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Thomsen-Friedenreich-related carbohydrate antigens in normal adult human tissues: a systematic and comparative study

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Abstract A broad variety of normal human tissues were examined for the expression of Thomsen-Friedenreich (TF)-related histo-blood group antigens, TF (Galβ1-3Gal*N*Acα1-R), Tn (TF precursor, Gal*N*Acα1-R), sialosyl-Tn (NeuAcα2-6Gal*N*Acα1-R), considered to be useful in cancer diagnosis and immunotherapy, and sialosyl-TF, the cryptic form of TF. These antigens or, more correctly, glycotopes, were determined by immunohistochemistry with at least two monoclonal antibodies (mAbs) each (except sialosyl-TF) as well as by lectin histochemistry. For a better dissection of sialosyl-TF and TF glycotopes, tissue sections were pretreated with galactose oxidase or the galactose oxidase-Schiff sequence. Staining with mAbs appeared to be more restricted than with the lectins used. Distribution patterns among normal epithelia were different for all four antigens. These antigens were also detected in some non-epithelial tissues. They can be classified in the following sequence according to the frequency of their occurrence in normal tissues: sialosyl-TF > >sialosyl-Tn >Tn >TF. Most of the positively staining sites for TF, Tn, and sialosyl-Tn are located in immunologically privileged areas. The complex results obtained with anti-TF mAbs (after treatment of the tissue sections with sialidase from *Vibrio cholerae*) and the lectins amaranthin and jacalin revealed a differential distribution of the subtypes of sialosyl-TF [NeuAcα2-3Galβ1-3Gal*N*Acα1-R and Galβ1-3 (NeuAcα2-6)Gal*N*Acα1-R] in normal human tissues. From our data it can be inferred that TF, Tn, and sialosyl-Tn are promising targets for a cancer vaccine.

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Introduction

Thomsen-Friedenreich-related antigens (TFRA) are a group of biosynthetically linked so-called histo-blood group carbohydrate antigens (Clausen and Hakomori 1989). The epitope (glycotope) structure of Thomsen-Friedenreich antigen (TF) is Galβ1-3Gal*N*Acα1-R (Kim and Uhlenbruck 1966). The precursor of TF is Tn antigen, whose glycotope is Gal*N*Acα1-R (Springer and Desai 1974). The cryptic forms of TF and Tn are defined as sialosyl-TF (s-TF) and sialosyl-Tn (s-Tn), respectively (Desai and Springer 1979; Kurosaka et al. 1983). TF, Tn, and s-Tn have been reported as oncofetal antigens expressed on epithelial cells (Springer 1984; Kjeldsen et al. 1988). Employing monoclonal antibodies (mAbs) and lectins, they have been examined in experimental and clinical studies for tumor diagnosis (Itzkowitz et al. 1990; Springer et al. 1990). Since they are strongly expressed on the surface of tumor cells and are able to induce humoral as well as cellular immune responses (Fung et al. 1990), there is reason to assume that these carbohydrate structures provide one or more suitable determinants for a successful tumor vaccine (Springer et al. 1995). To this end, comprehensive knowledge of the occurrence of TFRA in normal adult human tissues is of the utmost importance. Several groups have examined normal tissues in the context of their studies (Mandel et al. 1991; Philipsen et al. 1991; Therkildsen et al. 1994), but a systematic and comprehensive analysis is still lacking. The study of s-TF has only recently become possible by a combination of lectin staining with conventional histochemistry (Sata et al. 1990). Furthermore, Tn shares terminal Gal*N*Ac with blood group A antigen. Therefore, possible cross-reactivities of their respective mAbs and lectins had to be considered (Hirohashi et al. 1985).

The objectives of this study were: (1) to determine the extent to which all four TFRA (TF, s-TF, Tn, s-Tn) are present (or absent) in normal adult human tissues; (2) to compare several reagents of similar nominal specificity in their reactivity with these tissues; (3) to obtain more accurate information about the distribution of TF and s-

TF through the combination of immunostaining and lectin staining with galactose oxidase or the galactose oxidase-Schiff sequence treatments; and (4) to examine possible cross-reactivities of the reagents with blood group A or B antigen. Our task was facilitated by the availability of a newly developed mAb to TF (Karsten et al. 1995). In addition, a mAb to a related but not yet fully defined epitope containing *N*-glycolylneuraminic acid (NeuGc), 3E1.2 (Devine et al. 1991), was included.

Materials and methods

Tissue specimens

Normal human tissues were obtained from immediately fixed surgical specimens or following autopsies performed less than 10 h after death. The surgical specimens included (number of cases in parentheses): skin (5), submandibular gland (2), esophagus (1), stomach (2), descending colon (4), liver (4), gall-bladder (2), pancreas (2), trachea (1), lung (3), kidney (3), bladder (4), prostate gland (1), uterus (2), cervix (2), breast (5), testis (1), nerve (4), spleen (3), lymph nodes (4), thyroid gland (1), skeletal muscle tissue (1), and synovial tissue (1). Autopsy specimens were: jejunum (2), ileum (2), heart (1), brain (3), meninges (1), thymus gland (1), and adrenal gland (1). Only autopsy specimens maintaining good histological preservation were included here. All tissue samples were fixed in 10% buffered formalin and embedded in paraffin.

Reagents

Specifications of mAbs and lectins employed in this study are listed in Table 1. A78-G/A7 (abbreviated G/A7, Karsten et al. 1995) and 12A8-C7-F5 (abbreviated 12A8, Springer et al. 1988; identical to BaGS-6) were mAbs from the authors; G/A7 was used as supernatant, and 12A8 was diluted from ascites. HH8 (Clausen et al. 1988), TKH2 (Kjeldsen et al. 1988), and 3F1 (H. Clausen, unpublished) were kindly contributed by Dr. H. Clausen (Copenhagen, Denmark). TEC-02 (purified antibody, Dráber 1987) was provided by Dr. P. Dráber (Prague, Czech Republic) and 3E1.2 (Devine et al. 1991) by Dr. F.-G. Hanisch (Cologne, Germany). The remaining reagents were purchased from the following sources: B72.3 from BioGenex (San Ramon, USA); monoclonal anti-A (81 FR2.2) and anti-B (3E7) from Dako (Hamburg, Germany), and HE-24 and HE-29 from Biogenesis (Bournemouth, UK); biotinylated lectins (peanut agglutinin, PNA; *Helix pomatia* agglutinin, HPA; amaranthin, *Amaranthus caudatus* agglutinin, ACA; jacalin, *Artocarpus integrifolia* agglutinin, AIA) from Vector Laboratories (Burlingame, USA).

