Minireviews

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Tn antigens and their significance in oncology

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Tn and sialyl-Tn carbohydrate structures, first identified in glycophorins of persons with the rare Tn syndrome, were found to be present on the surface of most cancer cells. In this article, the studies on Tn and sialyl-Tn antigens as diagnostic and prognostic tumor markers and as immunogens in vaccines for cancer immunotherapy are shortly reviewed.

The Tn syndrome

It was found over 30 years ago that erythrocytes of some persons show a mixed-field polyagglutinability, i.e. agglutination of a portion (5–95%) of red blood cells (called Tn erythrocytes) by most normal human sera [1, 2]. The Tn erythrocytes were also agglutinated by some lectins (e.g. from *Salvia sclarea*), but not by peanut agglutinin reacting with another known type of polyagglutinable erythrocytes carrying Thomsen-Friedenreich (TF)[1] determinants. It was shown soon that Tn erythrocytes have glycophorins with truncated oligosaccharide chains. Glycophorins are the major sialoglycoproteins of erythrocyte membranes. They contain multiple O-linked oligosaccharide chains, with the tetrasaccharide NeuAc-α2-3Galβ1-3[NeuAcα2-6]GalNAcα1- as the major component. The mentioned TF polyagglutinable erythrocytes contain desialylated glycophorins with Galβ1-3GalNAcα1- chains (reviewed in [3]). In glycophorins of Tn erythrocytes the O-glycans are not galactosylated, and the Tn glycophorins express GalNAcα1-Ser/Thr (Tn) and NeuAcα2-6GalNAcα1-Ser/Thr (sialyl-Tn, sTn) structures [3–6]. The Tn polyagglutinability is an acquired long-lasting syndrome. It is found rarely, in less than 1 per 100 000 persons.

As expected, the Tn erythrocytes lack the 3-β-D-galactosyltransferase(3βGal-transferase) activity [7, 8]. The expression of Tn structures and lack of 3βGal-transferase activity were also found in a variable proportion of other cells (platelets, lymphocytes, myeloid cells, and hematopoietic progenitors) of Tn individuals [9–12]. The available data led to the conclusion that the enzyme deficiency arises from a somatic stem cell mutation resulting in clonal penetration of the mutated cells into the various hematopoietic lineages. However, the recent results draw attention to the possibility that an allele of 3βGal-transferase gene in persons with the Tn syndrome may be persistently but reversibly repressed instead of being mutated. This possibility was indicated by the reactivation of expression of 3βGal-transferase in cloned enzyme-deficient T cells from a Tn patient by such inducers of gene expression as 5-azacytidine or sodium *n*-butyrate [13].

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[1]Abbreviations: 3βGal-transferase, 3-β-D-galactosyltransferase; KLH, keyhole limpet hemocyanin; MAb, monoclonal antibody; OSM, ovine submaxillary mucin; sTn, sialyl-Tn; TF, Thomsen-Friedenreich.

The persons with the Tn syndrome frequently manifest hematological disorders (hemolytic anemia, thrombopenia, leukopenia, etc.) which are most likely caused by autoimmune reactions involving natural anti-Tn antibodies commonly present in human sera ([1] and refs. in [13]).

Occurrence of Tn and sTn determinants in normal tissues

In normal individuals the Tn and sTn structures are present in "cryptic" (i.e. substituted by other sugars) form in all glycoproteins carrying O-linked oligosaccharide chains. In some of poly-O-glycosylated glycoproteins (glycophorins, leukosialins, mucins) only a minor amount of Tn/sTn structures is present in the unsubstituted form due to the microheterogeneity of glycosylation [14–16]. The exceptions are some animal mucins in which NeuAc-GalNAc- or GalNAc-chains are dominant structures, e.g. ovine submaxillary mucin (OSM, sTn antigen) [15] or armadillo submandibular glycoprotein (Tn antigen) [17].

The interest in Tn and sTn antigens and in reagents detecting these structures has greatly increased when these antigens were found to be commonly present in human carcinomas [18].

