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The sialyl-α2,6-lactosaminyl-structure: Biosynthesis and functional role

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Sialylation represents one of the most frequently occurring terminations of the oligosaccharide chains of glycoproteins and glycolipids. Sialic acid is commonly found α2,3- or α2,6-linked to galactose (Gal), α2,6-linked to N-acetylgalactosamine (GalNAc) or α2,8-linked to another sialic acid. The biosynthesis of the various linkages is mediated by the different members of the sialyltransferase family. The addition of sialic acid in α2,6-linkage to the galactose residue of lactosamine (type 2 chains) is catalyzed by β-galactoside α2,6-sialyltransferase (ST6Gal.I). Although expressed by a single gene, this enzyme shows a complex pattern of regulation which allows its tissue- and stage-specific modulation. The cognate oligosaccharide structure, NeuAcα2,6Galβ1,4GlcNAc, is widely distributed among tissues and is involved in biological processes such as the regulation of the immune response and the progression of colon cancer. This review summarizes the current knowledge on the biochemistry of ST6Gal.I and on the functional role of the sialyl-α2,6-lactosaminyl structure.

Keywords: **glycosyltransferases, sialyltransferases, α2,6-sialylation, ST6Gal.I, CD22, CDw75**

Biosynthesis of the sialyl-α2,6-lactosaminyl linkage: The β-galactoside α2,6 sialyltransferase

The disaccharide Ga1β1,4GlcNAc (lactosamine) is a common constituent of thc antennae of N-linked oligosaccharide chains of glycoproteins, but may be also present in O-linked chains and in glycolipids. The β-galactoside α2,6 sialyltransferase (2.4.99.1, ST6Gal.I) is the only sialyltransferase so far identified able to catalyze the α2,6-sialylation of lactosamine.

ST6Gal.I was initially purified in a soluble form from bovine colostrum [1,2], and successively in a membrane-bound form from rat [3,4] and human liver [5]. Like other glycosyltransferases, ST6Gal.I is a type II membrane glycoprotein with a short NH_2-terminal citosolic domain, a hydrophobic transmembrane domain, a stem domain and a COOH-terminal catalytic domain exposed to the luminal side of Golgi cisternae [6]. The hydrophobic transmembrane domain, along with adjacent luminal and cytoplasmic sequences, contributes to specify the Golgi retention signal [7–10].

The first ST6Gal.I cDNA clone has been isolated from a rat liver cDNA library [11]. The rat gene spans at least 80 kb of genomic DNA and contains 11 exons [12] (Figure 1). The use of physically distinct promoters and the alternative splicing of the primary transcripts give rise to three major size classes mRNAs [13,14]. A first 4.7 kb form is comprised of 8 exons (−1, 0 and 1–6) and is expressed in many tissues. A second, 4.3 kb species, is typically expressed at a very high level by the liver. This form shares exons 1–6 with the 4.7 kb form but lacks exons −1 and 0. The two forms share also the translational start point, within exon 2, and the translational stop point, inside exon 6, yielding identical protein products. Along with the 4.7 kb form, rat kidney expresses three 3.7 kb transcripts formed by the association of one, two or three kidney-specific exons with exons 4–6 [12,15,16]. The functional significance (if any) of these kidney-specific forms,

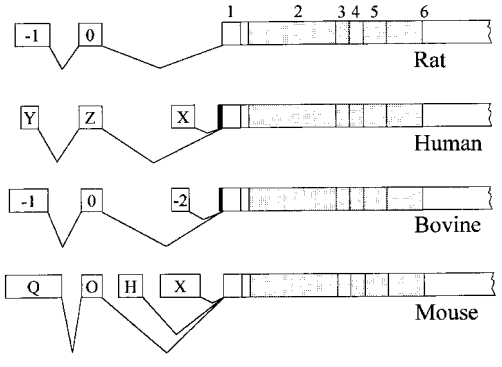

Figure 1. Schematic representation of the major ST6Gal.I transcripts expressed by mammalian species. The different mRNA forms are generated by joining different 5′-noncoding exons with exon I. Homologous exons are aligned vertically. The gray area represents the protein coding domain. The black area upstream of exon I in the human and bovine transcripts represents a tissue-specific sequence present in forms lacking upstream untranslated exons.

which are devoid of enzymatic activity, is obscure. After the isolation of a partial cDNA of human ST6Gal.I from submaxillary gland [17], full length cDNA clones were isolated from a placenta [18] and from a B-lymphoblastoid cell line Daudi cDNA library [19]. The human gene maps to chromosome 3 (q21–q28) [20] and shares with the rat gene the basic organization. All the mRNA species so far identified share exons 1–VI, homologous to the corresponding rat exons. In the placenta cDNA two additional 5′-untranslated exons, namely exons Y and Z, homologous to rat exons −1 and 0, are located 5′- of exon I. The Daudi cDNA lacks exons Y+Z but contains the 5′-untranslated exon X, for which there is no known rat counterpart. The transcript of the Y+Z-type occurs in many tissues, while that of the X-type is specific for mature B-lymphocytes and B-lymphoblastoid cell lines [20]. A third type of transcript, corresponding to the rat liver 4.3 kb form, has been cloned from the hepatoma cell line HepG2 [21]. This form, which contains a short sequence upstream of exon I, probably represents the major normal liver transcript but it has been detected also in normal and cancer colonic tissues [22] and in some colon cancer cell lines [23]. Minor isoforms containing other 5′-untranslated exons have been reported [24]. ST6Gal.I cDNA clones have been isolated also from mouse [25], chick embryo [26], and bovine tissues [27]. The mouse ST6Gal.I gene maps on chromosome 16 [28]. The 5′-untranslated exons of mouse transcripts show similarity and differences with the human forms [29]. An "housekeeping" form contains exons Q and O, homologous to human exons Y and Z respectively while the liver expresses a specific transcript containing exon H [30] and B lymphocytes produce specific forms containing exons X_1, X_2 or X_3 [29]. Bovine ST6Gal.I shares with other species the basic enzyme and genomic structure. Three families of transcripts homologous to the three major human forms have been described, but their tissue distribution is remarkably different [27]. A schematic representation of the transcript families expressed by mammalian species is provided in Figure 1.