Immunohistology and lectin histochemistry

Tissue sections were stained using the avidin-biotin complex (ABC) method with a commercial kit (Vectastain ABC Elite kit, Vector Laboratories). Paraffin sections, 4-µm-thick, were deparaffinized and rehydrated through a graded ethanol series. Endogenous peroxidase activity was destroyed by a 30-min treatment with 0.3% H_2O_2 in methanol. Non-specific binding sites were inhibited with normal rabbit serum, which had been absorbed with neuraminidase-treated red blood cells from blood group 0 individuals. After washing with phosphate-buffered saline (PBS), sections were incubated with mAbs or biotinylated lectins in appropriate dilutions overnight at 4° C. The thoroughly washed sections (except those stained with biotinylated lectins) were treated with biotinylated anti-mouse immunoglobulin antiserum for 30 min, and thereafter with the ABC complex at room temperature. Color development during incubation with the peroxidase substrate (diaminobenzidine) was controlled under the microscope with positive sections from colon carcinoma. Counterstaining was done with hematoxylin. Negative controls were incubated with a comparable dilution of mouse IgM and IgG1 (MOPC 104 E and MOPC 31C, respectively; Sigma, Deisenhofen, Germany) instead of the mAbs. For the immunohistochemical detection of sialosyl-TF, sections were incubated with neuraminidase from *Vibrio cholerae* (Serva, Heidelberg, Germany) at a concentration of 0.02 U/ml in PBS containing 0.01 M Ca^{++} for 1 h at room temperature to remove NeuAc, washed, and reacted with anti-TF mAb. Erythrocytes present in vessels in the tissue sections functioned as an internal positive control for sialosyl-TF.

Tissue sections from kidney were additionally treated with the avidin-biotin blocking kit (Vector Laboratories) to block endogenous biotin (Wood and Warnke 1981). Briefly, sections were incubated with the avidin blocking solution for 15 min after the routine step with normal serum, rinsed with PBS, and incubated with the biotin blocking solution for another 15 min.

Since neither the blood group type of the tissue donors nor their secretor status was known to us, we applied anti-A and anti-

Table 1 Thomsen-Friedenreich-related carbohydrate epitopes and monoclonal antibodies (*mAb*) or lectins used in this study

Antigen	Epitope structure	MAb or lectin
Thomsen-Friedenreich (TF)	Galβ1-3Gal*N*Acα1-R	A78-G/A7 HH8 Peanut agglutinin (PNA) Amaranthin (ACA) Jacalin (AIA)
Sialosyl-TF	Galβ1-3(NeuAcα2-6)Gal*N*Acα1-R and (NeuAcα2-3)Galβ1-3Gal*N*Acα1-R	A78-G/A7[a] Jacalin
	(NeuAcα2-3)Galβ1-3Gal*N*Acα1-R	Amaranthin
Tn	Gal*N*Acα-R	12A8-C7-F5 TEC-02 *Helix pomatia* agglutinin (HPA)[b]
Sialosyl-Tn	NeuAcα2-6Gal*N*Acα1-R	TKH2 B72.3 3F1
A	Gal*N*Acα1-3(Fucα1-2)Galβ1-3Glc*N*Ac-R	81 FR2.2 HE-24
B	Galα1-3(Fucα1-2)Galβ1-3Glc*N*Ac-R	3E7 HE-29

[a] After treatment of the tissue sections with neuraminidase from *Vibrio cholerae*

[b] Binds to other αGal*N*Ac-carrying structures, including blood group A

B mAbs to sections of each specimen and examined red blood cells and endothelia for reactivity. In the event of a positive reaction, we looked for possible staining of red blood cells and endothelia by the mAbs or the lectins similar to that visualized by anti-A or anti-B, indicating cross-reactivity with blood group A or B. HPA reactivity was considered HPA-specific in the absence of staining with anti-A. Tissues which became reactive towards anti-TF mAbs only after neuraminidase treatment (and also positive for amaranthin and jacalin), but not by anti-TF mAb alone, were considered to express sialosyl-TF.

In the case of positive results for both TF and s-TF, these glycotopes were differentiated by means of pretreatments with galactose oxidase or the galactose oxidase-Schiff sequence according to Sata et al. (1990) as follows. Sections were treated either with galactose oxidase (Sigma, 5 U/ml overnight at 37° C) or with galactose oxidase followed by Schiff reagent (15 min) as described by Schulte and Spicer (1983) before incubation with anti-TF antibody, amaranthin, or jacalin. Resistance of amaranthin and jacalin staining to the galactose oxidase-Schiff sequence ascertained the presence of s-TF (Sata et al. 1990).

Results

The immunohistological and lectin histochemical results are summarized in Table 2, a selection of stained tissue sections is shown in Figs. 1–9. The staining results of G/A7, amaranthin, and jacalin, in combination with neuraminidase, galactose oxidase or the galactose oxidase-Schiff sequence are listed in Table 3.

Distribution of TF-related antigens

Sialosyl-TF (“cryptic” TF) was detected in many normal epithelial, nerve, and lymphoid tissues. Tn and s-Tn were present predominantly in epithelial cells. As opposed to sialosyl-TF, sialosyl-Tn, and Tn, TF was only very rarely detected in normal cells of the body. Staining was found in parts of the sebaceous gland, the pancreatic duct, on apical membranes of renal distal and collecting tubules, macrophages in lymphoid organs, and spermatides. The extent of binding sites for TF, sialosyl-TF, Tn, and sialosyl-Tn in normal human epithelial tissue was ranked according to the present study as follows: sialosyl-TF > >sialosyl-Tn >Tn >TF.

Comparison of antibodies and lectins of similar specificity

TF antigen

The two mAbs against TF, G/A7 and HH8, revealed very similar staining patterns. G/A7 bound, in addition to the tissues mentioned above, to parts of white matter of the brain. PNA reacted with many epithelial and some non-epithelial cells.

Sialosyl-TF antigen

Although this antigen was broadly distributed, the staining patterns in terms of the number of stained cells and the localization in cells in the same tissues varied with the detection method employed as follows. First, anti-TF mAbs after neuraminidase treatment or jacalin stained all lymphocytes in the sections, whereas only the paracortical zone and follicular centers of lymph nodes and T cell zones in the spleen were positive for amaranthin. Second, jacalin stained thyroid epithelial cells, but amaranthin and anti-TF mAb after neuraminidase did not. Third, small intestine, descending colon, and gall-bladder epithelial cells bound in the supranuclear cytoplasm with anti-TF mAb after neuraminidase treatment, at the luminal surface for amaranthin, and strongly with goblet cells and the cytoplasm of epithelial cells for jacalin. Fourth, in the testis, amaranthin reacted only with spermatids, whereas both jacalin and anti-TF mAb after neuraminidase reacted with all cells of the seminiferous tubules and the interstitial cells.

Tn antigen

HPA, which is not strictly specific for Tn, reacted with epidermis and many glandular epithelia except prostatic, uterine, and breast. It also stained macrophages similarly to PNA. Anti-Tn mAbs 12A8 and TEC-02 were similar with respect to their reactivity with a limited number of epithelial and the absence of binding to non-epithelial tissues. However, uterine endometrium and interstitial cells of the testis were stained with 12A8 but not with TEC-02, whereas basal cells of the epitdermis were positive for TEC-02 and negative for 12A8 (see Table 2).

Sialosyl-Tn antigen

Two mAbs, B72.3 and TKH2, stained most tissues similarly; only two epithelial tissues showed different positive localization. The third mAb, 3F1, presented more reactivity with epithelial tissues, trachea, and prostate (Table 2).

In all cases, lectin reactivity with normal tissues, especially with epithelial cells, was less specific than staining with mAbs.

