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Invited Review

The Thomsen-Friedenreich (TF) antigen: a critical review on the structural, biosynthetic and histochemical aspects of a pancarcinoma-associated antigen

F.-G. Hanisch[1] and S.E. Baldus[2]

Institutes of [1]Biochemistry and [2]Pathology, University of Cologne, Cologne, Germany

Summary. Within the family of blood-group related carbohydrate antigens the Thomsen-Friedenreich (TF) antigen (or T antigen) is an outstanding member by attracting scientific interest for more than 65 years and by having retained its significance as object of current biomedical research; in particular, as a pancarcinoma-associated antigen. In accordance with its constant or even growing attraction scientists have searched for specific reagents which would allow the unambiguous and sensitive detection of the Thomsen-Friedenreich antigen on cells or tissues. While at the beginning, immunohistochemical work on TF antigen expression was restricted by the limited specificity of plant lectins (peanut lectin) a significant progress has been possible since the introduction of the hybridoma technique. The respective monoclonal antibodies display distinct fine specificities and cellular staining patterns in immunohistochemistry and have contributed to controversial discussions on the organ-characteristic and tumor-associated expression of the TF antigen in some organs. It is the aim of this survey to summarize in the context of its structural and biosynthetic aspects the current knowledge on the tissue expression of the TF antigen as based on the use of peanut agglutinin and monoclonal antibodies and to discuss the findings with regard to their biomedical relevance, in particular, with emphasis on their value in tumor diagnosis.

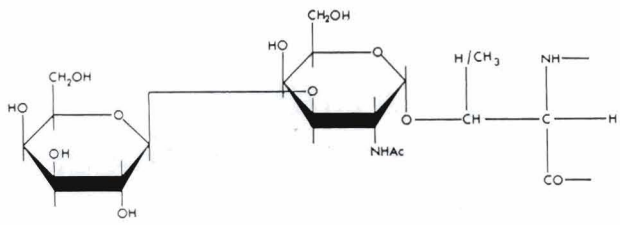
Key words: Thomsen-Friedenreich (TF) antigen, T antigen, Peanut agglutinin (PNA), Monoclonal antibody (mAb)

Offprint requests to: Prof. Dr. Franz-Georg Hanisch, Institute of Biochemistry, University of Cologne, Joseph-Stelzmann-Str. 52, 50931 Cologne, Germany

What is known about the structure of the TF antigen?

For several reasons it is advisable to start this survey with a few comments on the chemical nature of the antigen and its structural aspects. Since the work of Thomsen (1927) and his disciple Friedenreich (1930) on the panagglutination of virally transformed erythrocytes, it is known that the «3rd agglutinin» present in all human sera, besides and independent of the iso-agglutinins of the ABO system, binds to a membrane-bound receptor. In 1958, G.F. Springer and O. Mäkelä could independently show that the sialidase of myxo-viruses not only destroyed the respective viral receptors on red blood cells, but also the human blood group antigens M and N. Klenk and Uhlenbruck (1960) could later on localize the TF antigen-mediated agglutination to a membrane-bound glycoprotein which is now called glycophorin. The first structural elucidation of TF-active carbohydrates came, however, from inhibition studies on anti-TF-mediated agglutination using sialidase-treated brain gangliosides (Kim and Uhlenbruck, 1966). The most potent inhibitor was characterized as a disaccharide of the structure Galß1-3GalNAc. This disaccharide forms a cryptic component of the blood-group M and N tetrasaccharides on human glycophorin (Thomas and Winzler, 1969). Although there are cross-reactive structural elements on gangliosides (asialo-GM_1, Gg4Cer, structure 2 in Fig. 1), the TF antigen is a protein-bound carbohydrate. The TF disaccharide on human glycophorin A is linked O-glycosidically to the hydroxy amino acids serine or threonine (structure in Fig. 1) (Tomita et al., 1978). It has to be emphasized at this point that there is an important structural difference between the ganglioside-linked TF disaccharide, which is bound in a ß-anomeric configuration to subterminal sugars, and the protein-linked TFα disaccharide. Meanwhile, we know that there are many other protein-carriers of the TFα antigen which is an exposed or

Structure 1



Galβ1-3GalNAcα-O-Ser(Thr)

Structure 2

Galβ1-3GalNAcβ1-4Galβ1-4Glcβ-Cer

Fig. 1. Thomsen-Friedenreich disaccharide on human glycophorin A (structure 1) and asialo-GM_1, Gg4Cer (structure 2).

cryptic, sialic acid masked core structure on blood cell glycoproteins like leukosialin (CD43 antigen) (Nakada et al., 1995), and also on a variety of mucin-type glycoproteins like epiglycanin (Codington et al., 1975) or MUC1 (Fischer et al., 1984a; Hanisch et al., 1995).

The exposition of the TFα cryptantigen occurs in a tumor-associated manner

Initiation of O-glycosylation of proteins occurs in the cis-Golgi by the action of at least two independent UDP-GalNAc:peptide N-acetyl-galactosaminyl-transferases (Elhammer and Kornfeld, 1986; Sorensen et al., 1995). Contrasting to N-glycosylation these enzymes do not show a strict specificity towards a peptide motif, but instead are affected in substrate binding by critical amino acids in the vicinity of putative glycosylation sites (O'Connell et al., 1992). The first product of O-glycosylation, GalNAcα-O-Ser/Thr, which is identical to the TN antigen, forms the substrate of ß3-galactosyl-transferase, α3-N-acetylgalactosaminyltransferase, and ß3- or ß6-specific N-acetylglucosaminyltransferases. These enzymes convert the core-GalNAc into a series of di- or trisaccharides classified as core 1 to core 6 (Fig. 2) (Schachter, 1986; Hanisch et al., 1993). The synthesis of core 1, which is identical to the Thomsen-Friedenreich antigen, depends, accordingly, on a variety of factors: on the availability of the substrate, core-GalNAc, on the

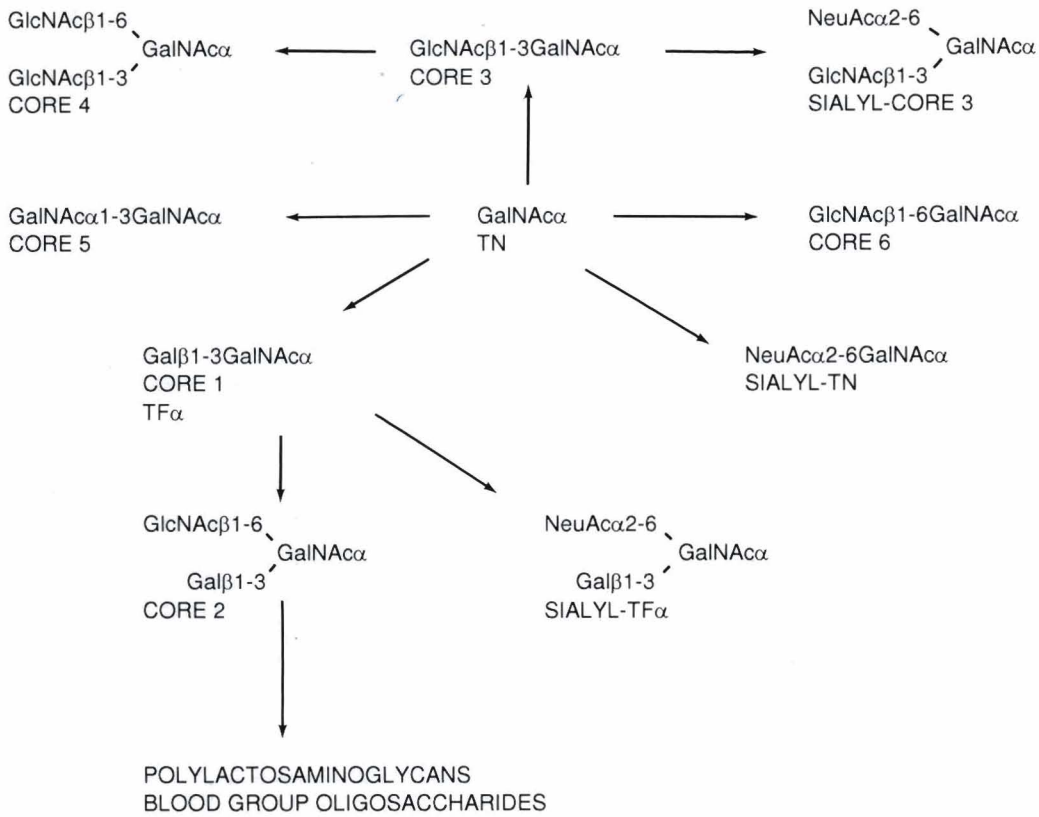


Fig. 2. Biosynthesis of core antigens.

activity of UDP-Gal:GalNAc-R-ß-galactosyltransferase, on the activities of competing glycosyltransferases acting on the same substrate position (core 3 synthesis, Fig. 2) or on enzymes that modify the core 1-disaccharide by further glycosylation (core 2 synthesis, Fig. 2). Core 1-disaccharide is generally a precursor of more complex carbohydrate chains reaching sizes of up to 20 monosaccharides and containing also α-glycosidically-linked L-fucose and sialic acids. Besides these blood group active megalosaccharides, a common type of O-linked chain is found in sialylated derivatives of core 1. Introduction of sialic acid into core 1 occurs in two positions of the substrate: at 6-GalNAc and at 3-Gal. Both types of sialylation represent biosynthetic stop signals by preventing further chain elongation of the 3- or 6-GalNAc branches. In the light of the above outlined biosynthetic background, TF antigen expression is a complex regulated epigenetic phenomenon. Among other factors it should depend on the cell-specific equipment of glycosyltransferases varying from species to species, exhibiting a genetic polymorphism within a species and organ specificity within an individual. In adult humans TF antigen is generally masked by further monosaccharide substitution, in particular, by sialylation. However, there exist a few exceptions, as for example glycophorin A on aged erythrocytes or MUC1 on human milk fat membranes (Glöckner et al., 1976). It is the merit of G.F. Springer to have revealed that TF antigen can become exposed during transformation and thus may be regarded as a potential tumor-associated antigen in a variety of human carcinomas (Springer et al., 1974, Springer and Desai, 1977; Springer, 1984). Initially detected by the reduced levels of natural anti-TF in the sera of cancer patients, the antigen has been found as a soluble substance in secretions and in the circulation as well as on carcinoma cells (Samuel et al., 1990). The pan-carcinoma-associated expression of TF antigen indicates that the transformation of epithelial cells is accompanied by alterations of the glycosylating machinery in the Golgi. Truncation of carbohydrate chains and a reduction of glycosylation is generally observed on tumor-derived glycoproteins. In particular, O-glycosylation of mucins is strongly affected by these alterations as demonstrated by enzymatic and structural studies in colorectal and mammary carcinoma models (Brockhausen et al., 1991, 1995; Vavasseur et al., 1994; Yang et al., 1994; Hanisch et al., 1996). While the positions and densities of O-glycosylation on mucins remain largely invariable during carcinogenesis (Nishimori et al., 1994; Stadie et al., 1995), TF expression should result from defective core elongation or changes in the core synthesis. In a model system of colonic carcinogenesis a biosynthetic switch from the organic characteristic formation of core 3 to core 1 and core 2 has been revealed (Brockhausen et al., 1991). In a different way the preferential core 1 synthesis in mammary carcinoma cells may originate in part from a deletion of the ß6-acetylglucosaminyl-transferase, which is responsible for the core 2 synthesis (Fig. 2). The latter has been revealed by enzymatic analyses (Brockhausen et al., 1995) and corroborated on the chemical level by structural studies of O-linked carbohydrate chains (Hull et al., 1989; Hanisch et al., 1996). In different cellular models the normally expressed polylactosamine-type oligosaccharides as found on mucins of lactating breast epithelia were shown to be truncated to the level of di- or trisaccharides (Hull et al., 1989; Hanisch et al., 1996). The preferential expression of sialylated core-type oligosaccharides on breast cancer mucin points to an alternative mechanism of incomplete chain elongation based on enzymatic competition and the introduction of biosynthetic stop signals. Since α6-sialyltransferase acts on the same substrate position as ß6-N-acetylglucosaminyltransferase (Fig. 2) the relative activities of both enzymes should regulate the branch elongation at 6GalNAc. However, cancer-associated α6-sialylation leads in turn to a substitution of core1 or a masking of TF antigen. It follows that antigen expression on the core-type level (TF, sialyl-TF, Tn, sialyl-Tn) has to be critically evaluated in the biosynthetic context. In the mammary gland, for example, TF antigen expression on MUC1 apparently remains constant during carcinogenesis (Hanisch et al., 1996) possibly due to a compensation of two opposite effects: an increase of TF disaccharide synthesis caused by a deletion of ß-6-N-acetylglucosaminyltransferase and a decrease resulting from sialylation of the TF disaccharide.