Although widely expressed among tissues [31,32], ST6Gal.I may undergo dramatic variations of expression during development and differentiation or in response to precise stimuli. The main regulatory mechanisms appear to operate at the transcriptional level [13,14]. In rats, the sequences 5′-flanking exon 1 contain multiple negative and positive regulatory elements and consensus binding sequences for liver-specific transcription factors which ensure the liver-specific expression of the 4.3 kb transcript [33]. In humans, the region flanking the hepatic promoter contains consensus binding sites for HNF-1, NF-IL6, AP2 and APRF transcription factors, while the region 5′-flanking the B-lymphocyte promoter contains potential binding sites for AP1, AP2, NFκB, C/EBP and CREB [34], and the region 5′-flanking Exon Y contains consensus binding sequences for MZF-1, AML-la, AP-1, Spl and Oct-l [35]. The presence of regulatory elements has been reported also inside exon X (i.e. in the region 3′-flanking the promoter) [36]. The transcription of mRNA species differing in the 5′-untranslated regions allows the quantitative regulation of ST6Gal.I expression not only at the transcriptional level but also at the translational level. In fact, the hepatic transcript shows an higher translational efficiency *in vitro* [37]. Moreover, several post-translational modifications affecting enzyme activity have been described: i) the presence of the N-linked chains is necessary for the activity of the enzyme molecule, especially of the soluble form (see below) [38]; ii) the rat liver enzyme can form disulfide-bonded dimers which are devoid of activity [39]; iii) the rat liver enzyme exists in two variants differing for the presence of a Tyr or a Cys at position 123 [40]. The latter form, which possesses a lower catalytic efficiency, is encoded by a post-transcriptional modification of the RNA [40]; iv) both the Tyr- and the Cys-forms may be phosphorylated, but the functional significance of this modification is unclear [41]. These data provide the picture of an enzyme encoded by a single gene whose expression is precisely regulated in a tissue- and stage-specific manner, owing to the existence of multiple regulatory mechanisms.

In 1983 Jamieson and coworkers [42] reported that the expression of hepatic ST6Gal.I increased upon turpentine-induced inflammation and that the enzyme can be released from hepatocytes like an acute phase protein. Further studies demonstrated the release of a catalytically active peptide, missing 63 NH_2-terminal aminoacids [11,43], by the action of cathepsin-like proteolitic enzymes [44,45]. The physiological relevance of a glycosyltransferase in the extracellular fluids is unclear. The released portion of the enzyme, although catalytically active, could hardly mediate sialylation of extracellular substrates, owing to the absence (or extremely low concentration) of the sugar nucleotide donor. More likely appears the possibility that the soluble glycosyltransferase is involved in the recognition of endogenous or exogenous oligosaccharide structures (such as those on the surface of microorganisms), as a lectin-like molecule. The mechanisms regulating ST6Gal.I during inflammation have been investigated in the past years and are still currently under intense investigation. Glucocorticoids up-regulate ST6Gal.I in hepatocytes [46,47], fibroblasts [48] in cultured jejunum of suckling rats [49,50], and mouse neural cells [51]. Interestingly, in hepatocytes and small intestine glucocorticoids stimulate the transcription of the hepatic form, while in fibroblasts they stimulate the transcription of the 4.7 kb form, indicating that the same agonist activates different promoters in different cell types. The acute phase proteins have been grouped in two classes: class 1 which is induced by IL-1 and TNF-α, and class 2, which is induced by IL-6 [52]. In mice, the acute-phase induction of hepatic ST6Gal.I is controlled by the IL-6 pathway and is driven through the hepatic promoter, which results in the expression of the transcript containing exon H [53]. In this system, the inflammatory response elicited by bacterial lipopolysaccharide (LPS) through TNF-α production does not affect ST6Gal.I expression. By contrast, in human humbelical vein endothelial cells (HUVEC) LPS, IL-1

and TNF-α activate vascular endothelium and induce an increase of ST6Gal.I expression which, in turn, enhances the level of α2,6 sialylation of membrane glycoproteins such as E-selectin, ICAM-1 and VCAM-1, which may act as ligands for CD22 (see below) [54,55]. Interesting theories on the functional significance of the inflammation-dependent increase of α2,6-sialylation have been proposed by Gagneux and Varki [56]. First, many pathogens preferentially use α2,3-sialylated sugar chains as receptors on host cells [57]; for such a pathogen, the increased level of α2,6-sialylation during infection would reduce the number of available binding sites. Second, the sialyl α2,6-lactosaminyl epitope appears to play an endogenous functional role mainly in cells related to B-lymphocyte function (see below); the inflammation-induced α2,6-linked sialyl-residues would act as "decoy" receptors, preventing an incoming pathogen provided with receptors specific for α2,6-linked sialic acid, from interacting with cells where this oligosaccharide epitope plays critical roles of endogenous recognition. Limiting the up-regulation of ST6Gal.I to the time of inflammation would prevent the adaptation of pathogens to such defense mechanisms.

A number of other substances have been reported to affect the level of either ST6Gal.I mRNA or enzyme activity or both, in several model systems. For example, thyrotropin down-regulates the mRNA level of cultured thyroid cells [58], thus providing a likely explanation for the increased mRNA level observed in hypothyroid mice [59]; *n*-butyrate down regulates the ST6Gal.I mRNA level through a post-transcriptional mechanism [60]; interferon α-2β inhibits both mRNA level and enzyme activity in rat testis [61] while peroxisome proliferators reduce ST6Gal.I expression and glycoprotein sialylation in rat hepatocytes [62]. Among the substances affecting ST6Gal.I expression, ethanol deserves a special mention. One of the most reliable markers of acute ethanol abuse is represented by the reversible hyposialylation of transferrin (a serum glycoprotein bearing almost exclusively sialic acid α2,6-linked to galactose) [63]. Ethanol administration reduces ST6Gal.I activity in rat liver [64,65] and causes a reduced stability of the mRNA [66], thus providing an enzymatic basis for the phenomenon. By contrast, in the human colon carcinoma cell lines Caco-2 and HT29, ethanol enhances ST6Gal.I expression even after a short term treatment [67].