Discrimination between TF and s-TF

Since there are no reagents available which are specific for s-TF, an indirect technique had to be applied in order to discriminate between TF and s-TF. This consisted of a staining procedure with mAb G/A7, specific for TF, and the lectins amaranthin and jacalin with or without pretreatment of the tissue sections with galactose oxidase or the galactose oxidase-Schiff sequence (Sata et al. 1990). The staining by G/A7 was completely abolished by pre-

Table 2 Expression of TF, sialosyl-TF, Tn, and sialosyl-Tn in human adult normal tissues. [+ Positive, – negative, (+) weakly positive]

Tissues	TF			Sialosyl-TF			Tn			Sialosyl-Tn		
	G/A7	HH8	PNA	N′+ G/A7[a]	ACA	AIA	12A8	TEC-02	HPA	B72.3	TKH2	3F1
Epidermis												
Basal layer	–	–	–	+	+	+	–	+	–	–	–	–
Spinous layer	–	–	+	+	+	+	–	–	+	–	–	–
Granular layer	–	–	–	+	+	+	–	–	–	–	–	–
Non-epidermal cells	–	–	–	–	–	–	–	–	–	–	–	–
Hair follicles	–	–	+	+	+	+	–	–	+	–	–	–
Sebaceous gland	+	+	+	+	+	+	–	–	–	–	–	–
Sweat gland												
Ducts	–	–	+	+	+	+	–	–	+	+	+	+
Secretory cells	–	–	+	+	+	+	–	–	+	–	–	–
Submandibular gland												
Serous acini	–	–	–	–	+	+	+	+	+	(+)	(+)	(+)
Mucous acini	–	–	–	–	+[b]	+	–	–	+	+	+	
Ducts	–	–	–	–	+[b]	+	+	+	+	+	+	+
Myoepithelial cells	–	–	–	+	+	+	–	–	–	–	–	–
Esophagus												
Squamous epithelium	–	–	+	+	+	+	–	–	+	–	–	–
Mucous acini	–	–	–	+	+	+	–	–	–	–	–	–
Ducts	–	–	–	+	+	+	–	–	–	–	–	–
Stomach												
Foveolar epithelium	–	–	+[c]	+[c]	+[c]	+	(+)	(+)	+[c]	+[c]	+	+[c]
Fundus glands	–	–	+	–	+	+	(+)	(+)	+	+	+	+
Corpus glands	–	–	+	–	+	+	(+)	(+)	+	+	+	+
Jejunum and ileum												
Mucosa	–	–	+[c]	+[c]	+[b]	+	+[c]	+[c]	+[c]	+[c]	+[c]	+[c]
Descending colon												
Mucosa	–	–	+[c]	+[c]	+[b]	+	–	–	–	–	–	–
Gall bladder												
Mucosa	–	–	–	+[c]	+[b]	+	–	–	+[c]	+[c]	+	+[c]
Liver												
Hepatocytes	–	–	–	+[c]	+[c]	+[c]	–	–	–	–	–	–
Kupffer cells	–	–	–	+	+	+	–	–	–	–	–	–
Bile ducts	–	–	–	+	+	+	–	–	+[b]	(+)	(+)	(+)
Pancreas												
Ducts	+[b]	+[b]	+[b]	+[b]	+[b]	+	–	–	+[b]	–	–	–
Acini	–	–	–	+	+	+	–	–	–	–	–	–
Islets of Langerhans	–	–	–	+	+	+	–	–	–	–	–	–
Heart												
Endocardium	–	–	–	+	+	+	–	–	–	–	–	–
Myocardium	–	–	–	–	–	–	–	–	–	–	–	–
Mesothelium	–	–	–	–	–	–	–	–	–	–	–	–
Trachea and lung												
Serous gland	–	–	–	+	+	+	–	–	+	–	–	+
Mucous gland	–	–	+	+	+	+	–	–	+	–	–	+
Ciliated epithelium	–	–	–	+	+	+	–	–	+	–	–	+
Respiratory epithelium	–	–	–	+	+	+	–	–	–	–	–	–
Kidney												
Glomerulus	–	–	–	+	+	+	–	–	–	–	–	–
Bowman’s capsule	–	–	–	–	–	–	–	–	–	–	–	–
Proximal tubules	–	–	–	–	–	–	–	–	–	–	–	–
Distal tubules	+[b]	+[b]	+[b]	+[b]	+[b]	+	–	–	+[b]	–	–	–
Collecting ducts	+[b]	+[b]	+[b]	+[b]	+[b]	–	–	+[b]	–	–	–	
Bladder												
Urothelium	–	–	–	+	+	+	–	–	+	–	–	–
Prostate												
Glandular epithelium	–	–	+[b]	+	+	+	–	–	–	–	–	+
Uterus												

Table 2 (continued)

Tissues	TF			Sialosyl-TF			Tn			Sialosyl-Tn		
	G/A7	HH8	PNA	N′+ G/A7[a]	ACA	AIA	12A8	TEC-02	HPA	B72.3	TKH2	3F1
Corpus endometrium	–	–	–	+[b]	+[b]	+	+[b]	–	–	+[b]	+[b]	+
Cervix endometrium	–	–	–	+[b]	+[b]	+	+[b]	–	–	+[b]	+[b]	+
Cervix glandular cells	–	–	–	+[b]	+[b]	+	–	–	–	+	+	+
Mammary gland												
Ducts	–	–	–	+[b]	+[b]	+	–	–	–	–	–	–
Acini	–	–	–	+[b]	+[b]	+	–	–	–	–	–	–
Myoepithelial cells	–	–	–	+	+	+	–	–	–	–	–	–
Testis												
Spermatids	+	+	+	+	+	+	–	–	–	–	–	–
Spermatocytes	–	–	–	+	+	–	+	–	–	–	–	––
Spermatogonia	–	–	–	+	–	+	–	–	–	–	–	–
Sertoli's cells	–	–	–	+	–	+	–	–	–	–	–	–
Interstitial cells	–	–	–	+	–	+	+	–	–	+	+	+
Brain												
Cerebral cortexes	–	–	–	+	+	+	–	–	–	–	–	–
Cerebral medulla	+	–	+	+	+	+	–	–	–	–	–	–
Glial cells	–	–	–	+	+	+	–	–	–	–	–	–
Ependymal cells	–	–	–	+	+	+	–	–	–	–	–	–
Meninges												
Arachnoid membrane	–	–	–	+	+	+	–	–	–	–	–	–
Pacchionian granulations	–	–	–	+	+	+	–	–	–	–	–	–
Peripheral nerve												
Axons	–	–	+	+	+	+	–	–	–	–	–	–
Schwann cells	–	–	+	+	+	+	–	–	–	–	–	–
Perineurium	–	–	–	–	–	–	–	–	–	–	–	–
Thymus gland												
Epithelial reticular cells	–	–	–	–	–	–	–	–	–	–	–	–
Hassall's bodies	–	–	–	+	+	+	–	–	+	–	–	–
Lymphocytes	–	–	–	+	+[d]	+	–	–	–	–	–	–
Macrophages	+	+	+	+	+	+	–	–	+	–	–	–
Spleen												
Trabeculae	–	–	–	–	–	–	–	–	–	–	–	–
Reticular cells	–	–	–	–	–	–	–	–	–	–	–	–
Macrophages	+	+	+	+	+	+	–	–	+	–	–	–
Lymphocytes	–	–	–	+	+[d]	+	–	–	–	–	–	–
Lymph nodes												
Lymphocytes	–	–	–	+	+[d]	+	–	–	–	–	–	–
Macrophages	+	+	+	+	+	+	–	–	+	–	–	–
Reticular cells	–	–	–	–	–	–	–	–	–	–	–	–
Thyroid gland												
Epithelium	–	–	–	–	–	+[b]	–	–	–	–	–	–
Colloid	–	–	–	–	–	–	–	–	–	–	–	–
Adrenal gland												
Adrenal cortex	–	–	–	–	–	–	–	–	–	–	–	–
Adrenal medulla	–	–	–	–	–	–	–	–	–	–	–	–
Muscle tissues												
Smooth muscle	–	–	–	–	–	–	–	–	–	–	–	–
Skeletal muscle	–	–	–	–	–	–	–	–	–	–	–	–
Connective tissue	–	–	–	–	–	–	–	–	–	–	–	–
Synovial tissue	–	–	–	+	+	+	–	–	–	–	–	–
Endothelium	–	–	–	+	+	+	–	–	+[e]	+[e]	–	
Erythrocytes	–	–	–	+	+	+	–	–	+[e]	–	+[e]	–

