blood of malignant tumour cells that form metastasis and leukocytes that go into tissues require contact between selectins and their sialyl Lewis x and sialyl Lewis a (sLe^x and sLe^a respectively) decorated ligands. Endothelial cells have been shown to express sLe^x epitopes in lymph nodes and at sites of inflammation, and this is crucial for the selectin-dependent leukocyte traffic. Besides the ability to synthesize sLe^x on sialylated *N*-acetyllactosamine via the action of α(1,3)fucosyltransferase(s), endothelial cells can also degrade sLe^x to Lewis x through the action of α(2,3)sialidase(s). In addition, several epithelial tumors possess the machinery to synthesize sLe^x, which facilitates their adhesion to endothelial E- and P-selectin.

***Keywords*: metastasis, leukocytes, sialyl Lewis x, sialyl Lewis a, endothelial, carcinoma, glycans, selectin**

Introduction

Inflamatory reactions, such as organ transplant rejection, are characterized by lymphocyte infiltration into the tissue [1]. This extravasation of lymphocytes is initiated by the interaction of members of the selectin family and their ligands, which leads to a flow-dependent rolling of lymphocytes on endothelial surfaces [2–5]. Of the three identified selectins, L-selectin is expressed on leukocyte surfaces and it recognizes glycoprotein ligands on endothelium [6–8]. Three characterized mucin-like heavily O-glycosylated proteins; GlyCAM-1, CD34 and MAdCAM-1 are L-selectin ligands [9–11]. These ligands recognize L-selectin only when correctly posttranslationally glycosylated. So far only the important glycoforms of murine GlyCAM-1 have been characterized in detail and they have been shown to be α2,3 sialylated, α1,3 fucosylated and sulfated, *ie* sialyl Lewis x (sLe^x) and/or sulfated sLe^x, respectively [12–16]. L-selectin was first discovered as a molecule that guides lymphocytes to lymph nodes, and later, also to sites of inflammation [17]. Recently the mechanism of action of L-selectin has become even more complicated, because L-selectin was shown to participate in leukocyte tethering and initial rolling only within a very narrow window of vascular shear forces [18]. Furthermore, rapid proteolytical cleavage of L-selectin from the rolling cells is necessary before extravasation of lymphocytes can take place [19].

The two other members of the selectin adhesion molecule family, E- and P-selectin, are expressed on activated endothelium [3, 20]. Also, their glycoprotein ligands are active only when properly decorated with fucosylated oligosccharides. PSGL-1 is a mucin glycoprotein expressed on leukocyte surfaces and is a ligand for both these endothelial selectins [21]. It has recently been shown to carry O-linked glycans, of which small proportions carry sLe^x structures on a multiply fucosylated poly *N*-acetyllactosamine backbone, and directly on the core GalNAc, linked to the protein backbone [22]. The presence of sLe^x decorated glycans is not enough for selectin-dependent recognition. CHO cells express the functional form of the selectin ligand PSGL-1 only when they are co-transfected with a branching enzyme for O-linked glycans (Core 2 β(1-6)*N*-acetylglucosaminyltransferase, C2GnT) and α(1,3)fucosyltransferase [23]. Fucosylated and sialylated O-glycans with simple core 1 polylactosamines in CHO cells do not recognize P-selectin [23] which suggests that not all sLex-decorations on the PSGL-1 glycoprotein allow P-selectin recognition. ESL-1 is another leukocyte surface molecule binding only to

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E-selectin and ESL-1 carries only N-linked glycans [24]. Also, L-selectin and CD24 have been shown to act as ligands for endothelial selectins [25, 26]. All selectins and their sLe^x-containing ligands contribute to the very early capture and rolling phases of the extravasation and thereby offer a potential site for anti-inflammatory intervention.

Structure of sLe^x and related molecules

All selectin ligands are decorated by sLex-type glycans, but clearly this is not enough [27]. sLe^x is a tetrasaccharide NeuNAcα2-3Galβ1-4(Fucα1-3)GlcNAc. Early observations also suggested another closely related structure, sialyl Lewis a (sLe^a, NeuNAcα2-3Galβ1-3(Fucα1-4)GlcNAc), might act as a ligand [28–30]. The sialic acid can be replaced within sLe^x or Sle^a by a sulfate group and the affinity towards E-selectin increases [31, 32]. The glycans on murine GlyCAM-1 that recognize L-selectin carry sulfated sLe^x [16].