The sialyl-α2,6-lactosaminyl-linkage in cancer and differentiation

The level of expression of the sialyl-α2,6-lactosaminyl-structures and of ST6Gal.I show a strong dependence on neoplastic transformation and differentiation in several tissues. The accumulation of α2,6-sialylated lactosaminyl structures in glycolipids from tumors of various origin, including colon was formerly detected using specific monoclonal antibodies [68,69], but the most widely used tool for the investigation of the sialyl-α2,6-linkage is the lectin from *Sambucus nigra* (SNA) [70]. Using SNA or another α2,6-sialyl-specific lectin from *Tricosanthes japonica*, our and other groups demonstrated an up-regulation of the sialyl α2,6-lactosaminyl-linkage in colon cancer tissues [71–73]. A strong SNA reactivity has been proposed to be an indicator of a poor 5 years survival, independent of tumor grade and histological differentiation [74]. The possibility that the sialyl-Tn epitope (NeuAc α2,6-GalNAc), another structure recognized by SNA, plays a relevant role in determining the SNA reactivity of colon cancer tissues has been ruled out [75]. Consistent with the notion of an onco-developmentally regulated expression of the sialyl-α2,6-lactosaminyl linkage, a down regulation of SNA reactivity upon weaning has been described in rat [49,76,77] and pig [78] intestine.

The enzyme ST6Gal.I also shows an onco-differentiation-dependent pattern of regulation in several tissues and cell types. As demonstrated by our group, ST6Gal.I enzyme activity is specifically increased in the vast majority of human colorectal cancer specimens, compared with the normal surrounding mucosa [79]. Further studies confirmed this finding [80–82] and showed a further increased expression in metastasis, compared with primary tumors [81]. In the human colon cancer cell line Caco-2, the enterocytic differentiation is accompanied by an increase of ST6Gal.I activity up to 10 fold [83,84]. In fetal and newborn rat intestinal cells, the ST6Gal.I activity is very high but undergoes a dramatic down regulation after weaning [85], which parallels a decrease of the corresponding mRNA [49,86]. Together, these results indicate that colonic cells may express very high levels of ST6Gal.I activity in a precise state of differentiation, corresponding to that of fetal enterocytes. The molecular events at the basis of the onco-developmental regulation of ST6Gal.I in intestinal cells are still poorly understood. In fetal and newborn rat intestine, the major mRNA species associated with the very high levels of expression is the 4.3 kb "hepatic" transcript. After weaning the transcription of this form is switched off and the 4.7 kb transcript becomes the major species [86]. In human colon cancer cell lines, we observed a differential expression of the Y + Z and of the hepatic transcripts: the former was detected in all the cell lines tested while the latter was expressed only in some [23]. The dramatic increase of ST6Gal.I activity which follows the differentiation of Caco2 cells is supported by an accumulation of the hepatic transcript [23,84]. In surgical specimens the picture appears to be more complex. In fact, a tendency to accumulate ST6Gal.I mRNA, at least in some patients, has been detected by Northern analysis [87] and by RT-PCR [88,89]. Both the Y + Z and the hepatic transcripts are detectable in normal and cancer tissues but, although the latter appears to accumulate in many cases, both may be differentially expressed in cancer tissues [22], indicating that the neoplastic transformation may affect ST6Gal.I transcription through at least two promoters. The concomitant elevation of ST6Gal.I activity and SNA reactivity in colon cancer opens the following question: is the increase of SNA reactivity directly proportional to the level of ST6Gal.I

activity? We have recently established that the answer is no [22]. In fact, the SNA reactivity is, in some cases, much lower or higher than that expected on the basis of enzyme activity, suggesting the existence of other mechanisms of regulation.

In liver, the sialyl-α2,6-lactosaminyl-linkage and ST6Gal.I appear to be onco-developmentally regulated. In rat hepatoma, the ST6Gal.I activity is higher than in normal liver [90] while in human hepatoma, gangliosides terminating with the sialyl-α2,6-lactosaminyl-linkage accumulate [91]. An important insight into the mechanisms linking ST6Gal.I expression and cell transformation comes from a study on a transgenic mouse model of hepatocellular carcinoma [92]. Mice expressing the large T antigen of SV40 under the control of an hepatocyte specific promoter, spontaneously develop hepatocarcinoma and show a general increase of α2,6-sialylation of liver glycoproteins and an up-regulation of ST6Gal.I. The large SV40 T antigen binds the Rb protein, causing the release of the E2F transcription factor which, in turns, activates the transcription of several genes involved in DNA synthesis and cell growth [93]. The effect of large T antigen on ST6Gal.I regulation suggests an intimate relationship between this enzyme and the cell cycle. The observed stimulation of ST6Gal.I activity in rodent fibroblasts overexpressing *ras* oneogenes [94,95] is consistent with this notion. In fact, *ras* controls the extent of phosphorylation of Rb protein by regulating the level of cyclin D1 whose partner CDK4 phosphorylates Rb, causing the release of E2F [96]. By contrast, the hepatic transcript has been reported to be down-regulated in an ascitic hepatoma cell line [97]. The developmental pattern of ST6Gal.I regulation in rat liver is the opposite of that described in intestine: in the newborn, the gene expression is low and results in the transcription of the 4.7 kb transcript [86].