[a] G/A7 after neuraminidase treatment
[b] Present along the luminal surface
[c] Present in the supranuclear cytoplasm or Golgi region. Cases to which neither [b]nor [c]has been assigned were mainly diffusely stained
[d] Not all lymphocytes positive
[e] In individuals of blood group A

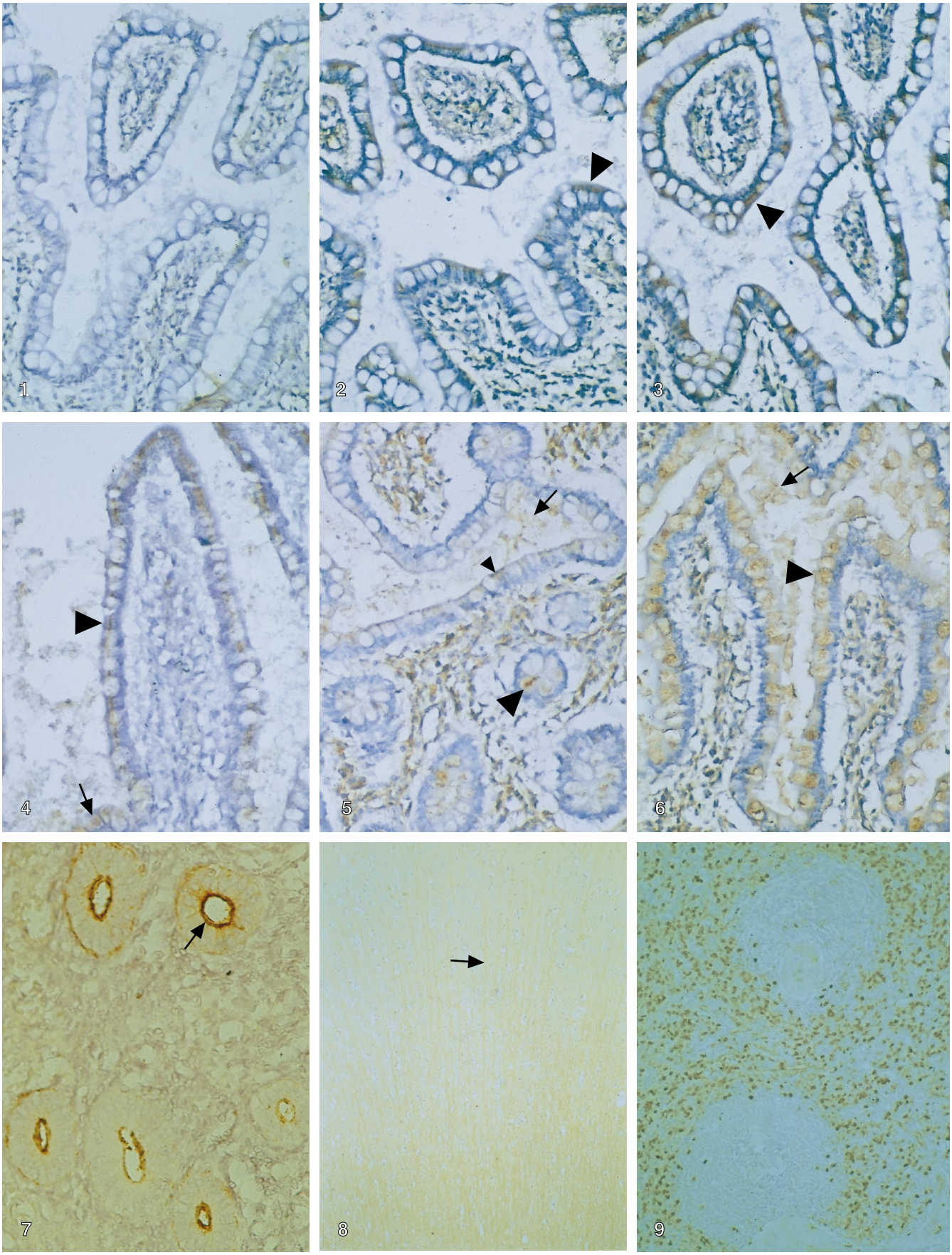
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Table 3 Staining of the monoclonal anti-TF antibody G/A7, amaranthin (*ACA*), and jacalin (*AIA*) in normal tissues after pretreatment with galactose oxidase (*GO*) or the galactose oxidase-Schiff sequence (*GO-S*). [*N´* neuraminidase, + positive, – negative, (+) weakly positive, *n.d.* not done]

Tissues	GO G/A7	GO-S ACA	GO-S AIA	N′ G/A7	N′ GO+G/A7	N′ GO-S+G/A7	GO N′+G/A7	GO-S N′+G/A7
Epidermis								
Basal layer	–	+	+	+	n.d	n.d	n.d	n.d
Spinous layer	–	+	+	+	n.d	n.d	n.d	n.d
Granular layer	–	+	+	+	n.d	n.d	n.d	n.d
Cornified layer	–	+	+	+	n.d	n.d	n.d	n.d
Non-epidermal cells	–	+	+	+	n.d	n.d	n.d	n.d
Sebaceous gland	–	+	+	+	n.d	n.d	n.d	n.d
Pancreas								
Ducts	–	+	+	+	–	–	+	+
Acini	–	+	(+)	+	–	–	(+)	(+)
Islets of Langerhans	–	(+)	+	+	–	–	(+)	(+)
Kidney								
Glomerulus	–	+	+	+	–	–	+	+
Bowman's capsule	–	–	–	–	–	–	–	–
Proximal tubules	–	–	–	–	–	–	–	–
Distal tubules	–	+	+	+	–	–	+	+
Collecting ducts	–	+	+	+	–	–	+	+
Testis								
Spermatids	–	+	+	+	n.d	n.d	–	n.d
Spermatogonia	–	–	+	+	n.d	n.d	–	n.d
Sertoli's cells	–	–	+	+	n.d	n.d	–	n.d
Interstitial cells	–	–	+	+	n.d	n.d	–	n.d
Brain								
Cerebral cortexes	–	+	+	+	n.d	n.d	–	n.d
Cerebral medulla	–	+	+	+	n.d	n.d	–	n.d
Glial cells	–	+	+	+	n.d	n.d	–	n.d
Thymus gland								
Epithelial reticular cells	–	–	–	–	n.d	n.d	n.d	n.d
Hassall's bodies	–	+	+	+	n.d	n.d	n.d	n.d
Lymphocytes	–	+[a]	+	+	n.d	n.d	n.d	n.d
Macrophages	–	+	+	+	n.d	n.d	n.d	n.d
Spleen								
Trabeculae	–	–	–	–	–	–	–	–
Recticular cells	–	–	–	–	–	–	–	–
Macrophages	–	+	+	+	–	–	+	+
Lymphocytes	–	+[a]	+	+	–	–	(+)	(+)
Lymph nodes								
Lymphocytes	–	+[a]	+	+	n.d	n.d	n.d	n.d
Macrophages	–	+	+	+	n.d	n.d	n.d	n.d
Reticular cells	–	–	–	–	n.d	n.d	n.d	n.d
Endothelium	–	+	+	+	–	–	(+)	(+)
Erythrocytes	–	+	+	+	–	–	(+)	(+)