What is known about the carrier proteins of TF antigen?

While TF disaccharide is an ubiquituous core structure found in a cryptic manner on many glycoproteins, the exposed expression as TF antigen is restricted to specific carrier proteins, cells and organs. The antigen has been found on erythropoietic and lymphoid cells as component of their major sialoglycoproteins glycophorin or leukosialin, respectively (Fan et al., 1990; Nakada et al., 1995). Also a series of serum glycoproteins have been analyzed to contain the antigen, regularly (Vaith et al., 1978) or on a subfraction consisting of aged, partially desialylated glycoproteins destined for sequestration by the hepatic binding protein-mediated endocytosis of hepatocytes (Ashwell and Morell, 1974).

Among normal adult epithelia only the breast tissue is known to express TF antigen in high activities. The carrier protein in the mammary gland has been identified as a mucin (Shimizu and Yamauchi, 1982; Fischer et al., 1984a), which is a subclass of glycoproteins characterized by their high molecular mass, their high carbohydrate content (>50% per weight), the preponderance of O-glycosidically-linked, structurally complex oligosaccharides and the property of repetitive peptide elements (Carraway and Fregien, 1995). On the basis of primary structures of these tandem repeats the existence of eight distinct mucin species (MUC1 to

Table 1. TF-reactive lectins and monoclonal antibodies grouped according to their fine specificities.

GROUP	DESIGNATION	(GROUP) SPECIFICITY	REFERENCE
1	Peanut (*Arachis hypogaea*) lectin	Galß1-3GalNAcα/ß	Uhlenbruck et al., 1969; Lotan et al., 1975
	49H8		Rahman and Longenecker, 1982
	155H7; 170H82		Longenecker et al., 1987
	A78-G/A7		Karsten et al., 1995
	HB-T1	Not classified	DAKO
	RS1-114		Stein et al., 1989
	AH8-258		Chembiomed
	HT-8		Metcalfe et al., 1984
2	161H4	Galß1-3GalNAcα	Longenecker et al., 1987
	HH8		Clausen et al., 1988
	Jacalin (*Artocarpus integrifolia* lectin)		Mahanta et al., 1990
3	BW835	V(Galß1-3GalNAcα)TSA on MUC1	Hanisch et al., 1995

MUC8) has been revealed (Gendler et al., 1988; Gum et al., 1989, 1990; Porchet et al., 1991; Toribara et al., 1993; Bobek et al., 1993; Shankar et al., 1994). Most of these MUC-species represent secretory mucins with an organ-characteristic expression, with the exception of MUC1, which is found as a membrane-integrated protein on most epithelial cells and in different organs. In the mammary gland MUC1 is the only MUC-species expressed and is detectable on epithelial membranes, including also the milk fat globule membranes, and as a soluble protein in the casein fraction of human milk (Hanisch et al., 1985). In the intestine, TF antigen is normally not expressed; however, it becomes detectable during progression of cancer. In the colon, five distinct MUC-species, MUC1, MUC2, MUC3, MUC4 and MUC6 (Ogata et al., 1992; De Bolos et al., 1995) have been identified on the basis of their mRNAs as possible carriers of TF antigen. While MUC2 represents the major mucin of the normal colon, its expression decreases significantly during carcinogenesis (Ogata et al., 1992). On the other hand, MUC1 mRNA was found in all colonic tissues and the levels were the same for normal and cancer tissue. mRNA determination, however, is of restricted reliability due to the reported polydispersity of mucin mRNA. On the contrary, an immunochemical quantification of the core proteins is in turn affected by the degree of glycosylation. Carbohydrate antigens may increase tumor-associated and independent of the core protein synthesis (Hanski et al., 1995). The situation is even more complicated in the stomach, where the expression of four to six distinct MUC species, MUC1, MUC2, MUC3, MUC4, MUC5 and MUC6, has been reported (Ho et al., 1995). MUC6 represents an organ-characteristic mucin species with decreased levels in gastric cancers, while MUC2, MUC3 and MUC4 are expressed cancer-associated.

A critical evaluation of TF-specific reagents

A multitude of carbohydrate-specific proteins including lectins and antibodies has been claimed to bind specifically to the TF antigen. A critical evaluation of these reagents, however, reveals that the fine specificities of anti-TF antibodies and lectins show striking differences and can be classified according to the sizes and structural aspects of the epitopes (Table 1). There is a large group of reagents defining the TF disaccharide without discriminating between the anomeric configuration of the linkage to the aglycon. This group comprizes the lectin from *Arachis hypogaea* (PNA) (Uhlenbruck et al., 1969; Lotan et al., 1975; Neurohr et al., 1982), monoclonal antibodies generated to artificially linked synthetic TF antigens (Longenecker et al., 1987) and a series of monoclonal antibodies to the natural antigen (Rahman and Longenecker, 1982; Karsten et al., 1995). Their common feature is a cross-reactivity of the natural TFα and synthetic TFß antigen or asialo-GM_1 (Gg4Cer). The second group includes the more specific reagents distinguishing between TFα and TFß antigens. Among the plant lectins the agglutinin from *Artocarpus integrifolia* (jacalin) has been revealed to bind specifically to the TFα disaccharide (Mahanta et al., 1990). The lack of cross-reactivity with the ß-anomer has been reported for hybridoma antibodies 161H4 (Longenecker et al., 1987) and HH8 (Clausen et al., 1988). A third group of TF-specific reagents should exist, when assuming that portions of the aglycon could be included in the epitope confering some degree of carrier specificity to the antibody. There is so far only one site-specific TF reagent, monoclonal antibody BW835, that has been reported to recognize TFα disaccharide in linkage to threonine within the peptide motif VTSA of MUC1 tandem repeats (Hanisch et al., 1995). Moreover, different affinities for the various reagents have to be considered. The binding constants of lectins are generally lower compared to antibodies by one to two orders of magnitude and a considerable degree of cross-reactivity to related carbohydrates, i.e. ß-galactosides limit the specificity of lectins like PNA. The peanut lectin, for example, has been demonstrated to cross-react to α-lactose, Galß1-4Glcα and Galß1-3GlcNAcß (Neurohr et al., 1982). Other lectins from *Bauhinia purpurea* (BPA), *Maclura pomifera* (MPA) and *Sophora japonica* (SJA) bind to TF antigen, but also to another mucin core-type antigen, Tn. MPA and SJA like PNA additionally react with Galß1-4GlcNAc as well as

Galß1-3GlcNAc (Wu, 1984).

Natural anti-TF in the sera of most vertebrates consists of a family of related isoantibodies which are claimed to be generated in response to surface antigens of the intestinal flora. While at least a fraction of these natural anti-TF antibodies may be regarded as only cross-reactive anti-ß-galactosyl antibodies and the existence of genuine anti-TF antibodies in human or other mammalian sera (Kania et al., 1980) has not been proven, specific experimentally induced antisera have been reported to define the antigen (Hanisch et al., 1983). These polyclonal antisera, however, suffer from the disadvantage that their qualities are difficult to reproduce with regard to specificity and avidity. The hybridoma technology has overcome these problems. However, each monoclonal antibody, although classified as antigen specific, exhibits a distinct fine specificity and immunohistochemical staining pattern. As will be outlined below, controversial conclusions have been drawn with regard to TFα antigen expression in some organs, when different hybridoma antibodies were used for immunostaining.

Immunohistochemical and cytological investigations: a survey

Immunohistological and cytological investigations on the TF antigen during the last decades have been mainly based on the use of three kinds of reagents: PNA, polyclonal antibodies and a series of monoclonal antibodies. Additionally, other lectins like *Artocarpus integrifolia* agglutinin (jacalin; Mahanta et al., 1990) or *Amaranthus caudatus* agglutinin (amaranthin, Boland et al., 1992; Sata et al., 1992), the latter cross-reacting with its sialylated form, were only rarely used. Jacalin, for example, has been described to be a marker of tissue histiocytes (Urdiales-Viedma et al., 1995) like PNA (Howard and Batsakis, 1982).

Although cross-reactive to other ß-galactosides, the most applied lectin in immunohistochemical studies has been the peanut lectin. Numerous studies have been performed on most normal tissues and their neoplastic as well as non-neoplastic alterations. These results are summarized here, being aware of the restricted binding specificity of PNA (see above). The generation of monoclonal antibodies like 49H8, HH8, HB-T1, A78-G/A7 or BW835 has given the opportunity to reexamine the expression patterns of TF antigen. In some organs, which were investigated by both PNA and Tα/ß-specific monoclonal antibodies, different binding patterns were observed, as for example in the colorectum (Yuan et al., 1986; Cao et al., 1995) and bladder (Langkilde et al., 1992b). In this review, we will focus on the gastrointestinal tract and mammary gland and alterations of TF antigen expression during neoplastic and non-neoplastic alterations of the histological structure of these organs. These organ sites are of special interest, since they frequently develop malignant tumors.

Furthermore, numerous works emphasized the biological relevance of TF antigen expression during carcinogenesis in these organs and in their metastases. Table 2 gives a summary of the results regarding other adult human tissues in normal histology as well as histopathology.

However, considering the quantity of histological and cytological studies performed during the last years, it cannot claim completeness. Results obtained by TF-specific monoclonal antibodies as well as by staining with PNA are shown. Since peanut agglutinin binds to many normal histological structures, the staining pattern of monoclonal antibodies is of special interest. MAb RS1-114 was reported to stain mammary glands and kidney tubules, whereas normal colon, lung, ovary, liver, salivary gland, skeletal muscle, skin, spleen and pancreas were non-reactive (Stein et al., 1989). In a comparative study on urinary bladder carcinomas, mAb HH8 (Clausen et al., 1988), a polyclonal anti-TF and PNA were investigated (Langkilde et al., 1992b). The authors described a cross-reactivity of PNA with protein-bound carbohydrates other than TF antigen in immunochemical experiments. In a parallel immunohistochemical study, staining of HH8 and PNA did not correlate (Langkilde et al., 1992a). According to the latter study, HH8, but not PNA, is a predictor of recurrence. PNA binding showed a correlation to a high mean nuclear volume of carcinoma cells. Other antibodies, like BW835 or A78-G/A7 have been used in screening of different tissues. BW835 stained all mammary carcinomas tested, and the majority of specimens of ovarian, prostate, stomach, colon, pancreas and lung cancer (Pfleiderer et al., 1992). A78-G/A7 has been shown (Karsten et al., 1995) to react with the majority of mammary and colorectal carcinomas (see below). In an extensive study Cao et al. (1996) investigated human normal adult tissues using PNA and TF-specific mAbs HH8 and A78-G/A7. Some normal structures were stained both by PNA and by the mAbs (sebaceous glands, pancreatic ducts, distal tubules and collective ducts of the kidney, spermatids, cerebral medulla and macrophages in thymus, spleen and lymph nodes), whereas other structures lacked TF epitopes defined by HH8 and A78-G/A7, although they were PNA positive (epidermal layers and hair follicles, sweat glands, gastrointestinal mucosae, mucous bronchial glands, prostatic glandular epithelium, axons and Schwann cells of peripheral nerves).