Apart from the cells of the immune system, which will be discussed in detail in the next section, an oncodifferentiation-dependent modulation of ST6Gal.I was reported in other tissues and relative malignancies, such as breast cancer [98], acute myeloid leukemia [99], choriocarcinoma [100] and some types of brain tumors [101]. A large percentage of primary and metastatic gastric carcinomas show an increased reactivity with anti-CDw75 antibodies (see below) [102], and an elevation of ST6Gal.I mRNA [103]. Differentiation of HL-60 cells *in vitro* is accompanied by a down regulation of both ST6Gal.I expression and α2,6-sialylated sugar chains [104], which is driven by the modulation of the Y+Z form [35]. On the contrary, normal bone-marrow myeloid cells display an increased α2,6-sialylation during the late stages of maturation that has been proposed to govern the release of cells in the blood [105].

The onco-differentiation dependent modulation of the sialyl-α2,6-lactosaminyl-linkage does not necessarily imply a role of such a structure in determining the phenotype of the cells. In principle, thc presence of α2,6-linked sialic acid could modulate biological interactions in two non mutually exclusive ways: first, by masking the underlying sugar chains (i.e. lactosaminic sequences), hindering their interaction with galactose-specific lectins (galectins); second, by direct interaction with specific sialic acid-binding lectins (siglecs). The relevance of the first mechanism is highlighted by the relationship between number of terminal lactosaminic sequences and metastatic potential [106]. An example of the second mechanism is provided by the CD22-mediated interactions, which will be discussed later. Whatever the mechanism, a contribution of α2,6-linked sialic acid in determining the phenotype of cancer cells has been inferred on the basis of the already mentioned clinical studies and of the experimental studies which will be reviewed below. It has been observed that colon cancer cell lines growing adherently and not expressing ST6Gal.I activity, contain subpopulations of loosely adherent cells expressing ST6Gal.I [107]. This observation is suggestive of a role of α2,6-linked sialic acid in reducing cell-cell and cell-matrix interactions, but an apparently conflicting indication has been provided by studies on Ehrlich ascites tumor cells [108,109]. The ST6Gal.I activity displayed by established human colon cancer cell lines is usually much lower than that of fresh surgica1 specimens, but when the cells are grown as nude mice xenografts their ST6Gal. I activity increases in most cases, reaching values close to those of surgical samples [110]. Two non mutually exclusive mechanisms have been proposed to explain this phenomenon: i) thc selection of subpopulations provided with an *in vivo* growth advantage; and ii) the adaptation of cells to *in vivo* growth. A close relationship between α2,6-sialylation and metastasis is indicated by the observation that in a mouse model, metastatic colon cancer cell lines express a specific increase of SNA-reactive glycoproteins and of ST6Gal.I enzyme activity, compared with poorly metastatic variants [111]. Rat fibroblasts transformed with *ras* oncogene show, in general, an SNA-positive phenotype. Compared with SNA-positive cells, subclones expressing an SNA-negative phenotype displayed a lower invasive potential *in vitro*, a stronger homotypic aggregation and a different morphology [112]. By contrast, a spontaneous mutant of the highly metastatic murine lymphoma cell line MDAY-2, shows, in concomitance with a dramatic increase of ST6Gal.I mRNA and enzyme activity, a reduced metastatic potential and a slower growth at the subcutaneous site of inoculation [113]. These data should be considered in the light of the following two points. First, the overexpression of a given glycosyltransferase might result in different biological effects in different cell type, possibly depending on the set of glycoprotein acceptors expressed by the cell. Second, a relationship between an altered α2,6-sialylation and a given phenotype does not necessarily imply a causal relationship. A direct approach to circumvent this problem has been followed by genetic engineering with the ST6Gal.I cDNA the colon cancer cell lines SW48 and SW948, not expressing the endogenous gene [114,115]. The analysis of the transfected clones has so far revealed striking cell-type-specific morphological and functional alterations. In fact, ST6Gal.I expression induces a flat morphology and a stronger adhesion to collagen type IV in SW948 transfectants, but not in SW48 clones (Chiricolo M and

Dall'Olio F. unpublished observation). In a different model system (human glioma cells), transfection with ST6Gal.I has been reported to result in marked morphological changes and decreased invasiveness [116].

The sialyl-α2,6-lactosaminyl-linkage in the immune system

As discussed in detail below, a transgenic mouse model carrying an homozygous inactivation of the ST6Gal.I gene shows mainly immunological alterations [117], thus indicating the pivotal role of α2,6-sialylated structures in the regulation of immune response.

CDw75 is a cell surface epitope expressed by the majority of B cells and, at a much lower level, by a subpopulation of T cells. A cDNA able to induce CDw75 expression when transfected in COS cells was found to be homologous to previously cloned ST6Gal.I cDNAs [19]. Although initially thought to be a cell surface α2,6-sialyltransferase, CDw75 was demonstrated to be an ST6Gal.I-dependent carbohydrate epitope formed by sialyl-α2,6-lactosaminyl-structures [118–121]. CDw75 expression coincides with maturation of B-cells; it begins at the stage of surface Ig+ (sIg+) and ceases with terminal differentiation into plasma cells. Along with CDw75, other ST6Gal.I-dependent cell differentiation antigens, such as HB6, HB4 and CD76, have been identified [122]. The molecular basis of CDw75 expression appears to rely on ST6Gal.I regulation. In fact, the B lymphoblastoid cell lines expressing CDw75 express also high levels of the ST6Gal.I mRNA containing the 5′ untranslated exon X [20], indicating a specific transcriptional activation of the B-lymphocyte promoter. The antibodies recognizing α2,6-sialylated antigens display a differential pattern of reactivity in B and T lymphocytes. This is due to the fact that the recognition is dependent on the number of polylactosaminic structures (i.e. the number of Galβ1,4G1cNAc disaccharide units) underlying the α2,6-linked sialic acid; in contrast to B cells, T cells express mainly polylactosaminylated sialoglycans [123].