[a] Not all lymphocytes positive

Figs. 1–9 Reactivity of a selection of human adult normal tissues with monoclonal antibodies and lectins to Thomsen-Friedenreich-related antigens

Figs. 1–6 Normal ileum from the same tissue block

Fig. 1 A78-G/A7 (TF) does not stain the mucosa (200×)

Fig. 2 12A8 (Tn) stains the supranuclear cytoplasm (*arrowhead*) of columnar cells (200×)

Fig. 3 B72.3 (s-Tn) also stains the supranuclear cytoplasm (*arrowhead*) of columnar cells (200×)

Fig. 4 *Helix pomatia* agglutinin (αGal*N*Ac-reactive lectin) stains the supranuclear cytoplasm (*arrowhead*) of columnar cells and some goblet cells (*arrow*) at the lower crypts (200×)

Fig. 5 Amaranthin (2,3-linked s-TF lectin) stains the apical membrane (*small arrowhead*) of columnar cells and secretion (*arrow*), but only some goblet cells (*big arrowhead*) at the lower crypts (200×)

Fig. 6 Jacalin (s-TF lectin) stains goblet cell vacuoles (*arrowhead*) and secretion (arrow, 200×)

Fig. 7 Pancreas: A78-G/A7 stains the luminal surface (*arrow*) of the ducts (200×)

Fig. 8 Brain: A78-G/A7 stains radial columns (*arrow*, 100×)

Fig. 9 Spleen: amaranthin stains T lymphocytes (100×)

treatment with galactose oxidase. Amaranthin and jacalin, which stain both TF and cryptic TF (s-TF), remain reactive in the case of s-TF even after galactose oxidase-Schiff sequence treatment (see Table 3). In our experiments, we additionally treated the sections with neuraminidase and galactose oxidase or the galactose oxidase-Schiff sequence in various orders under otherwise identical conditions (Table 3). The results suggest that sialic acid may only partially protect Galβ1-3GalNcα1 from the action of galactose oxidase.

Comparison of mAbs and lectins with respect to their possible cross-reactivity with blood group AB antigens

We observed that indeed HPA and TKH2 cross-reacted with blood group A. G/A7, HH8, 12A8, TEC-02, B72.3, and 3F1 showed no cross-reactivity with blood group A and B antigens.

During these studies we made the unexpected observation that anti-A and anti-B mAbs also stained some non-epithelial and non-endothelial cells, e.g., spermatids, regardless of the blood group of the individual.

The epitope reacting with mAb 3E1.2 (TF-unrelated, NeuGc-containing) was even more broadly distributed than s-TF; it was found in epithelial tissues, muscular tissue (skeletal muscle, cardiac muscle, and smooth muscle), nerve tissue (cortex and medulla of brain and peripheral nerve), lymphoid tissue, and endothelium, but not in connective tissue.

Discussion

The distribution of TFRA in malignant tissues has attracted considerable attention (Springer 1984). In contrast, systematic investigations of their occurrence in normal tissues are scarce. Binding sites of lectins recognizing TFRA, such as PNA, HPA (*Helix pomatia* agglutinin, binding to αGal*N*Ac), DBA (*Dolichos biflorus* agglutinin, αGal*N*Ac and Gal*N*Acα1-3Gal*N*Ac), VVA (*Vicia villosa* agglutinin, αGal*N*Ac, βGal*N*Ac, and Gal*N*Acα1-3Gal), or SBA (soybean agglutinin, αGal*N*Ac, βGal*N*Ac, and Gal*N*Acα1-3Gal) in normal tissues have been described in previous studies (Spicer and Schulte 1992; Danguy et al. 1994; Mcmahon et al. 1994), but most of these lectins have dual or multiple specificities, and therefore one has also to expect that these lectins may bind to carbohydrate structures other than TFRA in tissue sections (Ito and Hirota 1992). For example, the carbohydrate specificity of PNA is not strictly confined to the Galβ1-3Gal*N*Ac disaccharide; in fact, this lectin recognizes other types of terminal Gal residues (Swamy et al. 1991). HPA can bind αGal*N*Ac in Tn determinant (Gal*N*Acα1-Ser/Thr), Forssman disaccharide (Gal*N*Acα1-3Gal*N*Ac), and A determinant [Gal*N*Acα1-3 (Fucα1-2)Galβ1-3,4Glc*N*Ac] (Wu and Sugii 1991). Thus, distribution patterns of TFRA based exclusively on lectin binding are not sufficiently exact. Our study provides the first systematic and comprehensive overview on the occurrence of TFRA in normal human tissues and compares the reactivities of several reagents (mAbs and lectins) for these structures in the same material. By including galactose oxidase or the galactose oxidase-Schiff sequence pretreatment according to Sata et al. (1990), s-TF could be detected in the presence of TF. S-TF occurs extensively in normal tissues, especially in epithelial cells. TF, Tn, and s-Tn, which are widely accepted tumor antigens, were found in our study to be quite differently expressed in normal tissues. Among these glycotopes, TF is the one which occurred only in a few normal epithelial cells, whereas s-Tn was found in most cells of this type, and the expression of Tn was intermediate. We have noted that TF, Tn, and s-Tn are also expressed in some non-epithelial cells.