Immunohistology of the mammary gland

In immunohistochemical investigations of carcinomas of the mammary gland, Springer et al. (1975) for the first time described TF antigen to be expressed as tumor antigen using natural anti-TF from human serum. These early findings were confirmed by others (Klein et al., 1979; Newman et al., 1979; Seitz et al., 1984), who observed no labeling of normal breast epithelium by PNA without prior sialidase treatment of the tissue samples. Correspondingly, binding of peanut

Table 2. Expression of TF antigen in normal human tissues and their pathologically altered counterparts (excluded are gastrointestinal and mammary tissues).

ORAN	REAGENT	NORMAL TISSUE	PATHOLOGICALLY ALTERED TISSUE	REFERENCE
Squamous epithelia				
Skin	PNA	Keratinocytes	Actinic keratosis, M. Bowen, basal cell carcinomas (in part after S'ase)	Schaumburg-Lever et al., 1986
	PNA	N.S.	Basal cell carcinomas (96%)	Vigneswaran et al., 1987
	HH8 (mab)	Spinous cells	Psoriasis: spinous and some basal cells	Dabelsteen et al., 1990
Oral epithelium	PNA	Most strongly in basal cells	Dysplasia and squamous cell carcinoma	Saku and Okabe, 1989
	PNA	Most strongly in spinous cells	Leukoplakia with dysplasia and squamous cell carcinomas	Vigneswaran et al., 1990
	HH8 (mab)	Basal cell (after S'ase)	N.S.	Mandel et al., 1991
Bronchopulmonary tract				
Lung	PNA	Basal, mucous, goblet and Clara cells, pneumocytes II	Adenocarcinoma stronger than squamous cell carcinoma	Honda et al., 1986
	RS1-114 (mab)	Non-reactive	Carcinomas (69% not classified)	Stein et al., 1989
	PNA	Serous and mucous cells of bronchial glands	N.S.	Castells et al., 1992
Digestive glands				
Salivary glands	PNA	Acinar and ductal cells (secretions)	N.S.	McMahon et al., 1989
	PNA	Serous and mucous cells and ducts (after S'ase of F'ase)	N.S.	Ito et al., 1989
	PNA	Serous and mucous cells	N.S.	Vigneswaran et al., 1989
	HH8 (mab)	Myoepithelial cells secretions	Mucoepidermoid carcinoma	Fonseca et al., 1994
	HH8 (mab)	Myoepithelial cells, basal cells	Pleomorphic adenomas, adenoid cystic carcinomas: modified myoepithelial and epithelial cells	Therkildsen et al., 1994, 1995
Pancreas	PNA	Exocrine part (very faintly)	Some carcinoma specimen and cell lines	Raedler et al., 1983
	PNA	Acinar cells (generally in non-secretors, in secretors only after F'ase/G'ase)	N.S.	Ito et al., 1988a,b
	PNA	Some acini, ducts and mucus	Chronic pancreatitis and adenocarinoma	Ching et al., 1988
	HH8 (mab)	Acinus cells, after S'ase also centroacinar cells and ductal secretions	N.S.	Philipsen et al., 1991
	AH9-16 (mab)	Acini, some ducts	Adenocarcinoma (48%)	Itzkowitz et al., 1991
	PNA	Ducts non-reactive	Adenocarcinoma (70%)	Shue et al., 1993
Liver/gall bladder				
	PNA	Gallbladder body and neck, cyst duct epithelium	N.S.	Karayannopoulou and Damjanov, 1987
	PNA	Kupffer cells, bile ducts	Cholangiocarcinoma	Rhodes et al., 1988
	PNA	Bile ducts non-reactive	Hepatocellular carcinoma (16%), cholangiolar carcinoma (89%)	Zhang et al., 1989
	PNA	Kupffer cells, bile ducts	Hepatocellular carcinoma	Murakami et al., 1992
	PNA	Bile ducts	Cholangiocarcinoma	Yamashita et al., 1993
	PNA	Gallbladder epithelia cells (stronger after S'ase)	N.S.	Madrid et al., 1994
Urinary tract				
Kidney	PNA	Podocytes	Adenocarcinoma (hypernephroma)	Vierbuchen et al., 1980
	PNA	Distal tubules and collecting ducts	N.S.	Holthöfer et al., 1981; Faraggiana et al., 1982
	PNA	Distal tubules	Oncocytoma, carcinomas of different ypes	Ulrich et al., 1985
	PNA	Distal tubules and collecting ducts	Wilms tumor, malignant rhabdoid tumors	Takagi et al., 1987
	PNA	Collecting ducts	Oncocytoma and carcinomas (after S'ase)	Ortmann et al., 1988
	PNA	Collecting ducts	Chromophobe cell carcinoma (after S'ase)	Ortmann et al., 1991
	PNA	Distal tubules	N.S.	Ivanyi and Olsen, 1991

Bladder	PNA	Urothelium (after S'ase)	Transitional cell carcinoma (TCC)	Coon et al., 1982
	PNA	Urothelium (after S'ase)	TCC (more strongly after S'ase)	Lehman et al., 1984
	Rabbit pab	Urothelium (after S'ase)	TCC (correlated to recurrence)	Ohoka et al., 1985
	PNA	Non-reactive	TCC (correlated to invasiveness)	Limas and Lange, 1986
	PNA	N.S.	TCC (correlated to DNA content)	Orntoft et al., 1988
	PNA/A584	Urothelium (after S'ase)	TCC (correlated to aggressiveness)	Blasco et al., 1988
	PNA	N.S.	TCC (after S'ase decrease before recurrence)	Yamada et al., 1988
	PNA	N.S.	TCC (correlated to low atypia)	Langkilde et al., 1989
	PNA/HH8	HH8 non-reactive	TCC ($HH8^+$ 29%, PNA^+ 53%)	Langkilde et al., 1992a,b
Female genital tract				
Cervix uteri	PNA	Epithelial cells	N.S.	Schulte et al., 1985a
	PNA	Glandular epithelium	Dysplasia and carcinoma	Bychkov and Toto, 1986
	PNA	Squamous, columnar and metaplastic epithelium	Intraepithelial and invasive neoplasms	Byrne et al, 1989a,b
Uterus	PNA	Glands	Adenocarcinoma (more strongly after S'ase)	Yen et al., 1988
	PNA	Proliferative epithelium	Adenocarcinoma	Aoki et al., 1990
Fallopian tube	PNA	Secretory cells (after S'ase), some ciliated cells	N.S.	Schulte et al., 1985b
Ovary	PNA	N.S.	Yolk sac tumor positive, dysgerminoma and choriocarcinoma negative	Teshima et al., 1984
	PNA	N.S.	Carcinomas of different histological types	Friedlander et al., 1988
	HT-8 (mab)	Non-reactive	Serous and mucinous neoplasms	Ghazizadeh et al., 1990
	PNA	N.S.	Serous, mucinous, endometrioid and clear cell neoplasms	Bychkov et al., 1991; Sasano et al., 1991
	PNA	N.S.	Sex cord-stromal cell tumors negative	Bychkov et al., 1992
Male genital tract				
	PNA	N.S.	Embryonal and yolk sac tumors positive; teratomas variable; seminomas negative	Teshima et al., 1984
Testicle	PNA	N.S.	Embryonal carcinomas, yolk sac tumors, teratomas, seminomas negative	Kosmehl et al., 1989
Prostate	PNA	Glands and ducts (after S'ase)	Benign nodular hyperplasia (after S'ase), adenocarcinoma	Ghazizadeh et al., 1984; Chastoney et al., 1986
	PNA	Glands weakly or negative	Dysplasia and adenocarcinoma	Drachenberg and Papadimitriou., 1995
Endocrine organs				
Thyroid gland	PNA	Non-reactive	Some follicular and papillary carcinomas	Sobrinho-Simoes and Damjanov., 1986
	PNA	Follicular cells	Follicular adenomas and carcinomas, some papillary carcinomas	Gonzalez-Campora et al., 1988
	PNA	N.S.	Medullary carcinomas (esp. after S'ase)	Morghen and Carpenter, 1991
Parathyroid gland	PNA	Non-reactive	Adenoma, hyperplasia, carcinoma	Thiele et al., 1986
Pituitary gland	PNA	FSH-cells	Adenomas	Behncken and Saeger, 1991
	PNA	Probably FSH-cells	Prolactin-producing adenomas	Kurosaki et al., 1995
Adrenal glands	PNA	N.S.	Phaeochromocytoma	Morghen and Carpenter, 1991
Haemato-lymphatic organs and derived cells				
Bone marrow	PNA	N.S.	B cell subpopulation in regenerating marrow	Hogemann et al., 1985; van den Doel et al., 1988
	PNA	Non-reactive	Negative	Schumacher et al., 1991
	PNA	Monocytes, macrophages centroblasts, plasma cells	Some types of leukemia and non-Hodgkin lymphoma	Erber et al., 1992
	PNA	Plasma cells	Plasmocytoma	Slupsky et al., 1993
	PNA	Megakaryocytes (after S'ase)	N.S.	Baldus et al., 1994
	PNA	Macrophages	N.S.	Baldus et al., 1995

Lymph nodes	PNA	Germinal centre B-cells	Follicular lymphoma	Rose et al., 1981
	PNA	N.S.	Hodgkin/Reed-Sternberg (HRS) cells	Möller, 1982
	PNA	Some follicular center cells	Follicular hyperplasia and lymphoma	Ree and Hsu, 1983
	PNA	N.S.	HRS cells weakly or not stained	Strauchen, 1984
	PNA	N.S.	HRS cells	Hsu and Joffe, 1984
	PNA	Macrophages	HRS cells	Ree and Kadin, 1985
	PNA	Interdigitating reticulum cells	Histiocytosis X	Ree and Kadin, 1986
	PNA	Macrophages/histiocytes	HRS cells (85.7%)	Ree et al., 1989
Tonsils	PNA	B cell subpopulation ($sIgD^+$, $CD23^+$)	N.S.	Nahm et al., 1989
Peripheral blood	PNA	Lymphocytes	Leukemic cells	Reisner et al., 1979
	PNA	Platelet gp Ib (after S'ase)	N.S.	Moroi and Jung, 1984; Baldus et al., 1995;
	PNA	$CD34^+$ cells (after S'ase)	N.S.	Thiele et al., 1995
Bone and joints				
Articular cartilage	PNA	Interterritorial matrix	N.S.	Hoedt-Schmidt et al., 1993
Central nervous system				
Brain	PNA	N.S.	Only glioblastomas scarcely positive; astrocytomas, oligodendrogliomas, ependymomas negative	Wang et al., 1989
	PNA	N.S.	Benign (mainly after S'ase), and malignant ependymoma	Kuratsu et al., 1990

S'ase: sialidase treatment; F'ase: fucosidase treatment; G'ase: galactosidase treatment; mab: monoclonal antibody; N.S.: not studied; pab: polyclonal antibody.

agglutinin to human milk-fat globules was observed after hydrolysis of sialic acid (Newman and Uhlenbruck, 1977; Fischer et al., 1984a). Morphologically differentiated carcinomas were more reactive than undifferentiated (Newman et al., 1979; Howard et al., 1981; Boecker et al., 1984; Walker, 1985; Skutelsky et al., 1988), and PNA binding sites were inversely correlated to proliferation of tumor cells measured by Ki-67 (Di Stefano et al., 1991). A transition from luminal to cytoplasmic staining was observed during neoplastic transformation (Newman et al., 1979; Howard et al., 1981; Franklin, 1983; Louis et al., 1983). In other studies, a significant correlation of PNA binding sites and estrogen receptors was postulated (Klein et al., 1981a, 1983; Boecker et al., 1984). This issue was discussed very controversially in later publications. Some were in favour of this hypothesis (Helle and Krohn, 1986; Di Stefano et al., 1991), others denied a significant correlation (van der Linden et al., 1985; Stanley et al., 1986). The inconsistent expression of PNA binding sites in estrogen receptor-positive carcinomas may be caused by an induction of galactosyltransferases by estradiol and prolactin, as described in rat mammary gland and in a tumor model (Ip and Dao, 1978). In an investigation of human mammary carcinoma cells, only a combination of estradiol and progesterone could increase PNA binding, whereas administration of a single steroid hormone did not exert a significant effect (Daxenbichler et al., 1986).