The CD22 antigen is a cell surface sialic acid-binding lectin of the immunoglobulin superfamily [124], physically associated with the B cell antigen receptor (BCR), which is expressed on the surface of sIg+ B lymphocytcs, and specifically recognizes thc structure: NeuAcα2,6Galβ1,4G1cNAc [125,126]. According to the new nomenclature of animal sialic binding lectins, CD22 is referred to as Siglec-2 [127]. Upon BCR crosslinking, CD22 undergoes phosphorylation of tyrosine residues, triggering a cascade of biochemical events leading ultimately to a down-regulation of the immune response [128]. The nature of CD22 as a negative co-receptor is confirmed by the phenotype of CD22 knock out mice, which shows an augmented immune response and an increased serum concentration of autoantibodies [129]. However, in given circumstances, CD22 may transduce stimulatory signals as well [125,130]. CD22 shows a strict specificity for α2,6-sialylated lactosaminyl-structures, regardless of the underlying core structure (N-linked, O-linked or gangliosides), with a preference for multivalent ligands [131–133]. Many CD22-based cellular interactions have been reported to occur among B-lymphocytes and between B-lymphocytes and other cell types. For example, CD22 on B cells interacts with CD45RO (an isoform of the leukocyte-specific receptor-linked phosphotyrosine phosphatases) on T lymphocytes [134,135] and with CDw75 on B-lymphocytes [135]. The CD45RA isoform, which is expressed on the surface of mature naive T-lymphocytes is, in turn, a preferred substrate for ST6Gal.I, accounting for the stronger SNA-reactivity displayed by medullar thymocytes [136]. The expression of binding sites for CD22 on endothelial cells can be enhanced by LPS and inflammatory cytokines (IL-1 and TNF-α) through an ST6Gal. I-mediated sialylation of adhesion molecules such as E-selectin, ICAM-1 and VCAM-1 (see above) [54,55]. Unlike other mammalian lectins which are specific for relatively uncommon structures, CD22 binds a sequence commonly expressed by many glycoproteins and cell lines. The problem of the fine regulation of CD22-based interactions is still open but at least two mechanisms might play a role. First, it has been demonstrated that the α2,6-sialylation of N-linked chains of CD22, catalyzed by ST6Gal.I, abrogates the binding ability of the lectin [137,138]. If one considers that the developmental stage of B-lymphocytes characterized by CD22 expression coincides with a high ST6Gal.I expression supported by transcription of the X-form, a simple model could be hypothesized in which the high α2,6-sialylation of CD22 on B-lymphocytes inhibits the binding with α2,6-sialylated molecules on other lymphocytes, thus preventing undesired cellular interactions. Moreover, CD22 is inactivated by this mechanism on the surface of resting B-cells but is readily activated upon B-cell stimulation [139]. Second, IgM and haptoglobin (but not other α2,6-sialylated glycoproteins) are able to inhibit CD22 binding at the concentration found in plasma [55]. Thus, CD22-mediated interactions would be allowed in lymph and lymphoid organs, where the concentration of the above mentioned glycoproteins is lower, and inhibited in plasma. This picture is made even more complicated by the presence of a cell surface form of ST6Gal.I in B lymphocytes which has been reported to be able to sialylate a subset of surface molecules on B lymphocytes [140].

Important insights into the biological function of the sialyl α2,6-lactosaminyl-linkage can be obtained by the study of the transgenic mouse model carrying an homozygous inactivation of the ST6Gal.I gene [117]. Although these mice appear to be normal, they show a markedly reduced proliferation of B lymphocytes in response to LPS or IgM crosslinking or CD4O ligation, and a severely impaired ability in mounting an antibody response against both T-independent and T-dependent antigens. Interestingly, this defect can be rescued by the addition of interleukin-4. Together, the alterations of B cells function displayed by these mice cannot be simply explained by a reduced number of CD22-ligands interactions but are consistent with alterations of the intracellular transduction pathways involved in cell activation.

Concluding remarks

The fact that ST6Gal.I-deficient mice are viable and appear to be largely normal indicates that ST6Gal.I deficiency is compatible with a grossly normal development, while the alterations of the immune functions point to a peculiar role of this enzyme and of the cognate sugar linkage in the immune system. Nevertheless, more subtle alterations of other biological functions could be present, but are more difficult to demonstrate. The highly conserved very complex pattern of tissue- and stage-specific regulation shown by ST6Gal.I is consistent with a fundamental role of this enzyme. The detailed study of ST6Gal.I-genetically modified cell lines and of transgenic animals deficient for tissue-specific forms of ST6Gal.I, will provide an important new insight into the biology of the sialyl-α2,6-lactosaminyl-linkage.