Antibodies and lectins recognizing Tn and sTn structures

In initial studies on Tn antigens, lectins and human "natural" antibodies were used. The well known lectins from S. sclarea, Vicia villosa (both anti-Tn) and Helix pomatia (anti-Tn/TF) were most frequently applied. The recently characterized anti-Tn lectin from Moluccella laevis [19] is also a promising reagent. During the last decade several anti-Tn and anti-sTn monoclonal antibodies have been obtained and applied in cancer studies [15, 20–28]. Various immunogens are used for the MAbs production (Tn erythrocytes, cancer cells, asialo-agalacto-glycophorin, OSM). The antibodies differ in fine specificity. There are anti-Tn MAbs which cross-react or do not cross-react with sTn antigen. Some anti-Tn MAbs recognize GalNAc-α-residues independently of its linkage position and cross-react with blood group A antigen which is an oligosaccharide with nonreducing terminal GalNAcα-residue. Other MAbs are more specific and recognize only those GalNAc-

α-residues which are linked to Ser/Thr residues and react with all or most Tn glycoproteins. Finally, there are MAbs which recognize GalNAcα-Ser/Thr residues in the context of the surrounding structure present in the immunogen used. Such MAbs show a distinct preference for the Tn antigen used for immunization over other Tn glycoproteins [27]. Some MAbs (if not most of them) require epitopes containing clusters of GalNAcα-residues linked to adjacent Ser or Thr residues [26], whereas anti-Tn lectins are able to react also with single GalNAcα-residues [16, 19]. Therefore, it should be realized that the results obtained with different anti-Tn/sTn reagents may show quantitative (or even qualitative) differences dependent on the specificity and affinity of the reagent used.

Presence of Tn and sTn antigens in cancer cells

Most studies were performed by immunohistochemical staining of tissue sections with the use of anti-Tn or anti-sTn monoclonal antibodies [15, 20, 21, 23, 24, 28–40], or V. villosa lectin [24, 32, 37, 38, 41]. The occurrence of Tn/sTn antigens in a large variety of tumor cells and their absence in normal tissues is more striking than those observed with other tumor-associated antigens. The Tn and sTn antigens have been found (usually both, in various proportions) in most cases of breast, ovary and digestive tract cancers, and in a high proportion of lung, urinary bladder and uterine cervix cancers (Table 1). The expression of these antigens may be higher in metastatic lesions than in the primary tumor [29, 37]. The Tn/sTn antigens have been also found in some non- or pre-malignant lesions, e.g colorectal polyps in which sTn antigen (but not Tn) was selectively expressed by polyps with a greater malignant potential [42]. In most studies the normal tissues were not stained, or were stained weakly. However, the Tn/sTn antigens were found in fetal tissues which indicates that they belong to a group of oncodevelopmental antigens [24].

Some differences in the expression of Tn/sTn antigens (frequency of positive specimens, intensity of staining, distribution on the cell surface) in cancer tissues were shown to be dependent on the histological type of tumor, degree of differentiation, depth of invasion, tumor grade and location, etc. [24, 28, 30, 33, 35, 38, 39]. For example, the antigens were found

Table 1
Detection of Tn and sTn antigens in human tumor cells

Type of cancer	sTn and/or Tn[a] from – to	References
Breast carcinoma	++ – +++	15, 18, 20, 21, 23, 29, 30, 40
Lung cancers	+ – +++	15, 18, 20, 21, 23
Digestive tract cancers		
pancreas	++ – +++	15, 18, 20, 28, 31, 32
stomach	++ – +++	15, 18, 20, 23, 28, 35, 38
colon	++ – +++	15, 20, 21, 23, 24, 28, 31
liver	n.d.	15, 28
Urinary bladder carcinoma	+ – +++	34, 41
Gynecological cancers		
ovary	++ – +++	23, 33, 39
uterine cervix	+ – ++	36, 37
Melanoma	n.d. – +	23, 29
Sarcoma	n.d.	29

[a]The content of Tn and/or sTn antigens is given as frequency of the positive staining of specimens from different patients: +++, over 90%; ++, 50–90%; and +, below 50%; n.d., not detectable.

in most specimens of adenocarcinoma, large cell and squamous carcinoma of the lung, but were not detectable in the small cell lung carcinoma [20]. Despite some discrepancies between the results of different studies, most authors agree that expression of Tn and, particularly, of sTn antigen, is correlated with a poor prognosis in some types of cancer or premalignant lesions.

Serum levels of sTn antigen were found to be increased in patients with ovarian and other gynecological cancers [43–45] and digestive tract cancers [46]. In ovarian cancer the sTn showed the highest sensitivity and specificity when compared with other serum markers (sialyl-Le^x, CA19-9, CA125, CEA, tissue polypeptide antigen) and is considered to be an independent predictor of poor prognosis [43, 45].

The Tn and sTn determinants of cancer cells are located mostly in mucins which are high molecular mass poly-O-glycosylated glycoproteins. The metabolic events leading to the expression of Tn and sTn antigens in cancer are apparently different than in the Tn syndrome. The level of 3βGal-transferase in colonic cancerous tissues and cell lines and in normal colonic tissue was found to be similar [47]. Most

probably the regulation of mucin oligosaccharide biosynthesis in normal cells is different from that in malignant cells.