TF, defined by the two mAbs used here, was expressed among epithelia only in the sebaceous gland, in the pancreatic duct, and in apical membranes of renal distal and collecting tubules. These stainings were prevented by galactose oxidase pretreatment. Our results are in agreement with other authors who described the absence of TF in selected tissues like uterus, colon, urothelium or squamous cells (Ravn and Jensen 1988; Mandel et al. 1991; Itzkowitz 1992; Langkilde et al. 1992). Philipsen et al. (1991), Therkildsen et al. (1994), and Carneiro et al. (1994) reported the presence of TF in acinar cells of pancreas, in salivary glands, and in the stomach, respectively, employing mAbs HH8 and HB-T1. We have not seen TF in these tissues, either with mAb G/A7, HH8 or HB-T1. This has to be further examined with special consideration of the secretor status of the individuals and in frozen sections of these tissues as well. MAbs against TF stained some non-epithelial tissues in our study, including spermatids, macrophages, and parts of the white matter of the brain. The control sections with galactose oxidase pretreatment confirmed that G/A7 also bound to Gal*N*Acα1-3Gal*N*Ac in these tissues. Two groups reported earlier that nerve cells were stained with rabbit anti-TF serum or PNA, respectively (Örntoft et al. 1985; Apostolski et al. 1994). s-TF was detected either by means of anti-TF mAbs after removal of sialic acid by neuraminidase, or by the lectins amaranthin or jacalin. The problem of TF binding by amaranthin or jacalin was ruled out by a pretreatment with the galactose oxidase-Schiff sequence in the TF-positive tissues. s-TF is almost ubiquitously present in normal tissues, in agreement with the results of previous studies (Sata et al. 1990; Boland et al. 1991; Schetz and Anderson 1995). Sata and coworkers found that the reactivity of amaranthin with the left and the right colon was not identical (Sata et al. 1992). However, in our study some remarkable differences of the staining patterns between these three reagents in normal tissues were found. Amaranthin is reasonably specific for Galβ1-3Gal*N*Acα- and NeuAcα2-3Galβ1-3Gal*N*Acα1- (Rinderle et al. 1989). Jacalin binds to Galβ1-3Gal*N*Acα- and its NeuAcα2,3-linked and/or NeuAcα2,6-linked forms (Swamy et al.

1991; Maemura and Fukuda 1992). Pretreatment with neuraminidase from *V. cholerae* removes both 2,3- and 2,6-bound sialic acid residues (Watzlawick et al. 1992). Therefore, we hypothesize that the different staining patterns observed may reflect the occurrence of both subtypes of s-TF [NeuAcα2-3Galβ1-3Gal*N*Acα1-R and Galβ1-3(NeuAcα2-6)Gal*N*Acα1-R] and their respective sialyltransferases in changing ratios in the different cells and cellular regions. Biochemical investigations have provided data on the differential distribution of sialytransferases in various organs (Paulson et al. 1989). This can be extended to the cellular level within a given organ by histochemical localization of different glycosyltransferase products. Differences in the tissue distribution of α2,3- and α2,6-linked sialic acid in animal tissues and human tumor tissues have been detected by means of lectin histochemistry (Sata et al. 1989, 1991; Vierbuchen et al. 1995). In our study, all TF-positive tissues also expressed s-TF; in contrast, many tissues possessing s-TF did not react with anti-TF mAbs. This indicates that most normal cells have cryptic TF masked with sialic acid.

Tn expression in some epithelial tissues, for instance salivary gland, stomach, and uterus, has been described previously (Carneiro et al. 1994; Tashiro et al. 1994; Therkildsen et al. 1994). In the present study, other epithelial tissues like small intestine were also found to be positive for Tn. In addition, apparent differences in staining patterns between mAbs 12A8 and TEC-02 were observed, which may be explained by the fact that the latter recognizes a more extended epitope (Dráber 1987).

The presence of s-Tn in normal tissues has been described in previous studies, especially those employing mAb B72.3, although there are also reports claiming that B72.3 and TKH2 are non-reactive or only very weakly reactive with all normal tissues (Thor et al. 1986; Kjeldsen et al. 1988). Our results confirm findings that s-Tn is present in a considerable number of normal epithelial tissues and in interstitial cells of the testis (Stein et al. 1991). In addition, we found that the anti-s-Tn mAbs reacted with the upper gastrointestinal tract but not with the colonic epithelium. This may be explained by the fact that sialic acid residues are *O*-acetylated in colonic tissues (Ogata et al. 1995). Jass and colleagues found that 11 normal colon mucosae expressed *O*-acetylated s-Tn; the non-*O*-acetylated form was present only occasionally in single crypts of four cases, and strongly and diffusely in five mucosae (Jass et al. 1994). When *O*-acetylated sialic acid is converted to non-*O*-acetylated sialic acid by saponification through treatment with potassium hydroxide, normal colorectal goblet cells became immunoreactive with mAbs for s-Tn (Jass et al. 1995).

Inconsistent findings in different laboratories concerning the reaction of the lectins and mAbs to TFRA in normal and tumor tissues are not rare. These differences may be caused by various factors, including the quality of the tissue specimens, the diversity of techniques employed, the specificity and sensitivity of mAbs used, or the secretor status of the individual. First of all, the quality of the specimens significantly affects the detection of TFRA. Morphology appears to be a key indicator of specimen quality. Thus, the preservation of morphological detail in the sample is a prerequisite for examining these antigens. To mention one essential technical detail, normal serum used to block non-specific binding sites should be preabsorbed with neuraminidase-treated red blood cells in order to avoid anti-TF auto-antibody interference (Springer and Desai 1985). Another point to consider is the observed reactivity of pancreatic acinar cells and gastric mucins from non-secretors with PNA, but not from secretors (Ito et al. 1986; Macartney 1986). In general terms, we would like to stress that the combined use of different mAbs and their side-by-side comparison, if necessary in combination with specific glycosidase digestion procedures, are of great importance in obtaining more accurate results.

The presence of a given tumor antigen in certain normal tissues does not necessarily mean that this antigen cannot serve as a target for tumor diagnosis and immunotherapy. In fact, antigens localized in the cytoplasm or at the luminal side of epithelial surfaces of glands may be inaccessible or poorly accessible to mAbs injected in vivo or, alternatively, to active immunocytes (Pervez et al. 1989). Similarly, the parenchyma of the brain or spermatocytes of testis are not accessible as long as the blood-brain barrier and the blood-testis barrier are intact. Many of the binding sites for TFRA reagents in normal tissues described here are essentially restricted to such sites. In conclusion, from our data it can be inferred that TF, Tn, and sialosyl-Tn are promising targets for a cancer vaccine.

A different situation is represented by mAb 3E1.2. This antibody has been successfully used to monitor breast cancer in patient sera (Stacker et al. 1988), although it appears obvious that the epitope is expressed in normal tissues too ubiquitously to be of value for histopathological diagnosis. The main value of immunohistology may reside in its ability to provide basic information on whether a given antigen is expressed in healthy and pathological tissues, and where it is localized within the cell.

Finally, we would like to emphasize several interesting single facts observed during this study. For example, the anti-TF mAb, G/A7, stained macrophages in some organs such as lymph nodes, but not in connective tissues. In vitro we also found that macrophages after prolonged cultivation were positive for G/A7, whereas those from short term cultures cells were not (G. Pasternak and U. Karsten, unpublished). At present we cannot explain the cause and significance of these observations. Another very interesting and probably important finding is the fact that amaranthin reacted with most or all T lymphocytes in the spleen, but only with a subpopulation of thymic lymphocytes. This would suggest that the carbohydrate structure, NeuAcα2-3Galβ1-3Gal*N*Acα1-R, is only present on a subset of T lymphocytes and/or at a special stage of their differentiation. Third, TF (Galβ1-3Gal*N*Acα-) and s-TF (NeuAcα2-3Galβ1-3Gal*N*Acα1-)

are apparently expressed only in mature spermatids, but are absent in immature spermatocytes and spermatogonia. This will, however, to be addressed in further studies.