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References

1 Paulson JC, Beranek WE, Hill RL, *J Biol Chem* **252**, 2356–62 (1977).
2 Paulson JC, Rearick JI, Hill RL, *J Biol Chem* **252**, 2363–71 (1977).
3 Sticher U, Gross HJ, Brossmer R, *Biochem J* **253**, 577–80 (1988).
4 Weinstein J, de Souza U, Paulson JC, *J Biol Chem* **257**, 13835–44 (1982).
5 Sticher U, Gross HJ, Brossmer R, *Glycoconj J* **8**, 45–54 (1991).
6 Svensson EC, Soreghan B, Paulson JC, *J Biol Chem* **265**, 20863–8 (1990).
7 Colley KJ, Lee EU, Paulson JC, *J Biol Chem* **267**, 7784–93 (1992).
8 Dahdal RY, Colley KJ, *J Biol Chem* **268**, 26310–19 (1993).
9 Munro S, *EMBO J* **10**, 3577–88 (1991).
10 Wong SH, Low SH, Hong W, *J Cell Biol* **117**, 245–58 (1992).
11 Weinstein J, Lee EU, McEntee K, Lai PH, Paulson JC, *J Biol Chem* **262**, 17735–43 (1987).
12 Wen DX, Svensson EC, Paulson JC, *J Biol Chem* **267**, 2512–8 (1992).
13 O'Hanlon TP, Lau KM, Wang XC, Lau JT, *J Biol Chem* **264**, 17389–94 (1989).
14 Paulson JC, Weinstein J, Schauer A, *J Biol Chem* **264**, 10931–4 (1989).
15 O'Hanlon TP, Lau JT, *Glycobiology* **2**, 257–66 (1992).
16 Wang X, O'Hanlon TP, Young RF, Lau JT, *Glycobiology* **1**, 25–31 (1990).
17 Lance P, Lau KM, Lau JT, *Biochem Biophys Res Commun* **164**, 225–32 (1989).
18 Grundmann U, Nerlich C, Rein T, Zettlmeissl G, *Nucleic Acids Res* **18**, 667 (1990).
19 Stamenkovic I, Asheim HC, Deggerdal A, Blomhoff HK, Smeland EB, Funderud S, *J Exp Med* **172**, 641–3 (1990).
20 Wang X, Vertino A, Eddy RL, Byers MG, Jani-Sait SN, Shows TB, Lau JT, *J Biol Chem* **268**, 4355–61 (1993).
21 Aas-Eng DA, Asheim HC, Deggerdal A, Smeland E, Funderud S, *Biochim Biophys Acta* **1261**, 166–9 (1995).
22 Dall'Olio F, Chiricolo M, Ceccarelli C, Minni F, Marrano D, Santini D, *Int J Cancer* **88**, 58–65 (2000).
23 Dall'Olio F, Chiricolo M, Lau JT, *Int J Cancer* **81**, 243–7 (1999).
24 Lo NW, Lau JT, *Biochem Biophys Res Commun* **228**, 380–5 (1996).
25 Hamamoto T, Kawasaki M, Kurosawa N, Nakaoka T, Lee YC, Tsuji S, *Bioorg Med Chem* **1**, 141–5 (1993).
26 Kurosawa N, Kawasaki M, Hamamoto T, Nakaoka T, Lee YC, Arita M, Tsuji S, *Eur J Biochem* **219**, 375–81 (1994).
27 Mercier D, Wierinckx A, Oulmouden A, Gallet PF, Palcic MM, Harduin-Lepers A, Delannoy P, Petit JM, Leveziel H, Julien R, *Glycobiology* **9**, 851–63 (1999).
28 Kalcheva I, Elliott RW, Dalziel M, Lau JT, *Mamm Genome* **8**, 619–20 (1997).
29 Wuensch A, Huang RY, Ewing J, Liang X, Lau JT, *Glycobiology* **10**, 67–75 (2000).
30 Hu YP, Dalziel M, Lau JT, *Glycoconj J* **l4**, 407–11 (1997).
31 Kaneko Y, Yamamoto H, Colley KJ, Moskal JR, *J Histochem Cytochem* **43**, 945–54 (1995).
32 Kitagawa H, Paulson JC, *J Biol Chem* **269**, 17872–8 (1994).
33 Svensson EC, Conley PB, Paulson JC, *J Biol Chem* **267**, 3466–72 (1992).
34 Lo NW, Lau JT, *Glycobiology* **6**, 271–9 (1996).
35 Taniguchi A, Hasegawa Y, Higai K, Matsumoto K, *Glycobiology* **10**, 623–8 (2000).
36 Lo NW, Lau JT, *Glycobiology* **9**, 907–14 (1999).
37 Aasheim HC, Aas-Eng DA, Deggerdal A, Blomhoff HK, Funderud S, Smeland EB, *Eur J Biochem* **213**, 467–75 (1993).
38 Fast DG, Jamieson JC, McCaffrey G, *Biochim Biophys Acta* **1202**, 325–30 (1993).
39 Ma J, Colley KJ, *J Biol Chem* **271**, 7758–66 (1996).
40 Ma J, Qian R, Rausa FM, III, Colley KJ, *J Biol Chem* **272**, 672–9 (1997).
41 Ma J, Simonovic M, Qian R, Colley KJ, *J Biol Chem* **274**, 8046–52 (1999).
42 Kaplan HA, Woloski BM, Hellman M, Jamieson JC, *J Biol Chem* **258**, 11505–9 (1983).
43 Colley KJ, Lee EU, Adler B, Browne JK, Paulson JC, *J Biol Chem* **264**, 17619–22 (1989).
44 Lammers G, Jamieson JC, *Biochem J* **256**, 623–31 (1988).
45 Richardson K, Jamieson JC, *Comp Biochem Physiol B Biochem Mol Biol* **110**, 445–50 (1995).
46 Wang XC, O'Hanlon TP, Lau JT, *J Biol Chem* **264**, 1854–9 (1989).
47 Wang XC, Smith TJ, Lau JT, *J Biol Chem* **265**, 17849–53 (1990).
48 Vandamme V, Pierce A, Verbert A, Delannoy P, *Eur J Biochem* **211**, 135–40 (1993).
49 Hamr A, Vlasakova V, Kolinska J, *Biochim Biophys Acta* **1157**, 285–9 (1993).
50 Kolinska J, Zakostelecka M, Hamr A, Baudysova M, *J Steroid Biochem Mol Biol* **58**, 289–97 (1996).
51 Coughlan CM, Breen KC, *J Neurosci Res* **51**, 619–26 (1998).
52 Baumann H, Gauldie J, *Immunol Today* **15**, 74–80 (1994).