The Tn and sTn antigens as targets for cancer immunotherapy

The ubiquitous presence of Tn and sTn antigens in most carcinomas prompted the studies on their use as anti-tumor vaccines. Carbohydrates in general are not known to activate T cells, though there are some reports that they can cause T cell stimulation. The aim of the initial experiments was to find out whether the TF, Tn and sTn carbohydrate epitopes can be recognized by T cells and develop the cellular immune response which could act as a powerful defense against cancer. This question was first addressed 8 years ago by the group of Longenecker in Canada, and their animal model were mice challenged with the highly lethal transplantable mammary adenocarcinoma TA3-Ha cell line [48]. The TA3-Ha cells carry epiglycanin, a high molecular mass mucin-like glycoprotein containing multiple TF- and Tn-type O-glycans. Priming the mice with irradiated TA3-Ha cells or epiglycanin resulted in longer survival time after the subsequent challenge with live TA3-Ha cells in

comparison with the unprimed animals. More-over, delayed-type hypersensitivity was elicited and the effector T cells with the helper phenotype responded specifically to TF and Tn carbohydrate determinants in a major histocompatibility complex-restricted manner. The specificity of the response was established by using synthetic conjugates of several mono- and oligosaccharides with a carrier protein (human serum albumin or keyhole limpet hemocyanin). Similar results were obtained by the group of Hakomori who used asialoOSM (Tn antigen) [49] or synthetic dimeric Tn/lipopeptide conjugate [50] as immunogens. Immunization of mice with asialoOSM with Ribi adjuvant (containing monophosphoryl lipid A and *Bacillus Calmette-Guérin* cell wall skeleton — Ribi ImmunoChem, Hamilton, MT, U.S.A.) provided protection against lethal challenge of TA3-Ha cells, elicited production of IgG and IgM anti-Tn antibodies, induced the antigen-specific T cell response (CD4$^+$ IL-2-secreting T cells) and delayed type hypersensitivity detected by response to footpad injections with irradiated TA3-Ha cells. That the cellular immune response was related to carbohydrate Tn determinants was indicated by the cross-reactivity of the induced T cells with asialoOSM and TA3-Ha cells (asialoOSM and epiglycanin share Tn epitopes and have different polypetide cores) and by much lower effects obtained by immunization with deglycosylated OSM polypeptide.

The results obtained in mice encouraged the authors and other researchers to apply a similar strategy in clinical studies. O'Boyle *et al.* [51] immunized colorectal cancer patients at high risk of recurrence with partially desialylated OSM (Tn+sTn antigen), without and with adjuvants, and tested the level of anti-Tn and anti-sTn antibodies in their sera. There was no response in patients immunized without adjuvant, but in the presence of DETOX (containing *Mycobacterium phlei* cell wall skeleton) or *Bacillus Calmette-Guérin* adjuvants (Ribi ImmunoChem) 4/8 and 5/6 patients, respectively, responded with a significant increase in anti-Tn and anti-sTn antibodies. Toxicity was limited to inflammatory skin reactions at the site of vaccination, caused by the adjuvants. No delayed type hypersensitivity response was detected. The group of Longenecker, after the experience with synthetic TF-KLH antigen applied for treating the ovarian carcinoma patients [52], used the synthetic sTn hapten conjugated to KLH with DETOX adjuvant for immunizing the patients with metastatic breast cancer [53, 54]. Immunization was preceded by a single low dose of cyclophosphamide to inhibit suppressor cells. Similarly to the previous studies, the side effects were restricted to local cutaneous reactions and all patients developed IgM and IgG antibodies specific for sTn (reactive with sTn-KLH, sTn-human serum albumin and OSM, but not with TF antigens). The titers of complement-mediated cytotoxic antibodies, partially inhibited by synthetic sTn hapten, also were increased. The authors stress that it is too early for evaluation of clinical efficacy on the basis of a small pilot study. However, at the time the results were published, only three of the 12 patients treated had died from widespread cancer, five were alive for 12 or more months, and 4 patients were alive for 6 months after entry into the program.

The results of clinical studies showed unequivocally that the Tn/sTn antigens and adjuvants used can be administered safely and are recognized by the human immune system. At the present "state of the art", albeit the results seem to be promising, it is difficult to predict the beneficial effects of active Tn/sTn-specific immunotherapy in cancer patients. The immunological response is dependent on many factors, including cytokines which were found to be powerful adjuvants for the immune response to sTn antigen (OSM) in mice [55]. Efforts are undertaken to increase the immunogenecity of the carbohydrate vaccines by looking for more effective carrier proteins and adjuvants.

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