Glycosyltransferases in sLe^x synthesis

If sLe^x on glycans plays an essential role in the regulation of leukocyte extravasation, how is its expression regulated on endothelial cells? Starting from the very abundant core glycan sequence, *N*-acetyllactosamine, two enzymatic steps are required for the synthesis of sLe^x, the first one being the α(2,3)sialylation of terminal galactose [27]. To date four α(2,3)sialytransferases have been cloned and two of them are involved in the sLe^x synthesis [33–36]. After the *N*-acetyllactosamine has become sialylated the α(1,3)fucosyltransferase acts and transfers fucose from GDP-fucose to GlcNAc. Five human α(1,3)fucosyltransferases have been cloned and characterized in some detail. They seem to have slightly different, but overlapping specificities; four of them efficiently fucosylate sialylated acceptors, while Fuc-TIV prefers neutral acceptors [28, 37–43]. Fuc-TIII has two different activities (α(1,3) and α(1,4)fucosylation), leading to the generation of sLe^x and sLe^a, respectively.

sLe^x synthesis appears to be regulated by several processes. If the *N*-acetyllactosamine is α(2,6)sialylated then no further α(1,3)fucosylation takes place [44]. *N*-acetyllactosamine can also be α(1,2)fucosylated at the galactose, which leads to an H-type of blood group O-structure. Furthermore, if the *N*-acetyllactosamine is initially α(1,3)fucosylated at the GlcNAc, then α(2,3)sialytransferases are unable to form sLe^x structures. These examples show how complicated the synthesis of this relatively small oligosaccharide can be. The essential components of this process are therefore the availability of oligonucleotide donors (such as CMP-sialic acid or GDP-fucose) and the amount and activity of the corresponding glycosyltransferases within a given cell.

Expression, synthesis and degradation of sLe^x in endothelial cells

sLe^x glycans have been characterized both on normal peripheral blood granulocytes, as well as on certain carcinoma cells in the early 1980s [45], but it was not before the 1990s the sLe^x expression on endothelial cells was resolved. While endothelium normally does not express sLex glycans, there is one exception. Lymph node high endothelial venules (HEV) are lined by a special high endothelium that is a site for extravasation for lymphocytes. These HEVs were shown to express sLe^x strongly and thereby facilitate the L-selectin-dependent lymphocyte homing to lymph nodes [46–48].

After this demonstration of sLe^x expression in lymph node, high endothelium we wanted to investigate if normal vascular endothelial cells had the appropriate glycosyltransferases to synthesize sLe^x, and if this expression was under inflammatory regulation. The expression of sLe^x oligosaccharides was documented on cultured human umbilical endothelial cells (HUVECs) with flow cytometry and a panel of anti sLe^x mAbs [49]. Further evidence for the presence of sLe^x on HUVECs came from flow cytometry experiments where the cells were treated with a broad spectrum sialidase to remove sialic acid from the sLe^x glycans, and the resultant expression of Le^x antigen was analysed. As expected the expression of Le^x enhanced significantly after sialidase treatment, with a concomitant abolishment of reactivity with anti sLe^x antibodies [49]. Taken together these data suggest that *in vitro* cultured HUVECs express sLe^x related glycans.

Cultured endothelial cells expressed transcripts for at least one of the essential α(2,3)sialyltransferases (ST3N/ST3Gal II), and for several α(1,3)fucosyltransferases (Fuc-TIII, Fuc-TIV and Fuc-TVII), when they were analysed by Northern blots [49]. The presence of a given glycosyltransferase mRNA, however, does not directly provide evidence for the presence of the active enzyme. Thus, assays to detect enzyme activities were carried out. *N*-acetyllactosamine was used as the acceptor, CMP-[^{14}C]sialic acid as the donor and endothelial lysates as the source of the enzyme, and the structure of newly synthesized glycans was analysed by anion exchange and paper chromatography. HUVECs were shown to preferentially add sialic acid into a α(2,6) linkage, but the α(2,3) sialylated product was only obtained as a 10% yield [49], and its synthesis could not be enhanced by *in vitro* cytokine stimulations.