53 Dalziel M, Lemaire S, Ewing J, Kobayashi L, Lau JT, *Glycobiology* **9**, 1003–8 (1999).
54 Hanasaki K, Varki A, Stamenkovic I, Bevilacqua MP, *J Biol Chem* **269**, 10637–43 (1994).
55 Hanasaki K, Varki A, Powell LD, *J Biol Chem* **270**, 7533–42 (1995).
56 Gagneux P, Varki A, *Glycobiology* **9**, 747–55 (1999).
57 Karlsson KA, *Curr Opin Struct Biol* **5**, 622–35 (1995).
58 Grollman EF, Saji M, Shimura Y, Lau JT, Ashwell G, *J Biol Chem* **268**, 3604–9 (1993).
59 Helton TE, Magner JA, *Endocrinology* **134**, 2347–53 (1994).
60 Shah S, Lance P, Smith TJ, Berenson CS, Cohen SA, Horvath PJ, Lau JT, Baumann H, *J Biol Chem* **267**, 10652–8 (1992).
61 Bessler H, Singer R, Raanani P, Levinsky H, Lahav M, Cohen AM, *Biol Reprod* **53**, 1474–7 (1995).
62 Fayos BE, Bartles JR, *J Biol Chem* **269**, 2151–7 (1994).
63 Stibler H, Borg S, *Alcohol Clin Exp Res* **10**, 61–4 (1986).
64 Malagolini N, Dall'Olio F, Serafini-Cessi F, Cessi C, *Alcohol Clin Exp Res* **13**, 649–53 (1989).
65 Xin Y, Lasker JM, Lieber CS, *Hepatology* **22**, 1462–8 (1995).
66 Rao MN, Lakshman MR, *Metabolism* **48**, 797–803 (1999).
67 Malagolini N, Dall'Olio F, Turrini I, Cessi C, Serafini-Cessi F, *Alcohol Clin Exp Res* **18**, 1386–91 (1994).
68 Hakomori S, Patterson CM, Nudelman E, Sekiguchi K, *J Biol Chem* **258**, 11819–22 (1983).
69 Nilsson O, Lindholm L, Holmgren J, Svennerholm L, *Biochim Biophys Acta* **835**, 577–83 (1985).
70 Shibuya N, Goldstein IJ, Broekaert WF, Nsimba-Lubaki M, Peeters B, Peumans WJ, *J Biol Chem* **262**, 1596–601 (1987).
71 Dall'Olio F, Trere D, *Eur J Histochem* **37**, 257–65 (1993).
72 Sata T, Roth J, Zuber C, Stamm B, Heitz PU, *Am J Pathol* **139**, 1435–48 (1991).
73 Yamashita K, Fukushima K, Sakiyama T, Murata F, Kuroki M, Matsuoka Y, *Cancer Res* **55**, 1675–9 (1995).
74 Vierbuchen MJ, Fruechtnicht W, Brackrock S, Krause KT, Zienkiewicz TJ, *Cancer* **76**, 727–35 (1995).
75 Murayama T, Zuber C, Seelentag WK, Li WP, Kemmner W, Heitz PU, Roth J, *Int J Cancer* **70**, 575–81 (1997).
76 Taatjes DJ, Roth J, *Eur J Cell Biol* **46**, 289–98 (1988).
77 Taatjes DJ, Roth J, *Eur J Cell Biol* **53**, 255–66 (1990).
78 King TP, Begbie R, Slater D, McFadyen M, Thom A, Kelly D, *Glycobiology* **5**, 525–34 (1995).
79 Dall'Olio F, Malagolini N, Di Stefano G, Minni F, Marrano D, Serafini-Cessi F, *Int J Cancer* **44**, 434–9 (1989).
80 Gangopadhyay A, Perera SP, Thomas P, *Hybridoma* **17**, 117–23 (1998).
81 Gessner P, Riedl S, Quentmaier A, Kemmner W, *Cancer Lett* **75**, 143–9 (1993).
82 Kemmner W, Kruck D, Schlag P, *Clin Exp Metastasis* **12**, 245–54 (1994).
83 Dall'Olio F, Malagolini N, Serafini-Cessi F, *Biochem Biophys Res Commun* **184**, 1405–10 (1992).
84 Dall'Olio F, Malagolini N, Guerrini S, Lau JT, Serafini-Cessi F, *Glycoconj J* **13**, 115–21 (1996).
85 Dall'Olio F, Malagolini N, Di Stefano G, Ciambella M, Serafini-Cessi F, *Biochem J* **270**, 519–24 (1990).
86 Vertino-Bell A, Ren J, Black JD, Lau JT, *Dev Biol* **165**, 126–36 (1994).
87 Li M, Andersen V, Lance P, *Clin Sci (Colch)* **89**, 397–404 (1995).
88 Kudo T, Ikehara Y, Togayachi A, Morozumi K, Watanabe M, Nakamura M, Nishihara S, Narimatsu H, *Lab Invest* **78**, 797–811 (1998).
89 Petretti T, Kemmner W, Schulze B, Schlag PM, *Gut* **46**, 359–66 (2000).
90 Miyagi T, Koseki M, Tsuiki S, *Jpn J Cancer Res* **79**, 742–9 (1988).
91 Taki T, Yamamoto K, Takamatsu M, Ishii K, Myoga A, Sekiguchi K, Ikeda I, Kurata K, Nakayama J, Handa S, Matsumoto M, *Cancer Res* **50**, 1284–90 (1990).
92 Pousset D, Piller V, Bureaud N, Monsigny M, Piller F, *Cancer Res* **57**, 4249–56 (1997).
93 Nevins JR, *Nature* **358**, 375–6 (1992).
94 Easton EW, Bolscher JG, van den Eijnden DH, *J Biol Chem* **266**, 21674–80 (1991).
95 Le Marer N, Laudet V, Svensson EC, Cazlaris H, Van Hille B, Lagrou C, Stehelin D, Montreuil J, Verbert A, Delannoy P, *Glycobiology* **2**, 49–56 (1992).
96 Peeper DS, Upton TM, Ladha MH, Neuman E, Zalvide J, Bernards R, DeCaprio JA, Ewen ME, *Nature* **386**, 177–81 (1997).
97 Jain N, Sudhakar C, Das MR, *FEBS Lett* **317**, 147–51 (1993).
98 Recchi MA, Hebbar M, Hornez L, Harduin-Lepers A, Peyrat JP, Delannoy P, *Cancer Res* **58**, 4066–70 (1998).
99 Skacel PO, Edwards AJ, Harrison CT, Watkins WM, *Blood* **78**, 1452–60 (1991).
100 Fukushima K, Hara-Kuge S, Seko A, Ikehara Y, Yamashita K, *Cancer Res* **58**, 4301–6 (1998).
101 Kaneko Y, Yamamoto H, Kersey DS, Colley KJ, Leestma JE, Moskal JR, *Acta Neuropathol (Berl)* **91**, 284–92 (1996).
102 David L, Nesland JM, Funderud S, Sobrinho-Simoes M, *Cancer* **72**, 1522–7 (1993).
103 Petretti T, Schulze B, Schlag PM, Kemmner W, *Biochim Biophys Acta* **1428**, 209–18 (1999).
104 Taniguchi A, Higai K, Hasegawa Y, Utsumi K, Matsumoto K, *FEBS Lett* **441**, 191–4 (1998).
105 Le Marer N, Skacel PO, *J Cell Physiol* **179**, 315–24 (1999).
106 Passaniti A, Hart GW, *J Biol Chem* **263**, 7591–603 (1988).
107 Dall'Olio F, Malagolini N, Di Stefano G, Ciambella M, Serafini-Cessi F, *Int J Cancer* **47**, 291–7 (1991).
108 Roth J, Li WP, Knibbs RN, MacCallum DK, Song Z, Goldstein IJ, *Proc Natl Acad Sci USA* **91**, 11353–7 (1994).
109 Song Z, Varani J, Goldstein IJ, *Int J Cancer* **55**, 1029–35 (1993).
110 Dall'Olio F, Malagolini N, Serafini-Cessi F, *Int J Cancer* **50**, 325–30 (1992).
111 Bresalier RS, Rockwell RW, Dahiya R, Duh QY, Kim YS, *Cancer Res* **50**, 1299–307 (1990).
112 Le Marer N, Stehelin D, *Glycobiology* **5**, 219–26 (1995).
113 Takano R, Muchmore E, Dennis JW, *Glycobiology* **4**, 665–74 (1994).
114 Dall'Olio F, Chiricolo M, Lollini P, Lau JT, *Biochem Biophys Res Commun* **211**, 554–61 (1995).
115 Dall'Olio F, Mariani E, Tarozzi A, Meneghetti A, Chiricolo M, Lau JT, Facchini A, *Glycobiology* **7**, 507–13 (1997).
116 Yamamoto H, Kaneko Y, Rebbaa A, Bremer EG, Moskal JR, *J Neurochem* **68**, 2566–76 (1997).
117 Hennet T, Chui D, Paulson JC, Marth JD, *Proc Natl Acad Sci USA* **95**, 4504–9 (1998).
118 Bast BJ, Zhou LJ, Freeman GJ, Colley KJ, Ernst TJ, Munro JM, Tedder TF, *J Cell Biol* **116**, 423–35 (1992).