No prognostic relevance was ascribed to PNA staining of breast carcinomas (Barry et al., 1984). Using Mab 49H8 (Rahman and Longenecker, 1982), a worse prognostic impact of TF expression, i.e. an increased expression during tumor progression, has been reported (Wolf et al., 1988).

Several monoclonal TF-specific antibodies were highly reactive with mammary carcinomas (Rahman and Longenecker, 1982; Longenecker et al., 1987; Karsten et al., 1995) (Fig. 3). For example, A78-G/A7 stained 87% of 46 primary breast cancers tested and 91% of 23 regional metastases (Karsten et al., 1995). However, no extensive clinicopathological studies using these specific reagents have been performed up to now. A78-G/A7 exerted a minimal reactivity in luminal membranes and secretions of «normal» breast epithelium adjacent to tumors (19%). Using HH8 and HB-T, TF antigen was not expressed by normal breast epithelium; however, benign lesions like fibroadenoma and fibrocystic disease were reactive in a certain percentage (38% and 25%, respectively) (Reed et al., 1994). From 122 carcinomas, only 20% expressed the TF antigen. Additionally, none of 18 lymph node metastases exhibited any reactivity.

Future investigations will have to answer some of the open questions:

1. The correlation of TF antigen expression and estrogene/progesteron status of mammary carcinomas as well as the possible mechanisms of an induction of glycosyltransferase activity by these hormones.

2. The staining patterns of PNA and of the different mabs (A78-G/A7 vs. 49H8, HH8 and HB-T), are strikingly different according to the literature. These conflicting results challenge future comparative studies on a great number of benign and malignant breast lesions including a prognostic evaluation.

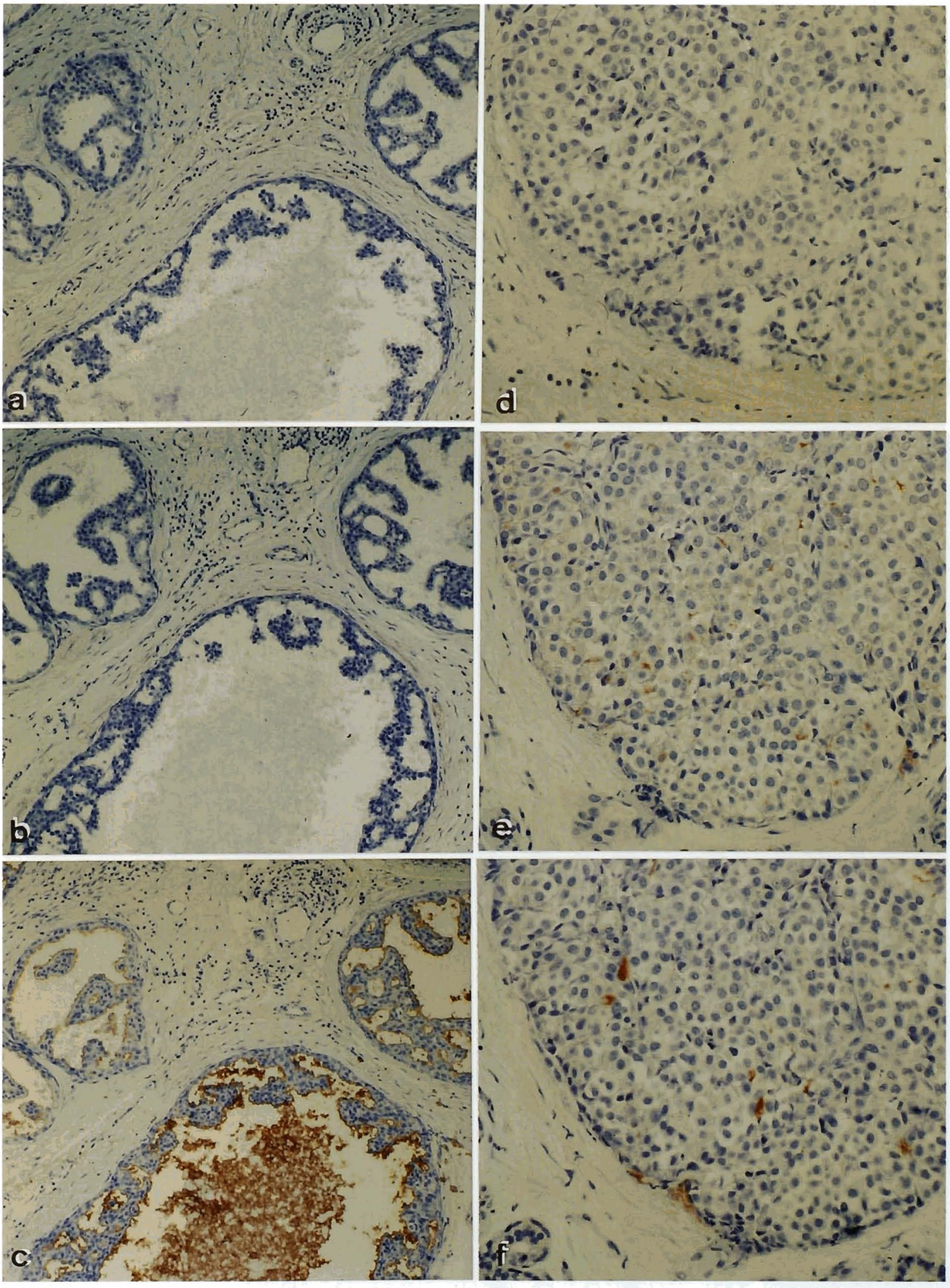


Fig. 3. Serial sections of two mammary carcinomas exhibiting different patterns of differentiation. a,d) PNA; b,e) mab A78-G/A7; c,f) mab BW835. In the carcinoma on the left side, only BW835 epitope is exposed (c), whereas the tumor on the right side shows A78-G/A7 as well as BW 835 epitope. PNA remains negative in both cases. a-c, x 330; d-f, x 660

Immunohistology of the colorectum

In the human colon and rectum, peanut agglutinin binding sites exhibited by adenocarcinomas were described for the first time in 1981 (Klein et al., 1981b). Working with a fluorescence-technique, the authors observed a complete lack of PNA staining even after sialidase treatment) in normal colon, whereas the so-called «transitional mucosa» surrounding carcinoma tissue contained some scattered positive goblet cells. Carcinoma cells as well as glandular mucus were heterogeneously stained. A strong reactivity of large bowel cancer was later confirmed by others using PNA (Boland et al., 1982a; Cooper, 1982; Kellokumpu et al., 1986; Rhodes et al., 1986; Campo et al., 1988; Calderó et al., 1989) or by using monoclonal antibodies (Yuan et al., 1986; Itzkowitz et al., 1989). TF antigen appears early during development of cancer, since also colorectal adenomas were reported to be PNA-reactive at the same time (Boland et al., 1982b; Cooper and Reuter, 1983; Rhodes et al., 1986). A change from cytoplasmic binding in mild or moderate dysplastic adenomas to a secretion-associated reactivity in severe dysplastic lesions was observed (Kellokumpu et al., 1986; Campo et al., 1988). In accordance with this, a change of PNA binding pattern during increase of nuclear:cell height ratio was reported (Orntoft et al., 1991). The Golgi staining, typical for grade I adenomas, decreased in favour of a luminal expression in grade II and III adenomas. PNA reactivity further increased with polyp size (>2 cm) and villous histology (McGarrity et al.,

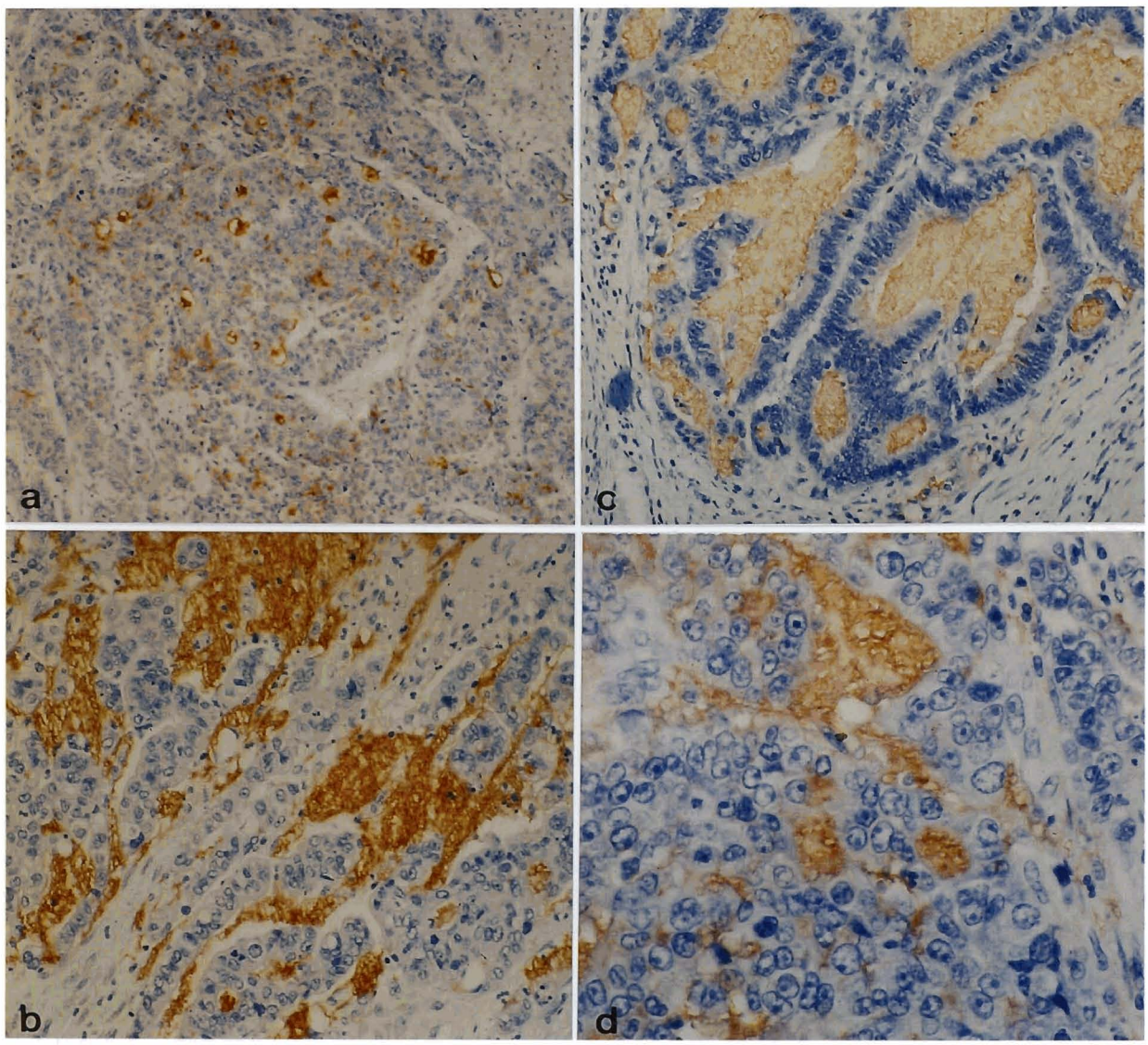


Fig. 4. Immunohistochemical investigation of colorectal carcinomas shows a strong expression of A78-G/A7 epitope **(a, d)** BW835 epitope **(b)** and PNA binding sites **(c)**, associated to secretions. a, x 330; b-d, x 660

1989). Amaranthin also reacted with colorectal adenomas and carcinomas (Sata et al., 1992). In summary, these data favour the assumption that TF antigen is a marker of carcinogenesis during progression of the adenoma-carcinoma sequence.