Resting endothelial cells could transfer fucose to both neutral *N*-acetyllactosamine and sialylated *N*-acetyllactosamine in an α(1,3) linkage to the GlcNAc. Interestingly, tumour necrosis factor (TNF)-stimulated endothelial cells could synthesize over four-times more sLe^x from sialylated *N*-acetyllactosamine than resting endothelial cells, indicating that proinflammatory signals can induce endothelial sLe^x synthesis [49]. Taken together these data suggested

that cultured endothelial cells have the capacity to synthesize sLe^x-type of glycans and the rate of synthesis can be further induced by inflammatory stimuli.

We were able to show a feedback loop in L-selectin dependent adhesion because cultured endothelial cells could also degrade the sLe^x motifs. This occurred primarily through the action of α(2,3)sialidase and to a minor extent through α(1,3)fucosidase activity. Besides the *de novo* expression of upregulated sLe^x expression which occurs at the start of an inflammation reaction it is necessary also to analyse the events leading to the termination of inflammation. The above *in vitro* evidence had already suggested that sLe^x is degraded by α(2,3)sialidase. A radiolabelled sLe^x glycan was synthesized, therefore, that was resistant to spontaneous β-elimination of the fucose residue using endothelial cell lysates it was shown that 75% of the sLe^x glycan was desialylated within 2 h [50]. A small proportion (6%) of the molecule experienced further defucosylation. No defucosylation was observed with the sialylated original molecule, indicating that the removal of sialic acid is the primary event in the degradation of sLe^x glycan by endothelial cells [50]. These data suggest that the synthesis and degradation of the sLe^x motif is under strict control and that it is a key regulator of extravasation and the consecutive lymphocyte homing and generation of inflammation.

Role of endothelial sLe^x in the induction of inflammation

To be able to interpret the role of endothelial sLe^x synthesis in the context of inflammatory reactions we characterized two organ transplant models in rats [1, 51]. Allogeneic kidney or heart grafts between different inbred rat strains are rejected within 5 days. The hallmark of allograft rejection is lymphocyte infiltration into the transplanted organ. One of the key questions in understanding this process is how and where do the lymphocytes get into the graft. We decided to analyse this lymphocyte traffic with the Stamper-Woodruff *in vitro* lymphocyte-endothelial binding assay. We showed that the adhesion of lymphocytes to kidney and heart allografts had already increased on the second postoperative day [51, 52]. This enhanced adhesion was solely due to an increased binding to capillary endothelium, as practically no other structure in the graft showed lymphocyte adhesion. Concomitant with the increased lymphocyte binding, the capillary endothelium, showed several morphological features common to high endothelium of lymphoid organs, as analysed by electron microscopy.

The previous observations indicated that capillary endothelium was the site of lymphocyte entry into the rejecting allografts. Of the endothelial adhesion molecules investigated, ICAM-1 was already expressed on the endothelium of normal grafts, and its expression was strongly enhanced during the rejection process without site-specific restriction [53]. VCAM-1 was not expressed on the endothelium of normal or syngeneic transplants, but its expression was induced during allograft rejection, not only in capillaries, but also occasionally on the endothelium of larger vessels. On the other hand, sLe^x showed a very restricted pattern of expression; endothelium was sLe^x negative both in control and syngeneic grafts, whereas capillary endothelium reacted strongly only in the allografts, but not in control organs [53]. Furthermore, only capillaries in the allografts, but not in the syngeneic grafts or normal tissues bound an L-selectin-IgG fusion protein, indicating that ligands for L-selectin were induced during rejection [53].

All the above mentioned data provided circumstantial evidence for a crucial role of sLe^x glycans in the generation of inflammatory infiltrates during transplant rejection. However, direct evidence for the role of fucose containing sLe^x glycans participating in lymphocyte extravasation to lymph nodes and to sites of inflammation was still needed. Five mammalian α(1,3)fucosyltransferases have been cloned and recently, the first mice deficient in one of these genes was constructed and characterized [28, 38, 41–47]. The Fuc-TVII knock-out mice resemble, to some extent, patients suffering from the leukocyte adhesion deficiency II (LAD II) syndrome. The leukocytes in the knock-out mice do not express active E- or P-selectin ligands, which leads to marked leukocytosis and defective leukocyte extravasation to sites of inflammation. Concomitantly, no functional endothelial ligands for L-selectin are expressed in these animals, leading to severe attenuation of lymphocyte homing to lymph nodes [33]. These Fuc-TVII knock-out mice provide the first direct evidence that fucose is an essential component of the L-selectin ligands together with sialic acid and sulfate.