119 Guy K, Andrew JM, *Immunology* **74**, 206–14 (1991).
120 Keppler OT, Moldenhauer G, Oppenlander M, Schwartz-Albiez R, Berger EG, Funderud S, Pawlita M, *Eur J Immunol* **22**, 2777–81 (1992).
121 Munro S, Bast BJ, Colley KJ, Tedder TF, *Cell* **68**, 1003 (1992).
122 De Lau WB, Kuipers J, Voshol H, Clevers H, Bast BJ, *J Immunol* **150**, 4911–9 (1993).
123 Kniep B, Schakel K, Nimtz M, Schwartz-Albiez R, Schmitz M, Northoff H, Vilella R, Gramatzki M, Rieber EP, *Glycobiology* **9**, 399–406 (1999).
124 Powell LD, Varki A, *J Biol Chem* **270**, 14243–6 (1995).
125 Law CL, Sidorenko SP, Clark EA, *Immunol Today* **15**, 442–9 (1994).
126 Sgroi D, Varki A, Braesch-Andersen S, Stamenkovic I, *J Biol Chem* **268**, 7011–8 (1993).
127 Crocker PR, Clark EA, Filbin M, Gordon S, Jones Y, Kehrl JH, Kelm S, Le Douarin N, Powell L, Roder J, Schnaar RL, Sgroi DC, Stamenkovic K, Schauer R, Schachner M, van den Berg TK, van der Merwe PA, Watt SM, Varki A, *Glycobiology* **8**, v (1998).
128 Doody GM, Justement LB, Delibrias CC, Matthews RJ, Lin J, Thomas ML, Fearon DT, *Science* **269**, 242–4 (1995).
129 O'Keefe TL, Williams GT, Davies SL, Neuberger MS, *Science* **274**, 798–801 (1996).
130 Tuscano J, Engel P, Tedder TF, Kehrl JH, *Blood* **87**, 4723–30 (1996).
131 Powell LD, Sgroi D, Sjoberg ER, Stamenkovic I, Varki A, *J Biol Chem* **268**, 7019–27 (1993).
132 Powell LD, Varki A, *J Biol Chem* **269**, 10628–36 (1994).
133 Powell LD, Jain RK, Matta KL, Sabesan S, Varki A, *J Biol Chem* **270**, 7523–32 (1995).
134 Sgroi D, Koretzky GA, Stamenkovic I, *Proc Natl Acad Sci USA* **92**, 4026–30 (1995).
135 Stamenkovic I, Sgroi D, Aruffo A, Sy MS, Anderson T, *Cell* **66**, 1133–44 (1991).
136 Baum LG, Derbin K, Perillo NL, Wu T, Pang M, Uittenbogaart C, *J Biol Chem* **271**, 10793–9 (1996).
137 Braesch-Andersen S, Stamenkovic I, *J Biol Chem* **269**, 11783–6 (1994).
138 Sgroi D, Nocks A, Stamenkovic I, *J Biol Chem* **271**, 18803–9 (1996).
139 Razi N, Varki A, *Proc Natl Acad Sci USA* **95**, 7469–74 (1998).
140 Gross HJ, Merling A, Moldenhauer G, Schwartz-Albiez R, *Blood* **87**, 5113–26 (1996).

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