With regard to the expression of PNA binding sites in normal (as well as hyperplastic or transitional) colorectal mucosa, controversial findings were reported. Whereas PNA receptors were described in the supranuclear portion of epithelial cells (Cooper, 1982; Kellokumpu et al., 1986; Campo et al., 1988), this observation was not confirmed by others (Rhodes et al., 1986). Calderó et al. (1989) and McGarrity et al. (1989) described a binding to goblet cells in the ascending colon only. Using a mab (AH9-16), Itzkowitz et al. (1989) did not obtain any staining of normal colonic membrane, whereas transitional mucosa was reactive in 47% of the specimens. A complete lack of TF reactivity in normal colorectum as well as in colon cancer was described in a study using mabs HH8 and HT-8 (Orntoft et al., 1990). Using amaranthin, which cross-reacts with NeuAcα2-3Galß1-3GalNAc, cryptic (i.e. sialylated) TF antigen could be visualized in goblet cells and enterocytes of surface and crypt epithelium of the right colon. In the left colorectum, only crypt enterocytes were reactive (Sata et al., 1992). Anti-TF mab did not react with any cells.

In ulcerative colitis, a change of PNA binding pattern was described. Instead of a binding to supranuclear cytoplasm, a reactivity of the glycocalix and/or apical portion of columnar cells was observed in cases with highly active inflammation (Cooper et al., 1987).

As a consequence of the different fine-specificities of PNA, polyclonal antibodies (pabs) and monoclonal antibodies (mabs) distinct staining patterns were revealed in comparative studies. In a study using PNA, a polyclonal anti-TF serum and mab AH8-258 specific for Galß1-3GalNAcα (Yuan et al., 1986), nearly 50% of the normal colorectal specimens were stained by the lectin, but a reaction with pab or mab was virtually absent. Generally, PNA showed the highest sensitivity in staining of hyperplastic and adenomatous polyps as well as carcinomas. On the contrary, the mab reactivity correlated with polyp size, histological size and dysplasia in adenomas (Yuan et al., 1986). In a recent comparative investigation (Cao et al., 1995), various mabs with different fine-specificities were employed. Normal and transitional mucosa were generally non-reactive. In carcinomas, mabs cross-reacting between TFα and TFß stained slightly more intensively than TFα- or especially TFß-specific mabs. Liver metastases showed positivity in a significantly higher percentage (91%) than primary carcinomas (60%). Additionally, TF-positive carcinomas developed liver metastases in a higher percentage than TF-negative tumors (57% vs. 14%). These results would fit in well with experimental data suggesting that the organotropism of liver metastasis may be mediated by a binding of tumor cells via exposed TF disaccharide to the asialoglycoprotein receptor of hepatocytes (Springer et al., 1983) or a receptor on Kupffer cells (Kolb-Bachofen et al., 1982). In a murine colon carcinoma model, liver metastasis after intrasplenic injection of tumor cell subpopulations was proportional to the degree of tumor cell-surface galactose expression (Yeatman et al., 1989). The reported evidence points to a general mechanism, since also the metastasis of murine sarcoma and lymphoma cells has been demonstrated in model systems to be mediated by the interaction of exposed galactose on tumor cells and cellular lectins in the liver (Beuth et al., 1988).

Mab BW835, recently characterized to bind to MUC1-bound TF antigen (Hanisch et al., 1995), was compared in a histochemical study with mab A78-G/A7 and mabs directed to the core peptides of MUC1 (HMFG2) or MUC2 (4F1) to elucidate a coexpression of TF antigen and one of the major MUC species in human colon (Baldus et al., submitted for publication) (Fig. 4). A strong correlation of TF- and MUC1-specific antibody binding was observed in the carcinoma samples, whereas MUC2-specific mab showed a distinct pattern of reactivity. TF- and MUC1-specific mabs including BW835 only faintly reacted in transitional mucosa, but showed a more intense staining of secretions during progression of the adenoma-carcinoma sequence. In contrast, MUC2 exhibited a predominantly perinuclear staining with a decrease of staining intensity associated with malignant transformation.

Recent biochemical investigations have supplied important insights into the expression of TF antigen and glycosyltransferases involved in its biosynthesis. TF antigen could be demonstrated by chemical analysis of mucin derived from ulcerative colitis and colonic adenocarcinomas after release by O-glycanase. In normal colonic mucin, TF antigen could only be detected after removal of sialic acid and fucose (Campbell et al., 1995). This fits well in with its detectability by histochemical means in most neoplasms and its concealment in normal tissue. Correspondingly, ß3-galactosyltransferase was increased in colorectal carcinoma tissues (Yang et al., 1994) as well as in a cellular model of colonic tumor progression derived from a patient suffering from familial polyposis coli (Vavasseur et al., 1994).

In summary the following points should be emphasized with reference to TF antigen expression in the colorectum:

1. PNA shows a broad spectrum of reactivity with neoplasms, whereas normal tissue exhibits no or weak expression of its binding site.

2. PNA binding patterns are different from those of monoclonal TF-specific antibodies. Its reactivity may (in part) be explained by its binding to Galß1-3GlcNAc (Lotan et al., 1975), which forms the type 1 blackbone of mucin-associated O-glycans and is strongly expressed even in normal colonic mucin (Podolsky, 1985). However, binding of mabs like A78-G/A7 and HH8 cannot be inhibited by this structure in immunochemical

assays (Karsten et al., 1995).

3. Regarding TF-specific mabs, conflicting results have been published. As an exception, mabs HH8 and HT8 did not stain colorectal carcinomas at all (Orntoft et al., 1990). Since increased TF antigen has recently be shown biochemically in mucin derived from ulcerative colitis and colonic adenocarcinoma (Campbell et al., 1995), the obvious discrepancy can only be explained by different fine-specificities and binding properties of the mabs or by the staining methods used in different laboratories.

4. A78-G/A7 reacts most strongly with liver metastases as well as primary colorectal carcinomas, which cause liver metastases (Cao et al., 1996). This observation has to be verified by investigating a greater number of cases. Thereby, a possible role of TF antigen as a predictor of prognosis and groups of «low risk» and «high risk» patients has to be evaluated.

5. Recent results suggest a correlation between the expression of MUC1 mucin protein cores and TF antigen in the colorectum (Baldus et al., unpublished) indicating that this mucin species may be regarded as the primary carrier of carcinoma-associated TF antigen and could form the basis of serodiagnostic assays.

Immunohistology of gastric tissues

In the stomach, an inhomogeneous reactivity of surface epithelial (Sato and Spicer, 1982), neck and antral glandular cells as well as of carcinomas was observed, whereas all parietal cells and most chief cells were negative (Kuhlmann et al., 1983). An additional staining of canalicular membranes of parietal cells was revealed and reactivity of carcinomas could be increased by sialidase digestion in most cases, especially in Hale positive and diffuse cell carcinomas (Fischer et al., 1984b). Cryptic TF antigen was also observed in intestinal-type tumors, whereas diffuse cell carcinomas remained weakly positive even after sialidase treatment (Bur and Franklin, 1985). Regarding the protein carrier of TF antigen, biochemical analysis has shown that PNA binding sites are expressed by the polymorphic epithelial mucin (MUC1) and a novel 24 kD glycoprotein derived from KATO-III human gastric carcinoma cells (Masuzawa et al., 1992). An α1-2fucosylated cryptic form of TF antigen has been detected in gastric surface epithelia of secretor individuals by mab MBr1 (specific for α1-2fucosylated TF antigen) or by PNA after fucosidase digestion (Okada et al., 1994). In intestinal metaplasia and cancer, the fucosylated antigen was suppressed or completely lost, whereas the expression of sialylated or unmasked TF antigen increased. A difference between intestinal- and diffuse-type carcinomas with regard to PNA binding was not observed (Sotozono et al., 1994). In another investigation on precursor lesions of gastric carcinomas using HB-T1, TF antigen was more prevalent in incomplete than in complete intestinal metaplasia. Also adenomatous and hyperplastic lesions exhibited TF reactivity. Foveolar epithelial and neck cells of antrum and corpus were reactive, as were mucous antral glands and the canalicular membranes of parietal cells (Carneiro et al., 1994). Previously, a staining of only 20% of gastric carcinomas had been reported by the same group using mabs HH8 and HB-T, which did not stain normal epithelium at all (David et al., 1992). Recently, Chung et al. (1996) investigated the relation (using mab HB-T) between expression of TF antigen and clinicopathological features as well as outcome in patients with gastric cancer: TF positivity was significantly correlated with serosal invasion, hepatic and lymph node metastasis or peritoneal dissemination. In stage III and IV disease, survival of patients exhibiting TF antigen was significantly worse.

Summarized, normal gastric surface epithelial and glandular cells as well as stomach cancers exhibit TF antigen expression recognized by mab HB-T as well as PNA, however, in a varying quantity. The antigen may be masked by α1-2-fucosylation or sialylation. It seems to be related to various clinicopathological characteristics and prognosis.

Perspectives

As is evident from Table 2, the majority of immunohistochemical studies on TF antigen expression has been performed by using the lectin from peanuts. The undesired cross-reactivities of this lectin to other ß-galactosides, however, cast a reflection on the reliability of the reported findings. Since more specific reagents are available now and the fine specificities of some monoclonal antibodies are known, there is a need to reinvestigate the tissue distribution of TF antigen in health and disease.

Particularly, only rarely the tissue distribution of TF antigen in healthy individuals has been studied in a comprehensive and systematic manner (Cao et al., 1996). This knowledge will be of the utmost importance, if the carbohydrate antigen is envisaged to serve as a determinant for the development of a successful tumor vaccine (McLean and Longenecker, 1991; MacLean et al., 1992; Yacyshyn et al., 1995). We should also know more about the serodiagnostic qualities of this antigen, since earlier studies have not considered the use of carrier-specific reagents for the evaluation of a tumor-associated TF antigen expression on distinct mucin species. In the same context the early observations made by Springer, i.e. the carcinoma-associated decrease of natural anti-TF activities in patients' sera, should be reevaluated by designing new assay systems discriminating between «specific anti-TF» and cross-reactive «anti-ß-galactoside» antibodies.

Finally, the pathobiological relevance of this antigen is revealed by its possible functional involvement in carbohydrate-mediated cell adhesion and organ-specific arrest of metastasizing tumor cells. Moreover to its association with metastasizing carcinoma cells in the liver (Cao et al., 1995), evidence has been obtained for

TF antigen on tumor cells to mediate their adhesion to hepatocytes (Springer et al., 1983). The molecular basis of this recognition process should lie in the specificity of the asialoglycoprotein receptor (Ashwell and Morell, 1974) or a related lectin on Kupffer cells (Kolb-Bachofen et al., 1982). In accordance with this, experimental liver metastasis has been inhibited by the infusion of galactose or arabinogalactan (Beuth et al., 1988). Clinical studies have been initiated and will hopefully corroborate these findings for colorectal carcinoma patients treated by perioperative infusions. The possible prevention of metastasis into the liver by infusion of carbohydrate inhibitors will challenge chemists and clinicians to synthesize and test more efficient polyvalent conjugates of ß-galactosides (including TF disaccharide) to reduce the high concentrations of low affinity inhibitors like galactose.

References

Aoki D., Nozawa S., Iizuka R., Kawakami H. and Hirano H. (1990). Differences in lectin binding patterns of normal endometrium and endometrial adenocarcinoma, with special reference to staining with *Ulex europeus* agglutinin I and peanut agglutinin. Gynecol. Oncol. 37, 338-345.

Ashwell G. and Morell A.G. (1974). The role of surface carbohydrates in the hepatic recognition and transport of circulating glycoproteins. Adv. Enzymol. 41, 99-128.