There is some controversy over whether rodent leukocytes synthesize sLe^x as it cannot be detected by anti-sLe^x antibody [54]. However, murine GlyCAM-1 secreted from lymph node endothelium bears sulfated sLe^x epitopes [13, 14, 16] and we have shown that capillary endothelium on rat kidney and heart allografts start to express sLe^x *de novo* during rejection episodes [53, 55]. The sLe^x-type structures on endothelium of rat lymph nodes or inflamed tissues react with the anti sLe^x mAbs. Furthermore, cultured rat endothelial cells have been shown to synthesize sLe^x glycans [50]. Yet again, the crucial role of α(1,3)fucosylated glycans on L, E- and P-selectin ligands have been shown by gene knock-out mice [33], suggesting that sLe^x-type glycans are expressed in the murine system. So, it is possible that sLe^x-type oligosaccharides are not expressed on rodent leukocytes, or that they have modifications that do not react with the presently available anti sLe^x mAbs.

Oligosaccharide antagonists for selectins in inflammation

Immediately after selectin ligands had been shown to contain sLe^x, a number of laboratories initiated projects aimed

at discovering putative selectin antagonists. Most of these approaches synthesized oligosaccharides, and the results from these preliminary animal experiments are very promising [56].

As the *in vitro* synthesis of sLe^x glycans is tedious and expensive, these glycans have been tested so far only in short-term inflammation models that involve a rapid influx of granulocytes into the site of inflammation. In the first model studied a snake venom used to cause P-selectin-dependent granulocytic inflammation in the lungs. By intravenous infusion of sLe^x, a ligand for P-selectin, the number of granulocytes accumulating in the lungs of rats was significantly decreased, with a concomitant reduction in the lung injury [57].

Reperfusion of an ischemic coronary or brain vascular bed enhances the tissue injury despite the reestablishment of blood flow to these tissues. This phenomenon, is termed reperfusion injury, and is characterized by the presence of extravasating granulocytes. As in the snake venom-induced granulocytosis, the exogenous sLe^x injected into blood circulation just before the initiation of reperfusion could in most cases dramatically reduce the granulocyte accumulation in ischaemic tissues and consequently decreased the reperfusion injury [58–65]. Sialylated *N*-acetyllactosamine (sLNn, see Figure 1) was a crucial control in most of these studies. The latter molecule lacks fucose compared to the sLe^x structure, and it was always without of any effects in these inflammation models.

Data from *in vivo* experiments with animals demonstrated the efficacy of very low concentrations of sLe^x in inhibiting short-term P-selectin mediated inflammation [57, 58, 62]. Even long-term inflammatory responses occurring within a few days could be inhibited by continuous infusion of anti L-selectin mAb to animals. L-selectin knockout mice or animals treated with anti L-selectin Mabs had normal differential counts of peripheral blood leukocytes [66, 67]. These data suggest that it might be possible to treat long term inflammations by inhibiting selectin-mediated leukocyte traffic with oligosaccharides antagonists.

All the above mentioned work had been done with monovalent sLe^x glycans. This is mainly due to the fact that multivalent sLe^x glycans were not available. Chemical synthesis cannot be used easily to generate very large oligosaccharides and even the enzymatic synthesis is tedious due to the need to purify appropriate glycosyltransferases, optimize the reaction conditions and purify the synthesized glycans. Previously it was known that the affinity of otherwise very low affinity oligosaccharide-lectin interactions could be enhanced by increasing the multivalency of the oligosaccharide component [68–72]. Therefore, a programme was initiated to enzymatically synthesize large complex multivalent sLe^x glycans, in order to analyse their potential capacity to inhibit selectin-dependent inflammation [72–76].