Not much is known about the nature of the glycoconjugates carrying TF and TF-related epitopes on tumor cells, and even less about these glycoconjugates in normal cells. So far, TF-positive, mucin-like fractions have been separated by means of affinity chromatography with PNA-agarose from human milk and gastric mucosa (Fischer et al. 1984a, b). Glycoproteins with molecular weights of approximately 120 and 70–80 kDa have been isolated on PNA-agarose columns from neuronal tissue (Apostolski et al. 1994). Since there are now mAbs with higher specificity than lectins available, a new approach to the identification of TF-carrying molecules is feasible.

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References

Apostolski S, Sadiq SA, Hays A, Corbo HM, Suturkova-Milosevic L, Chaliff P, et al. (1994) Identification of Gal(β1-3)Gal*N*Ac bearing glycoproteins at the nodes of Ranvier in peripheral nerve. J Neurosci Res 38:134–141

Boland CR, Chen YF, Rinderle SJ, Resau JH, Luk GD, Lynch HT, Goldstein IJ (1991) Use of the lectin from *Amaranthus caudatus* as a histochemical probe of proliferating colonic epithelial cells. Cancer Res 51:657–665

Carneiro F, Santos L, David L, Dabelsteen E, Clausen H, Sobrinho-Simões M (1994) T (Thomsen-Friedenreich) antigen and other simple mucin-type carbohydrate antigens in precursor lesions of gastric carcinoma. Histopathology 24:105–113

Clausen H, Hakomori S-I (1989) ABH and related histo-blood group antigens; immunochemical differences in carrier isotypes and their distribution. Vox Sang 56:1–20

Clausen H, Stroud M, Parker J, Springer GF, Hakomori S-I (1988) Monoclonal antibodies directed to the blood group A associated structure, galactosyl-A: specificity and relation to the Thomsen-Friedenreich antigen. Mol Immunol 25:199–204

Danguy A, Akif F, Pajak B, Gabius H-J (1994) Contribution of carbohydrate histochemistry to glycobiology. Histol Histopathol 9:155–171

Desai PR, Springer GF (1979) Biosynthesis of human blood group T-, N- and M-specific immunodeterminants on human erythrocyte antigens. J Immunogenet 6:403–417

Devine PL, Clark BA, Birrell GW, Laytin GT, Ward BG, Alewood PF, McKenzie IFC (1991) The breast tumor-associated epitope defined by monoclonal antibody 3E1.2 is an *O*-linked mucin carbohydrate containing *N*-glycolylneuraminic acid. Cancer Res 51:5826–5836

Dráber P (1987) The epitope of mouse embryonic antigen(s) recognized by monoclonal antibody TEC-02 is a carbohydrate carried by high-molecular-weight glycoconjugates. Cell Differ 21:119–130

Fischer J, Klein P-J, Farrar GH, Hanisch F-G, Uhlenbruck G (1984a) Isolation and chemical and immunochemical characterization of the peanut lectin-binding glycoprotein from human milk fat globule membranes. Biochem J 224:581–589

Fischer J, Uhlenbruck G, Klein P-J, Vierbuchen M, Fischer R (1984b) Characterization of glycoconjugates of human gastrointestinal mucosa by lectins. II. Lectin binding to the isolated glycoproteins of normal and malignant gastric mucosa. J Histochem Cytochem 32:690–696

Fung PYS, Madej M, Koganty RR, Longenecker BM (1990) Active specific immunotherapy of a murine mammary adenocarcinoma using a synthetic tumor-associated glycoconjugate. Cancer Res 50:4308–4314

Hirohashi S, Clausen H, Yamada T, Shimosato Y, Hakomori S-I (1985) Blood group A cross-reacting epitope defined by monoclonal antibodies NCC-LU-35 and -81 expressed in cancer of blood group 0 or B individuals: its identification as Tn antigen. Proc Natl Acad Sci USA 82:7039–7043

Ito N, Hirota T (1992) Histochemical and cytochemical localization of blood group antigens. Fischer, Stuttgart

Ito N, Nishi K, Nakajima M, Matsuda Y, Ishitani A, Mizumoto J, Hirota T (1986) Localization of blood group antigens in human pancreas with lectin-horseradish peroxidase conjugates. Acta Histochem Cytochem (Kyoto) 19:205–218

Itzkowitz SH (1992) Carbohydrate changes in colon carcinoma. APMIS [Suppl 27] 100:173–180

Itzkowitz SH, Bloom EJ, Kokal WA, Modin G, Hakomori S, Kim YS (1990) Sialosyl-Tn: a novel mucin antigen associated with prognosis in colorectal cancer patients. Cancer 66:1960–1966

Jass JR, Allison LJ, Edgar SG (1994) Monoclonal antibody TKH2 to the cancer-associated epitope sialosyl Tn shows cross-reactivity with variants of normal colorectal goblet cell mucin. Pathology 26:418–422

Jass JR, Allison LJ, Edgar SG (1995) Distribution of sialosyl Tn and Tn antigens within normal and malignant colorectal epithelium. J Pathol 176:143–149

Karsten U, Butschak G, Cao Y, Goletz S, Hanisch F-G (1995) A new monoclonal antibody (A78-G/A7) to the Thomsen-Friedenreich pan-tumor antigen. Hybridoma 14:37–44

Kim Z, Uhlenbruck G (1966) Untersuchungen über T-Antigen und T-Agglutinin. Z Immunol Forsch 130:88–99

Kjeldsen T, Clausen H, Hirohashi S, Ogawa T, Iijima H, Hakomori S-I (1988) Preparation and characterization of monoclonal antibodies directed to the tumor associated *O*-linked sialosyl-2-6 α-*N*-acetylgalactosaminyl (sialosyl-Tn) epitope. Cancer Res 48:2214–2220

Kurosaka A, Nakajima H, Funakoshi I, Matsuyama M, Nagayo T, Yamashina I (1983) Structures of the major oligosaccharides from a human rectal adenocarcinoma glycoprotein. J Biol Chem 258:11594–11598

Langkilde NC, Wolf H, Clausen H, Kjeldsen T, Ørntoft TF (1992) Nuclear volume and expression of T-antigen, sialosyl-Tn-antigen, and Tn-antigen in carcinoma of the human bladder. Cancer 69:219–227

Macartney JC (1986) Lectin histochemistry of galactose and *N*-acetyl-galactosamine glycoconjugates in normal gastric mucosa and gastric cancer and the relationship with ABO and secretor status. J Pathol 150:135–144

Maemura K, Fukuda M (1992) Poly-*N*-acetyllactosaminyl *O*-glycans attached to leukosialin: the presence of sialyl Le^X structures in *O*-glycans. J Biol Chem 267:24379–24386

Mandel U, Petersen OW, Sørensen H, Vedtofte P, Hakomori S-I, Clausen H, Dabelsteen E (1991) Simple mucin-type carbohydrates in oral stratified squamous and salivary gland epithelia. J Invest Dermatol 97:713–721

Mcmahon RFT, Panesar MJR, Stoddart RW (1994) Glycoconjugates of the normal human colorectum: a lectin histochemical study. Histochem J 26:504–518