Baldus S.E., Thiele J., Charles A., Hanisch F.-G. and Fischer R. (1994). Carbohydrate antigens of human megakaryocytes and platelet glycoproteins: a comparative study. Histochemistry 102, 205-211.

Baldus S.E., Thiele J., Park Y.-O, Charles A., Mross C., Hanisch F.-G., Zirbes T.K., Wickenhauser C. and Fischer R. (1995). Carbohydrate and peptide antigens in macrophage populations derived from human bone marrow and milk - and immunomorphological and immunochemical analysis. Histochem. J. 27, 630-638,

Barry J.D., Koch T.J., Cohen C., Brigati D.J. and Sharkey F.E. (1984). Correlation of immunohistochemical markers with patient prognosis in breast carcinoma. A quantitative study. Am. J. Clin. Pathol. 82, 582-585.

Behncken A. and Saeger W. (1991). Lectin-binding in pituitary adenomas and normal pituitaries. Pathol. Res. Pract. 187, 629-631.

Beuth J., Ko H.L., Schirrmacher V., Uhlenbruck G. and Pulverer G. (1988). Inhibition of liver tumor cell colonisation in two animal tumor models by lectin blocking with D-galactose or arabinogalactan. Clin. Exp. Metastas. 6, 115-120.

Blasco E., Torrado J., Belloso L., Arocena F., Gutiérrez-Hoyos A. and Cuadrado E. (1988). T-antigen: a prognostic indicator of high recurrence index in transitional carcinoma of the bladder. Cancer 61, 1091-1095.

Bobek L.A., Tsai H., Biesbrock A.R. and Levine M.J. (1993). Molecular cloning, sequence, and specificity of expression of the gene encoding the low molecular weight human salivary mucin (MUC7). J. Biol. Chem. 268, 20563-20569.

Boecker W., Klaubert A., Bahnsen J., Schweickhart G., Pollow K., Mitze M., Kreienberg R., Beck T. and Stegner H.-E. (1984). Peanut lectin histochemistry of 120 mammary carcinomas and its relation to tumor type, grading, staging, and receptor status. Virchows Arch. (A) 403, 149-161.

Boland C.R., Montogomery C.K. and Kim Y.S. (1982a). Alterations in human colonic mucin occurring with cellular differentiation and malignant transformation. Proc. Natl. Acad. Sci. USA 79, 2051-2055.

Boland C.R., Montgomery C.K. and Kim Y.S. (1982b). A cancer-associated mucin alteration in benign colonic polyps. Gastroenterology 82, 664-672.

Boland C.R., Martin M.A. and Goldstein I.J. (1992). Lectin reactivities as intermediate biomarkers in premalignant colorectal epithelium. J. Cell. Biochem. Suppl. 16G, 103-109.

Brockhausen I., Romero P.A. and Herscovics A. (1991). Glycosyltransferase changes upon differentiation of CaCo-2 human colonic adenocarcinoma cells. Cancer Res. 51, 3136-3142.

Brockhausen I., Yang J.M., Burchell J., Whitehouse C. and Taylor-Papadimitriou J. (1995). Mechanisms underlying aberrant glycosylation of MUC1 mucin in breast cancer cells. Eur. J. Biochem. 233, 607-617.

Bur M. and Franklin W.A. (1985). Lectin binding to human gastric adenocarcinomas and adjacent tissues. Am. J. Pathol. 119, 279-287.

Bychkov V. and Toto P.D. (1986). Wheat germ and peanut agglutinin binding to normal dysplastic and neoplastic cervical epithelium. Gynecol. Obstet. Invest. 21, 158-163.

Bychkov V., Doland J.R. and Reddy V.B. (1991). Lectin binding to common epithelial tumors of the ovaries. Gynecol. Obstet. Invest. 31, 166-171.

Bychkov V., Deligdisch L., Talerman A. and Reddy V.B. (1992). Lectin histochemistry of sex cord-stromal cell tumors and small cell carcinoma of the ovaries. Gynecol. Obstet. Invest. 34, 115-118.

Byrne P., Williams A. and Rollason T. (1989a). Studies of lectin binding to the human cervix uteri: I. Normal cervix. Histochem J. 21, 311-322.

Byrne P., Williams A. and Rollason T. (1989b). Studies of lectin binding to the human cervis uteri: II. Cervical intraepithelial neoplasia and invasive squamous carcinoma. Histochem. J. 21, 323-336.

Calderó J., Campo E., Ascaso C., Ramos J., Panadés M.J. and Rene J.M. (1989). Regional distribution of glycoconjugates in normal, transitional and neoplastic human colonic mucosa. Virchows Arch. (A) 415, 347-356.

Campbell B.J., Finnie I.A., Hounsell E.F. and Rhodes J.M. (1995). Direct demonstration of increased expression of Thomsen-Friedenreich (TF) antigen in colonic adenocarcinoma and ulcerative colitis mucin and its concealment in normal mucin. J. Clin. Invest. 95, 571-576.

Campo E., Condom E., Palacín A., Quesada E. and Cardesa A. (1988). Lectin binding patterns in normal and neoplastic colonic mucosa. Dis. Colon Rectum 31, 892-899.

Cao Y., Karsten U.R., Liebrich W., Haensch W., Springer G.F. and Schlag P.M. (1995). Expression of Thomsen-Friedenreich-related antigens in primary and metastatic colorectal carcinomas. Cancer 76, 1700-1708.

Cao Y., Stosiek P., Springer G.F. and Karsten U. (1996). Thomsen-Friedenreich-related carbohydrate antigens in normal adult human tissues: a systematic and comparative study. Histochem. Cell. Biol. 106, 197-207.

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

Carraway K.L. and Fregien N. (1995). Mucin structure and function: insight from molecular biology. Trends Glycosci. Glycotechnol. 7, 31-

44.

Castells M.T., Ballesta J., Madrid J.F., Martínez-Menárguez J.A. and Avilés M. (1992). Ultrastructural localization of glycoconjugates in human bronchial glands: the subcellular organization of N- and O-linked oligosaccharide chains. J. Histochem. Cytochem. 40, 265-274.

Chastoney P., Hurlimann J. and Gardiol D. (1986). Biological tissue markers in benign and malignant disease of the human prostate. Virchows Arch. (A) 410, 221-229.

Ching C.K., Black R., Helliwell T., Savage A., Barr H. and Rhodes J.M. (1988). Use of lectin histochemistry in pancreatic cancer. J. Clin. Pathol. 41, 324-328.

Chung Y.-S., Yamashita Y., Kato Y., Nakata B., Sawada T. and Sowa M. (1996). Prognostic significance of T antigen expression in patients with gastric carcinoma. Cancer 77, 1768-1773.

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

Codington J.F., Linsley K.B. and Jeanloz R.W. (1975). Immunochemical and chemical investigations of the structure of glycoprotein fragments obtained from epiglycanin, a glycoprotein at the surface of the TA3-Ha cancer cell. Carbohydrate Res. 40, 171-182.

Coon J.S., Weinstein R.S. and Summers J.L. (1982). Blood group precursor T-antigen expression in human urinary bladder carcinoma. Am. J. Clin. Pathol. 77, 692-699.

Cooper H.S. (1982). Peanut lectin-binding sites in large bowel carcinoma. Lab. Invest. 47, 383-390.

Cooper H.S. and Reuter V.E. (1983). Peanut lectin-binding sites in polyps of the colon and rectum. Lab. Invest. 49, 655-661.

Cooper H.S., Farano P. and Coapman R.A. (1987). Peanut lectin binding sites in colons of patients with ulcerative colitis. Arch. Pathol. Lab. Med. 111, 270-275.

Dabelsteen E., Broby-Johansen U., Jeppe-Jensen D. and Mandel U. (1990). Cell surface glycosylation patterns in psoriasis. APMIS 98, 221-228.

David L., Nesland J.M., Clausen H., Carneiro F. and Sobrinho-Simoes M. (1992). Simple mucin-type carbohdyrate antigens (Tn, sialosyl-Tn and T) in gastric mucosa, carcinomas and metastases. APMIS 100 (suppl. 27), 162-172.

Daxenbichler G., Duerken M., Marth C., Boeck G. and Dapunt O. (1986). Influence of steroids and retinoic acid on peanut-lectin binding of human breast cancer cells. J. Steroid Biochem. 24, 119-124.

De Bolos C., Garrido M. and Real F.X. (1995). MUC6 apomucin shows a distinct normal tissue distribution that correlates with Lewis antigen expression in the human stomach. Gastroenterology 109, 723-734.

Di Stefano D., Mingazzini P.L., Scucchi L., Donnetti M. and Marinozzi V. (1991). A comparative study of histopathology, hormone receptors, peanut lectin binding, Ki-67 immunostaining, and nucleolar organizer region-associated proteins in human breast cancer. Cancer 67, 463-471.

Drachenberg C.B. and Papadimitriou J.C. (1995). Aberrant pattern of lectin binding in low and high grade prostatic intraepithelial neoplasia. Cancer 75, 2539-2544.

Elhammer A. and Kornfeld S. (1986). Purification and characterization of UDP-N-acetylgalactosamine:polypeptide N-acetylgalactosaminyl-transferase from bovine colostrum and murine lymphoma BW 5147 cells. J. Biol. Chem. 261, 5249-5255.

Erber W.N., Asbahr H., Meyer B., Herrmann R.P. and Davies J.M. (1992). Peanut agglutinin (lectin from *Arachis hypogaea*) binding to hemopoietic cells: an immunophenotypic study using a biotin streptavidin technique. Pathology 24, 173-176.

Fan J.Q., Kanatani K., Andoh N., Yamamoto K., Kumagai H. and Tochikura T. (1990). Release of T-antigen, a carcinoma marker from native human cells, by endo-alpha-N-acetylgalactosaminidase of *Alcaligenes sp.* Biochem. Biophys. Res. Comm. 172, 341-347.

Faraggiana T., Malchiodi F., Prado A. and Churg J. (1982). Lectin-peroxidase conjugate reactivity in normal human kidney. J. Histochem. Cytochem. 30, 451-458.

Fischer J., Klein P.J., Farrar G.H., Hanisch F.-G. and 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., Klein P.J., Vierbuchen M., Skutta B., Uhlenbruck G. and Fischer R. (1984b). Characterization of glycoconjugates of human gastrointestinal mucosa by lectins. J. Histochem. Cytochem. 32, 681-689.

Fonseca I., Costa Rosa J., Félix A., Therkildsen M.H., Mandel U. and Soares J. (1994). SImple mucin-type carbohydrate antigens (T, Tn and sialosyl-Tn) in mucoepidermoid carcinoma of the salivary glands. Histopathology 25, 537-543.

Franklin W.A. (1983). Tissue binding of lectins in disorders of the breast. Cancer 51, 295-300.

Friedenreich V. (1930). The Thomsen hemagglutination phenomenon. Levin & Munksgaard. Copenhagen.

Friedlander M., Leary J. and Russell P. (1988). An evaluation of CA 125, CA1 and peanut lectin immunoreactivity in epithelial ovarian neoplasms: correlation with histopathological features, prognostic variables and patient outcome. Pathology 20, 38-44.

Gendler S., Taylor-Papadimitriou J., Duhig T., Rothbard J. and Burchell J. (1988). Highly immunogenic region of human polymorphic epithelial mucin expressed by carcinomas made up of tandem repeats. J. Biol. Chem. 263, 12820-12823.

Ghazizadeh M., Kagawa S., Izumi K. and Kurokawa K. (1984). Immunohistochemical localization of T antigen-like substance in benign hyperplasia and adenocarcinoma of the prostate. J. Urol. 132, 1127-1130.

Ghazizadeh M., Oguro T., Sasaki Y., Aihara K., Araki T. and Springer G.F. (1990). Immunohistochemical and ultrastructural localization of T antigen in ovarian tumors. Am. J. Clin. Pathol. 93, 315-321.