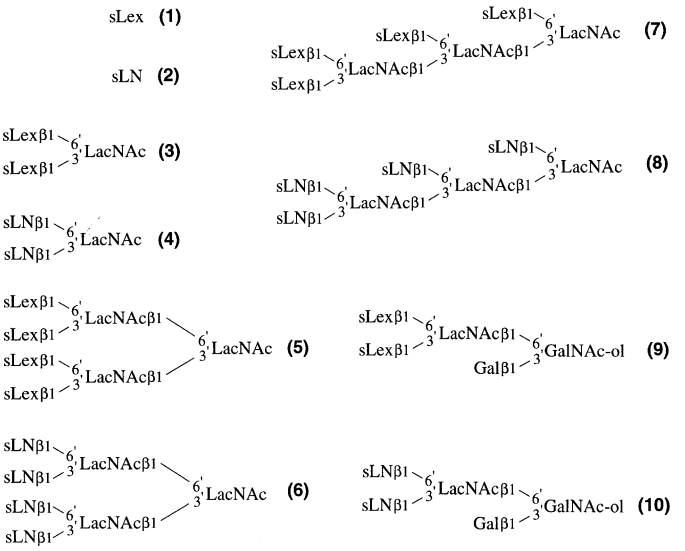

Figure 1. Structures of enzymatically synthesized glycans discussed in this review. sLe^x represents NeuNAca2-3Galb1-4(Fuca1-3)GlcNAc, sLN is NeuNAca2-3Galb1-4GlcNAc, and LacNAc represents Galb1-4GlcNAc.

The structures and the numbering of the glycans to be discussed are presented in Figure 1. A series of sLe^x glycans on a branched polylactoamine backbone was synthesized. Glycan **1** is sLe^x, glycan **3** is branched divalent sLe^x and glycan **5** is branched tetravalent sLe^x. Glycans **2**, **4**, and **6** represent the corresponding non-fucosylated sialylated *N*-acetyllactosamine (sLNs). The definitive structures of the glycans in Figure 1 were established by a number of techniques, such as high pH anion exchange (HPAEC) chromatography, nuclear magnetic resonance (NMR)-spectroscopy and matrix assisted laser desorption ionization (MALDI)-mass spectrometry [72–76].

The ability of this family of branched sLe^x- and sLN-glycans to inhibit L-selectin mediated adhesion of lymphocytes to endothelium of transplants undergoing acute-rejection was tested. All exogenous α(2,3)sialic acid- and α(1,3) fucose-containing polylactosamines (*ie* mono-, di- and tetravalent sLe^x, structures **1**, **3** and **5**, respectively) inhibited the L-selectin-dependent lymphocyte binding to graft capillary endothelium both in the kidney and heart transplantation models significantly [55, 74]. The potency of these molecules increased as the number of sLe^x-determinant in them increased, the tetravalent sLe^x (glycan **5**) being more superior to other branched glycans. *In vitro* data by others also suggest that oligosaccharide constructs bearing two sLe^x groups (divalent sLe^x structures) are five times better inhibitors of E-selectin dependent adhesion compared to monovalent sLe^x [70, 71]. None of the fucose-free structures, *ie* sLNs, whether mono-, di- or tetravalent (glycans **2**, **4** and **6**) inhibited the lymphocyte adhesion [55, 74]. These results support the concept of the crucial role of sLe^x and

α(1,3)fucosyltransferases, in the L-selectin-dependent extravasation of lymphocytes.

We synthesized also a tetravalent sLe^x on a linear backbone (glycan **7**). This glycan has the benefit that its synthesis is much simpler, requiring a smaller number of enzymatic reaction steps than the branched tetravalent sLe^x (glycan **5**) [76]. The linear tetravalent sLe^x was shown to be as effective as the branched one, IC_{50} values (concentration of glycan giving 50% inhibition of adhesion) were in the low nanomolar range. Once again the control glycan (**8**) lacking fucoses was without any effect. These tetravalent sLex glycans (**5** and **7**) consisting of 22 monosaccharides in a precisely defined array, probably represent the largest oligosaccharides so far enzymatically constructed [76]. We have also synthesized an O-linked divalent sLe^x (glycan **9**), which inhibits the L-selectin-dependent lymphocyte adhesion to capillary endothelium. It is a three-fold better inhibitor than the truncated divalent sLe^x molecule (glycan **3**) [75]. These data suggest that parts of these glycans other than the terminal sLe^x-determinant contribute to the selectin-dependent adhesion.