Ogata S, Ho I, Chen A, Dubois D, Maklansky J, Singhal A, Hakomori S-I, Itzkowitz SH (1995) Tumor-associated sialylated antigens are constitutively expressed in normal human colonic mucosa. Cancer Res 55:1869–1874

Örntoft TF, Mors NPO, Eriksen G, Jacobsen NO, Poulsen HS (1985) Comparative immunoperoxidase demonstration of T-antigens in human colorectal carcinomas and morphologically abnormal mucosa. Cancer Res 45:447–452

Paulson JC, Weinstein J, Schauer A (1989) Tissue-specific expression of sialyltransferases. J Biol Chem 264:10931–10934

Pervez S, Kirkland SC, Epenetos AA, Moor WJ, Evans DJ, Krausz T (1989) Effect of polarity and differentiation on antibody localization in multicellular tumour spheroid and xenograft models and its potential importance for in vivo immunotargeting. Int J Cancer 44:940–947

Philipsen EK, Jørensen M, Dabelsteen E (1991) Expression of blood group-related carbohydrate antigens in normal human pancreatic tissue. APMIS 99:931–940

Ravn V, Jensen H (1988) Thomsen-Friedenreich-related antigen in human endometrium. APMIS 96:552–558

Rinderle SJ, Goldstein IJ, Matta KL, Ratcliffe RM (1989) Isolation and characterization of amaranthin, a lectin present in the seeds of *Amaranthus caudatus*, that recognizes the T- (or cryptic T)-antigen. J Biol Chem 264:16123–16131

Sata T, Lackie PM, Taatjes DJ, Peumans W, Roth J (1989) Detection of the Neu5Ac(α2,3) Gal(β1,4) Glc*N*Ac sequence with the leukoagglutinin from *Maackia amurensis*: light and electron microscopic demonstration of differential tissue expression of terminal sialic acid in α2,3- and α2,6-linkage. J Histochem Cytochem 37:1577–1588

Sata T, Zuber C, Rinderle SJ, Goldstein IJ, Roth J (1990) Expression patterns of the T antigen and the cryptic T antigen in rat fetuses: detection with the lectin amaranthin. J Histochem Cytochem 38:763–774

Sata T, Roth J, Zuber C, Stamm B, Heitz PU (1991) Expression of α2,3-linked sialic acid residues in neoplastic but not in normal human colonic mucosa: a lectin-gold cytochemical study with *Sambucus nigra* and *Maackia amurensis* lectins. Am J Pathol 139:1435–1448

Sata T, Roth J, Zuber C, Stamm B, Rinderle SJ, Goldstein IJ, Heitz PU (1992) Studies on the Thomsen-Friedenreich antigen in human colon with the lectin amaranthin: normal and neoplastic epithelium express only cryptic T antigen. Lab Invest 66:175–186

Schetz JA, Anderson PA (1995) Glycosylation patterns of membrane proteins of the jellyfish *Cyanea capillata*. Cell Tissue Res 279:315–321

Schulte BA, Spicer SS (1983) Light microscopic histochemical detection of terminal galactose and *N*-acetylgalactosamine residues in rodent complex carbohydrates using a galactose oxidase-Schiff sequence and the peanut lectin-horseradish peroxidase conjugate. J Histochem Cytochem 31:19

Spicer SS, Schulte BA (1992) Diversity of cell glycoconjugates shown histochemically: a perspective. J Histochem Cytochem 40:1–38

Springer GF (1984) T and Tn, general carcinoma autoantigens. Science 224:1198–1206

Springer GF, Desai PR (1974) Common precursors of human blood group MN specificities. Biochem Biophys Res Commun 61:470–475

Springer GF, Desai PR (1985) Tn epitopes, immunoreactive with ordinary anti-Tn antibodies, on normal, desialylated human erythrocytes and on Thomsen-Friedenreich antigen isolated therefrom. Mol Immunol 22:1303–1310

Springer GF, Chandrasekaran EV, Desai PR, Tegtmeyer H (1988) Blood group Tn-active macromolecules from human carcinomas and erythrocytes: characterization of and specific reactivity with mono- and polyclonal anti-Tn antibodies induced by various immunogens. Carbohydr Res 178:271–292

Springer GF, Desai PR, Wise W, Carlstedt SC, Tegtmeyer H, Stein R (1990) Pancarcinoma T and Tn epitopes: autoimmunogens and diagnostic markers that reveal incipient carcinomas and help establish prognosis. In: Herbermann RB, Mercer DW (eds) Immunodiagnosis of cancer. Dekker, New York, pp 587–611

Springer GF, Desai PR, Spencer BD, Tegtmeyer H, Carlstedt SC, Scanlon EF (1995) T/Tn antigen vaccine is effective and safe in preventing recurrence of advanced breast carcinoma. Cancer Detection Prevention 19:374–380

Stacker SA, Thompson CH, Sacks NPM, Tjandra J, Lowe MG, Bishop J, Mckenzie IFC (1988) Detection of mammary serum antigen in sera from breast cancer patients using monoclonal antibody 3E1.2. Cancer Res 48:7060–7066

Stein R, Goldenberg DM, Mattes MJ (1991) Normal tissue reactivity of four anti-tumor monoclonal antibodies of clinical interest. Int J Cancer 47:163–169

Swamy MJ, Gupta D, Mahanta SK, Surolia A (1991) Further characterization of the saccharide specificity of peanut (*Arachis hypogaea*) agglutinin. Carbohydr Res 213:59–67

Tashiro Y, Yonezawa S, Kim YS, Sato E (1994) Immunohistochemical study of mucin carbohydrates and core proteins in human ovarian tumors. Hum Pathol 25:364–372

Therkildsen MH, Mandel U, Thorn J, Christensen M, Dabelsteen E (1994) Simple mucin-type carbohydrate antigens in major salivary glands. J Histochem Cytochem 42:1251–1259

Thor A, Ohuchi N, Szpak CA, Johnston WW, Schlom J (1986) Distribution of oncofetal antigen tumor-associated glycoprotein-72 defined by monoclonal antibody B72.3. Cancer Res 46:3118–3124

Vierbuchen MJ, Fruechtnicht W, Brackrock S, Krause KT, Zienkiewicz TJ (1995) Quantitative lectin histochemical and immunohistochemical studies on the occurrence of alpha(2,3)- and alpha(2,6)-linked sialic acid residues in colorectal carcinomas: relation to clinicopathologic features. Cancer 76:727–735

Watzlawick H, Walsh MT, Yoshioka Y, Schmid K, Brossmer R (1992) Structure of the *N*- and *O*-glycans of the A-chain of human plasma α2HS-glycoprotein as deduced from the chemical compositions of the derivatives prepared by stepwise degradation with exoglycosidases. Biochemistry 31:12198–12203

Wood GS, Warnke R (1981) Suppression of endogenous avidin-binding activity in tissues and its relevance to biotin-avidin detection systems. J Histochem Cytochem 29:1196–1204

Wu AM, Sugii S (1991) Coding and classification of D-galactose, *N*-acetyl-D-galactosamine, and β-D-Gal*p*-[1-3(4)]-—D-Glc*pN*ac, specificities of applied lectins. Carbohydr Res 213:127–143