Glöckner W.M., Newman R.A., Dahr W. and Uhlenbruck G. (1976). Alkali-labile oligosaccharides from glycoproteins of different erythrocyte and milk fat globule membranes. Biochim. Biophys. Acta 443, 402-413.

González-Campora R., Sánchez-Gallego F., Martin-Lacave I., Mora-Marín J., Montero-Linares C. and Galera-Davidson H. (1988). Lectin histochemistry of the thyroid gland. Cancer 62, 2354-2362.

Gum J.R., Byrd J.C., Hicks J.W., Toribara N.W., Lamport D.T.A. and Kim Y.S. (1989). Molecular cloning of human intestinal mucin cDNAs. J. Biol. Chem. 264, 6480-6487.

Gum J.R., Hicks J.W., Swallow D.M., Lagace R.L., Byrd J.C., Lamport D.T.A., Siddiki B. and Kim Y.S. (1990). Molecular cloning of cDNAs derived from a novel human intestinal mucin gene. Biochem. Biophys. Res. Comm. 171, 407-415.

Hanisch F.-G., Farrar G.H., Schmalisch R. and Uhlenbruck G. (1983). Immunohistochemistry of O-glycosidically linked Galß1-3GalNAc on fragments of human glycophorin A. Immunobiology 165, 147-160.

Hanisch F.-G., Uhlenbruck G., Dienst C., Stottrop M. and Hippauf E. (1985). Ca125 and Cal9-9: two cancer-associated sialylsaccharide antigens on a mucus glycoprotein from human milk. Eur. J. Biochem. 149, 323-330.

Hanisch F.-G., Chai W., Rosankiewicz J.R., Lawson A.M., Stoll M.S. and Feizi T. (1993). Core-typing of O-linked glycans from human gastric mucins. Eur. J. Biochem. 217, 645-655.

Hanisch F.-G., Stadie T. and Bosslet K. (1995). Monoclonal antibody BW835 defines a site-specific Thomsen-Friedenreich disaccharide linked to threonine with the VTSA Motif of MUC1 tandem repeats. Cancer Res. 55, 4036-4040.

Hanisch F.-G., Stadie T.R.E., Deutzmann F. and Peter-Katalínic J. (1996). MUC1 glycoforms in breast cancer. Cell line T47D as a model for carcinoma-associated alterations of O-glycosylation. Eur. J. Biochem. 236, 318-327.

Hanski C., Hanski M.-L., Zimmer T., Ogorek D., Devine P., Riecken E.O. (1995). Characterization of the major sialyl-Le^X-positive mucins present in colon, colon carcinoma, and sera of patients with colorectal cancer. Cancer Res. 55, 928-933.

Helle M. and Krohn K. (1986). Reactivity of a monoclonal antibody recognizing an estrogen receptor regulated glycoprotein in relation to lectin histochemistry in breast cancer. Virchows Arch. (A) 410, 23-29.

Ho S.B., Shekels L.L., Toribara N.W., Kim Y.S., Lyftogt C., Cherwitz D.L. and Niehans G.A. (1995). Mucin gene expression in normal preneoplastic, and neoplastic human gastric epithelium. Cancer Res. 55, 2681-2690.

Hoedt-Schmidt S., McClure J., Jasani M.K. and Kalbhen D.A. (1993). Immunohistochemical localization of articular cartilage proteoglycan and link protein in situ using monoclonal antibodies and lectin-binding methods. Histochemistry 99, 391-403.

Hogemann P.H.G., Veerman A.J.P., van Zantwijk C.H. and Huismans D.R. (1985). Peanut agglutinin distinguishes cALLA positive reactive bone marrow cells from leukaemic blasts. Br. J. Haematol. 59, 392-394.

Holthöfer H., Virtanen I., Pettersson E., Törnroth T., Alfthan O., Lindner E. and Miettinen A. (1981). Lectins as fluorescence microscopic markers for saccharides in the human kidney. Lab. Invest. 45, 391-399.

Honda T., Ono K., Katsuyama N., Nakayama J. and Akamatsu T. (1986). Mucosubstance histochemistry of the normal mucosa and epithelial neoplasms of the lung. Acta Pathol. Jpn. 36, 665-680.

Howard D.R. and Batsakis J.G. (1982). Peanut agglutinin: a new marker for tissue histiocytes. Am. J. Clin. Pathol. 77, 401-408.

Howard D.R., Ferguson P. and Batsakis J.G. (1981). Carcinoma-associated cytostructural antigenic alterations: detection by lectin binding. Cancer 47, 2872-2877.

Hsu S.-M. and Jaffe E. (1984). LeuM1 and peanut agglutinin stain the neoplastic cells of Hodgkin's disease. Am. J. Clin. Pathol. 82, 29-32.

Hull S.R., Bright A., Carraway K.L., Abe M., Hayes D.F. and Kufe D.W. (1989). Oligosaccharide differences in the DF3 sialomucin antigen from normal human milk and the BT-20 human breast carcinoma cell line. Cancer Comm. 1, 261-267.

Ip C. and Dao T.L. (1978). Effect of estradiol and prolactin on galactosyltransferase and α-lactalbumin activities in rat mammary gland and mammary tumor. Cancer Res. 38, 2077-2083.

Ito N., Nishi K., Nakajima M., Ohmura Y. and Hirota T. (1988a). Effects of α-L-fucosidase digestion on lectin staining in human pancreas. J. Histochem. Cytochem. 36, 503-509.

Ito N., Nishi K., Nakajima M., Ohmura Y. and Hirota T. (1988b). Effects of α-galactosidase on lectin staining in human pancreas. Histochemistry 89, 121-128.

Ito N., Nishi K., Nakajima M., Ohmura Y. and Hirota T. (1989). Histochemical analysis of the chemical structure of blood group-related carbohydrate chains in serous cells of human submandibular glands using lectin staining and glycosidase digestion. J. Histochem. Cytochem. 37, 1115-1124.

Itzkowitz S.H., Yuan M., Montgomery C.K., Kjeldsen T., Takahashi H.K., Bigbee W.L. and Kim Y.S. (1989). Expression of Tn, sialosyl-Tn, and T antigens in human colon cancer. Cancer Res. 49, 197-204.

Itzkowitz S., Kjeldsen T., Friera A., Hakomori S.-I., Yang U.-S. and Kim Y.S. (1991). Expression of Tn, sialosyl Tn, and T antigens in human pancreas. Gastroenterology 100, 1691-1700.

Ivanyi B. and Olsen T.S. (1991). Immunohistochemical identification of tubular segments in percutaneous renal biopsies. Histochemistry 95, 351-356.

Kania J., Uhlenbruck G., Janssen E., Klein P.J. and Vierbuchen M. (1980). Isolation, characterization and implications of anti-TF (Thomsen-Friedenreich) agglutinins from different sources. Immunobiology 157, 154-168.

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

Karayannopoulou G. and Damjanov I. (1987). Lectin binding sites in the human gallbladder and cystic duct. Histochemistry 88, 75-83.

Kellokumpu I., Karhi K. and Andersson L.C. (1986). Lectin-binding sites in normal, hyperplastic, adenomatous and carcinomatous human colorectal mucosa. APMIS 94, 271-280.

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

Klein P.J., Newman R.A., Mueller P., Uhlenbruck G., Citoler P., Schaefer H.E., Lennartz K.J. and Fischer R. (1979). The presence and significance of the Thomsen-Friedenreich antigen in mammary gland. II. Its topochemistry in normal, hyperplastic and carcinoma tissue of the breast. J. Cancer Res. Clin. Oncol. 93, 205-214.

Klein P.J., Vierbuchen M., Wuerz H., Schulz K.-D. and Newman R.A. (1981a). Secretion-associated lectin-binding sites as a parameter of hormone dependence in mammary carcinoma. Br. J. Cancer 44, 746-748.

Klein P.J., Osmers R., Vierbuchen M., Ortmann M., Ksaia J. and Uhlenbruck G. (1981b). The importance of lectin binding sites and carcinoembryonic antigen with regard to normal hyperplastic, adenomatous, and carcinomatous colonic mucosa. Rec. Res. Cancer Res. 79, 1-9.

Klein P.J., Vierbuchen M., Fischer J., Schulz K.-D., Farrar G. and Uhlenbruck G. (1983). The significance of lectin receptors for the evaluation of hormone dependence in breast cancer. J. Steroid Biochem. 19, 839-844.

Klenk E. and Uhlenbruck G. (1960). Über neuraminsäurehaltige Mucoide aus Menschenerythrocystenstroma, ein Beitrag zur Chemie der Agglutinogene. Z. Physiol. Chem. 319, 151-160.

Kolb-Bachofen V., Schlepper-Schäfer J., Vogell W. and Kolb H. (1982). Electron microscopic evidence for an asialoglycoprotein receptor on Kupffer cells: localization of lectin-mediated endocytosis. Cell 29, 859-866.

Kosmehl H., Langbein L. and Katenkamp D. (1989). Lectin histochemistry of human testicular germ cell tumors. Neoplasma 36, 29-39.

Kuhlmann W.D., Peschke P. and Wurster K. (1983). Lectin-peroxidase conjugates in histopathology of gastrointestinal mucosa. Virchows Arch. (A) 398, 319-328.

Kuratsu J., Sueyoshi N., Mihara Y. and Ushio Y. (1990). Localization and significance of peanut agglutinin-binding sites on ependymoma cells. Acta Neuropathol. 79, 634-639.

Kurosaki M., Hori T., Takata K., Kawakami H. and Hirano H. (1995). Peanut agglutinin binding to human prolactin-producing pituitary adenomas. Acta Neuropathol. 89, 475-482.

Langkilde N.C., Wolf H. and Orntoft T.F. (1989). Binding of wheat germ agglutinin and peanut agglutinin to human transitional cell carcinoma: correlation with histopathologic grade, invasion and DNA ploidy. Cancer 64, 849-853.

Langkilde N.C., Wolf H., Clausen H., Kjeldsen T. and Orntoft T.F. (1992a). Nuclear volume and expression of T-antigen, sialosyl-Tn-antigen and Tn-antigen in carcinoma of the human bladder. Cancer 69, 219-227.

Langkilde N.C., Wolf H., Clausen H. and Orntoft T.F. (1992b). Human urinary bladder carcinoma glycoconjugates expressing T- (Galß(1-3)GalNAcα-1-O-R) and T-like antigens: a comparative study using peanut agglutinin and poly- and monoclonal antibodies. Cancer Res. 52, 5030-5036.

Lehman T.P., Cooper H.S. and Mulholland S.G. (1984). Peanut lectin binding sites in transitional cell carcinoma of the urinary bladder. Cancer 53, 272-277.

Limas C. and Lange P. (1986). T antigen in normal and neoplastic urothelium. Cancer 58, 1236-1245.

Longenecker B.M., Willans D.J., MacLean G.D., Selvaraj S., Suresh M.R. and Noujaim A.A. (1987). Monoclonal antibodies and synthetic tumor-associated glycoconjugates in the study of the expression of Thomsen-Friedenreich-like and Tn-like antigens on human cancers. J. Natl. Cancer Inst. 78, 489-496.

Lotan R., Skutelsky E., Danon D. and Sharon H. (1975). The purification, composition, and specificity of the anti-T lectin from peanut (*Arachis hypogaea*). J. Biol. Chem. 250, 8518-8523.

Louis C.J., Sztynda T., Cheng Z.-M. and Wyllie R.G. (1983). Lectin binding affinities of human breast tumors. Cancer 52, 1244-1250.

MacLean G.D. and Longenecker B.M. (1991). Clinical significance of the Thomsen-Friedenreich antigen. Sem. Cancer Biol. 2, 433-439.

MacLean G.D., Bowen-Yacyshyn M.B., Samuel J., Meikle A., Stuart G., Nation J., Poppema S., Jerry M., Koganty R., Wong T. and Longenecker B.M. (1992). Active immunization of human ovarian cancer patients against a common carcinoma (Thomsen-Friedenreich) determinant using a synthetic carbohydrate antigen. J. Immunother. 11, 292-305.