The particularly high inhibitory potency of tetravalent glycans **5** and **7** for L-selectin is probably generated by the multiplicity of sLe^x epitopes, implying that one glycan may bind to several L-selectin molecules on lymphocyte surface. Monomeric receptors may become crosslinked on cell surface, like individual haemagglutinin trimers on intact influenza virus can be crosslinked by bivalent sialosides [77].

Of potential importance for the high affinity interactions is also the length of the saccharide chains linking the sLe^x determinants in tetravalent glycans **5** and **7**. These chains contain GlcNAcβ1-6Gal bonds, giving them extra length and flexibility. Such spacers between the binding epitopes are inserted into the molecule to enhance the possibility of multisite binding of the glycans. The data suggests that multiple-branched polylactosamines may prove themselves to be inhibitors of some saccharide-dependent recognition processes [55, 74, 76]. It is possible that multiple-branched or linear polylactosamines act as natural ligands in some of the processes.

A decrease in the inhibitory efficacy of glycans **5** and **7** was observed at the highest concentration used [55, 74, 76]. The explanation for this finding might be that too few L-selectin molecules are available for the glycan to bind in a multivalent manner at the high micromolar concentration. The decrease of binding efficiency at higher concentrations could also be due to an increasing level of glycan–glycan interaction [45].

sLe^x and sLe^a expression on epithelial carcinoma cells

In order to metastasize hematogeneously malignant cells have to first invade into blood vessels. Following their dissemination via the circulation they may adhere to the vascular endothelium, penetrate through the endothelium, and move into the surrounding tissue (extravasation) develop into a metastatic colony [78]. Structural analyses of tumour-associated carbohydrate antigens have shown them to be α(2,3)sialylated and α(1,3)fucosylated oligosaccharide such as sLe^x and sLe^a. Several malignant cells such as colon, gastric, pancreatic, lung, liver and ovarian carcinomas express these oligosaccharide structures [76–84]. Altered glycosylation of malignant cell surface lipids and proteins compared to those on the normal cell surface has been suggested to play a role in tumour metastasis.

Extensive protease treatments did not remove sLe^x and sLe^a epitopes completely from the surface of colon carcinoma cell lines COLO 205 and HT-29 suggesting that some of these determinants are lipid-linked [88]. The number of different sLe^x-carrying proteins on COLO 205 cells may be very limited, since very few protein bands were observed to interact with an anti-sLe^x antibody on Western blots (Majuri ML *et al*., unpublished data). High affinity glycoprotein ligands for P- and E-selectin, PSGL-1 and ESL-1 respectively have been characterized from leukocytes [21, 24], but their presence on carcinoma cells remains uncertain. No other glycoprotein ligands for selectins have yet been found on epithelium.

The replacement of sialic acid by sulfate in sLe^x and sLe^a results in a sulfo-Le^x and sulfo-Le^a glycan respectively, and these have been shown to recognize E-selectin [31, 32, 89]. Some of these glycans have been used as tumour markers for the monitoring the prognosis of tumour-bearing patients [90].

Adhesion of epithelial carcinoma cells to endothelium

Adhesion of epithelial cancer cells to activated endothelium mediated by E-selectin and its carbohydrate-decorated ligands is believed to be a major mechanism for carcinoma metastasis [30, 91–94]. This is further supported by *in vitro* studies of tumour cell interaction with endothelium under flow conditions. The rolling and adhesion of HT-29 colon carcinoma cells to IL-1-activated HUVECs were completely prevented by treatment of endothelial cells with anti-E-selectin mAb while anti-VCAM-1 antibody had no effect [95]. Studies performed with recombinant E-selectin, E-selectin cDNA transfectants and activated endothelial cells expressing E-selectin have confirmed that these malignant cells adhere to endothelium via E-selectin and sLe^x and/or sLe^a interactions [30, 96]. Activation of endothelial cells with inflammatory mediators such as interleukin-1 (IL-1) and TNF has been found to augment colon carcinoma cell adhesion [91, 93, 97, 98].

Synthesis of sLe^x and sLe^a in carcinoma cells

Several of the necessary glycosyltransferases for the synthesis of sLe^x can be expressed in carcinoma cells. We have shown that ST3N, Fuc-TIII, Fuc-TV and Fuc-TVI are

suitable candidates for the synthesis of the sLe^x and sLe^a epitopes in adenocarcinoma cells. The significant expression of Fuc-TIII in these tumour cells correlates with earlier studies [99] where the mRNA of Fuc-TIII was detected in most of the epithelial cancer cell lines tested. However, a our study we could clearly see higher amounts of FUC-TV and Fuc-TVI mRNAs in the tumour cells compared to the level of FT-IV mRNA [88]. This is in contrast to the previous results where Fuc-TIV mRNA was also abundantly detected in most of the epithelial cancer cell lines [99]. However, the role of Fuc-TIV in the synthesis of sLe^x or sLe^a is controversial, since it is not able to link fucose to sialylated *N*-acetyllactosamine or to sialyllacto-*N*-biose, respectively.

The increased adhesion of COLO 205 and HT-29 cells to E-selectin after TNF-stimulation of the tumour cells suggests that they have enhanced levels of the glycosyltransferases synthesizing sLe^x and sLa^a. Our results show that COLO 205 cells possess high $\alpha(1,3)$ and $\alpha(1,4)$fucosyltransferase activities towards sialylated acceptors when they are in the resting state and this can be further stimulated by TNF [88]. Concomitantly, HT-29 cells $\alpha(1,3)$fucosylates sialylated acceptors with a much lower efficiency, but expresses more $\alpha(2,3)$sialyltransferase activity than COLO 205 cells [88].

sLe^x and sLe^a in the metastasis of carcinoma cells

Several families of adhesion molecules have been identified to play a role in the extravasation process of metastasis [2–6, 8]. Due to the initiating role of selectins and ligands containing sLe^x and sLe^a in this process we analysed the role of these components in the generation of metastatic breast carcinoma lesions.

We analysed the expression of endothelial E- and P-selectin and epithelial (carcinoma) cell sLe^x and sLe^a expression in normal tissues as well as in primary and metastatic breast carcinoma lesions within individual patients. Endothelium in metastatic lesions expressed high levels of both E- and P-selectin [100]. While the normal breast epithelial cells do not express sLe^x or sLe^a, the epithelial expression of these epitopes was strongly enhanced in primary breast carcinoma lesions. Furthermore, the epithelial the expression of sLe^x and/or sLe^a was higher in metastatic breast carcinomas compared to primary breast carcinomas in the majority of patients [100]. Our data support the hypothesis that circulating carcinoma cells that express sLe^x and/or sLe^a have a higher probability to extravasate at sites where endothelium express E- and P-selectin, and thus to generate new metastastic growths.

The carcinoma cell lines adhere *in vitro* to endothelial selectins in a calcium-, sLe^x- and E-/P-selectin-dependent manner [30, 88, 101, 102]. The circumstantial evidence from human patient material presented here is further supported by the demonstration of a direct role for E-selectin in tumour metastasis in a murine transgenic model [103]. The role for enhanced endothelial sLe^x expression in solid breast tumours is not known, but a possible explanation could be the recruitment of tumour-infiltrating leukocytes to sites of metastasis. The interactions of sialylated Lewis antigens and selectins may be worth investigating in the future in relationship to metastatic spread after tumour surgery.

Future perspectives of sLe^x and sLe^a mediated extravasation

Recent observations have broadened the biological scope for investigating sLe^x-interactions, as oral streptococci have been shown to express sLe^x [104, 105]. Only a few years ago it was believed that bacteria could not express such oligosaccharides. The bacterial strains expressing sLe^x adhere to the inner surface of heart, *ie* endocardium and cause a severe, often life-threatening infection termed endocarditis.

sLe^x and sLe^a participate in the interactions of several cell types, such as lymphocytes, tumour cells and bacteria, which are necessary, but not the only events leading to inflammation, metastasis and infection respectively. Furthermore, enzymatically synthesized oligosaccharides might prove to be good candidates for anti-inflammatory, anti-metastatic and anti-infection agents.

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