Madrid J.F., Castells M.T., Martínez-Menárguez J.A., Avilés M., Hernández F. and Ballesta J. (1994). Subcellular characterization of glycoproteins in the principal cells of the human gallbladder. Histochemistry 101, 195-204.

Mäkelä O. and Cantell K. (1958). Destruction of M and N blood group receptors of human red cells by some influenza viruses. Ann. Med. Exper. Fenn. 36, 366-374.

Mahanta S.K., Sastry M.V. and Surolia A. (1990). Topography of the combining region of a Thomsen-Friedenreich-antigen-specific lectin jacalin (Artocarpus integrifolia agglutinin). A thermodynamic and circular-dichroism spectroscopic study. Biochem. J. 265, 831-840.

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

Masuzawa Y., Miyauchi T., Hamanoue M., Ando S., Yoshida J., Takao S., Shimazu H., Adachi M. and Muramatsu T. (1992). A novel core protein as well as polymorphic epithelial mucin carry peanut agglutinin binding sites in human gastric carcinoma cells: sequence analysis and examination of gene expression. J. Biochem. 112, 609-615.

McGarrity T.J., Peiffer L.P. and Abt A.B. (1989). Lectin histochemistry of adenomatous polyps. Cancer 64, 1708-1713.

McMahon R.T.F., Benbow E.W., Lofthouse A.K. and Stoddart R.W. (1989). Human salivary gland glycoconjugates: a lectin histochemical study. Histochem. J. 21, 663-674.

Metcalfe S., Springer G.F., Svennsen R.J. and Tegtmayer H. (1984). Monoclonal antibodies specific for human Thomsen-Friedenreich (T) and Tn blood group precursor antigens. In: Protides of the biological fluids. Vol. 32. Peters H. (ed). Pergamon. New York. pp 765-768.

Möller P. (1982). Peanut agglutinin: a useful tool for detecting Hodgkin cells in paraffin sections. Virchows Arch. (A) 396, 313-317.

Morghen M. and Carpenter F. (1991). Peanut lectin: a histochemical marker for phaeochromocytomas. Virchows Arch. (A) 419, 203-207.

Moroi M. and Jung S.E. (1984). Selective staining of human platelet glycoproteins using nitrocellulose transfer on electrophoresed protein and peroxidase-conjugated lectins. Biochim. Biophys. Acta 798, 295-301.

Murakami I., Sarker A.B., Hayashi K. and Agaki T. (1992). Lectin binding patterns in normal liver, chronic active hepatitis, and hepatocellular carcinoma. Acta Pathol. Jpn. 42, 566-572.

Nahm M.H., Takes P.A., Bowen M.B. and Macke K.A. (1989). Subpopulations of B lymphocytes in germinal centers. II. A germinal center B cell subpopulation expresses sIgD and CD23. Immunol. Lett. 21, 201-208.

Nakada H., Inoue M., Tanaka N., Wakamiya N. and Yamashina I. (1995). Expression of the T antigen on a T-lymphoid cell line, supT1. Glycoconj. J. 12, 356-359.

Neurohr K.J., Bundle D.R., Young N.M. and Mantsch H.H. (1982). Binding of disaccharides by peanut agglutinin as studied by ultraviolet difference spectroscopy. Eur. J. Biochem. 123, 305-310.

Newman R.A. and Uhlenbruck G. (1977). Investigation into the occurrence and structure of lectin-receptors on human and bovine erythrocyte, milk-fat globule and lymphocyte plasma-membrane glycoproteins. Eur. J. Biochem. 76, 149-155.

Newman R.A., Klein P.J. and Rudland P.S. (1979). Binding of peanut lectin to breast epithelium, human carcinomas, and a cultured rat mammary stem cell: use of the lectin as a marker of mammary differentiation. J. Natl. Cancer Inst. 63, 1339-1346.

Niikawa S., Yamada H., Sakai N., Ando T., Zhang W., Hara A. and Shimokawa K. (1992). Distribution of cellular carbohydrate moieties in human dysontogenetic brain tumors, especially in craniopharyngioma and epidermoid/dermoid. Acta Neuropathol. 85, 71-78.

Nishimori I., Johnson N.R., Sanderson S.D., Perini F., Mountjoy K., Cerny R.L., Gross M.L. and Hollingsworth M.A. (1994). Influence of acceptor substrate primary amino acid sequence on the activity of human UDP-N-acetylgalactosamine: polypeptide N-acetylgalactosaminyltransferase. Studies with the MUC1 tandem repeat. J. Biol. Chem. 269, 16123-16130.

O'Connell B.C., Hagen F.K. and Tabak L.A. (1992). The influence of flanking sequence on the O-glycosylation of threonine in vitro. J. Biol. Chem. 267, 25010-25018.

Ogata S., Uehara H., Chen A. and Itzkowitz S.H. (1992). Mucin gene

expression in colonic tissues and cell lines. Cancer Res. 52, 5971-5978.

Ohoka H., Shinomiya H., Yokoyama M., Ochi K., Takeuchi M. and Utsumi S. (1985). Thomsen-Friedenreich antigen in bladder tumors as detected by specific antibody: a possible marker of recurrence. Urol. Res. 13, 47-50.

Okada Y., Sotozono M.-A., Sakai N., Yonei T., Nakanishi S. and Tsuji T. (1994). Fucosylated Thomsen-Friendereich antigen in α-anomeric configuration in human gastric surface epithelia: an allogenic carbohydrate antigen possibly controled by the Se gene. J. Histochem. Cytochem. 42, 371-376.

Orntoft T.F., Petersen S.E. and Wolf H. (1988). Dual-parameter flow cytometry of transitional cell carcinomas. Quantitation of DNA content and binding of carbohydrate ligands in cellular subpopulations. Cancer 61, 963-970.

Orntoft T.F., Harving N. and Langkilde N.C. (1990). O-linked mucin-type glycoproteins in normal and malignant colon mucosa: lack of T-antigen expression and accumulation of TN and sialosyl-Tn antigens in carcinomas. Int. J. Cancer 45, 666-672.

Orntoft T.F., Langkilde N.C., Wiener H. and Ottosen P.D. (1991). Cellular localization of PNA binding in colorectal adenomas: comparison with differentiation, nuclear: cell height ratio and effect of desialylation. APMIS 99, 275-281.

Ortmann M., Vierbuchen M. and Fischer R. (1988). Renal oncocytoma. II. Lectin and immunohistochemical features indicating an origin from the collecting duct. Virchows Arch. (B) 56, 175-184.

Ortmann M., Vierbuchen M. and Fischer R. (1991). Sialylated glyco-conjugates in chromophobe renal cell carcinoma compared with other renal cell tumors. Indication of its development from the collecting duct epithelium. Virchows Arch. (B) 61, 123-132.

Pfleiderer P., Hanisch F.-G. and Boßlet K. (1992). New monoclonal antibody for the in vitro detection of breast carcinoma associated mucin. Second International Workshop on carcinoma-associated mucins. Cambridge (UK).

Philipsen E.K., Jorgensen M. and Dabelsteen E. (1991). Expression of blood-group-related carbohydrate antigens in normal human pancreatic tissue. APMIS 99, 931-940.

Podolsky D.K. (1985). Oligosaccharide structure of human colonic mucin. J. Biol. Chem. 260, 8262-8271.

Porchet N., Cong N.V., Dufosse J., Audie J.P., Guyonnet-Duperat V., Gross M.S., Denis S., Degand P., Bernheim A. and Aubert J.P. (1991). Molecular cloning and chromosome localization of a novel human tracheo-bronchial mucin cDNA containing tandemly repeated sequences of 48 base pairs. Biochem. Biophys. Res. Comm. 175, 414-422.

Raedler A., Schmiegel W.H., Raedler E., Arndt R. and Thiele H.-G. (1983). Lectin-defined cell surface glycoconjugates of pancreatic cancer cells and their nonmalignant counterparts. Exp. Cell Biol. 51, 19-28.

Rahman A.F.R. and Longenecker B.M. (1982). A monoclonal antibody specific for the Thomsen-Friedenreich cryptic T antigen. J. Immunol. 129, 2021-2024.

Ree H.J. and Hsu S.M. (1983). Lectin histochemistry of malignant tumors: I. Peanut agglutinin (PNA) receptors in follicular lymphoma and follicular hyperplasia. An immunohistochemical study. Cancer 51, 1631-1638.

Ree H.J. and Kadin M.E. (1985). Macrophage-histiocytes in Hodgkin's disease: the relation to clinicopathologic presentation and course of disease. Cancer 56, 333-338.

Ree H.J. and Kadin M.E. (1986). Peanut agglutinin: a useful marker for histiocytosis X and interdigitating reticulum cells. Cancer 57, 282-287.

Ree H.J., Neiman R.S., Martin A.W., Dallenbach F. and Stein H. (1989). Paraffin section markers for Reed-Sternberg cells. Cancer 63, 2030-2036.

Reed W., Bryne M., Clausen H., Dabelsteen E. and Nesland J.M. (1994). Simple mucins (T, sialosyl-T, Tn and sialosyl-Tn) are not diagnostic for malignant breast lesions. Anticancer Res. 14, 609-616.

Reisner Y., Biniaminov M., Rosenthal E., Sharon N. and Ramot B. (1979). Interaction of peanut agglutinin with normal human lymphocytes and with leukemic cells. Proc. Natl. Acad. Sci. USA 76, 447-451.

Rhodes J.M., Black R.R. and Savage A. (1986). Glycoprotein abnormalities in colonic carcinomata, adenomata and hyperplastic polyps shown by lectin peroxidase histochemistry. J. Clin. Pathol. 39, 1331-1334.

Rhodes J.M., Hubscher S., Black R., Elias E. and Savage A. (1988). Lectin histochemistry of the liver in biliary disease, following transplantation and in cholangiocarcinoma. J. Hepatol. 6, 277-282.

Rose M.L., Habeshaw J.A., Kennedy R., Sloane J., Wiltshaw E. and Davies A.J.S. (1981). Binding of peanut lectin to germinal centre-cells: a marker for B-cell subsets of follicular lymphoma? Br. J. Cancer 44, 68-74.

Saku T. and Okabe H. (1989). Differential lectin-bindings in normal and precancerous epithelium and squamous cell carcinoma of the oral mucosa. J. Oral Pathol. Med. 18, 438-445.

Samuel J., Noujaim A.A., MacLean G.D., Suresh M.R. and Longenecker B.M. (1990). Analysis of human tumor associated Thomsen-Friedenreich antigen. Cancer Res. 50, 4801-4808.

Sasano H., Saito Y., Nagura H., Kudo R., Rojas M. and Silverberg S.G. (1991). Lectin histochemistry in mucinous and serous ovarian neoplasms. Int. J. Gynecol. Pathol. 10, 252-292.

Sata T., Roth J., Zuber C., Stamm B., Rinderle S.J., Goldstein I.J. and Heitz P.U. (1992). Studies on the Thomsen-Friedenreich antigen in human colon with the lectin *Amaranthin*. Lab. Invest. 66, 175-186.

Sato A. and Spicer S.S. (1982). Ultrastructural localization of galactose in the glycoprotein of gastric surface cells with a peanut lectin conjugate. Histochem. J. 14, 125-138.

Schachter H. (1986). Biosynthetic controls that determine the branching and microheterogeneity of protein-bound oligosaccharides. Cell Biol. 64, 163-181.

Schaumburg-Lever G., Alroy J., Ucci A. and Lever W.F. (1986). Cell surface carbohydrates in proliferative epidermal lesions. II. Masking of peanut agglutinin (PNA) binding sites in solar keratoses, Bowen's disease, and squamous cell carcinoma by neuraminic acid. J. Cutan Pathol. 13, 163-171.

Schulte B.A., Poon K.C., Rao K.P.P. and Spicer S.S. (1985a). Lectin histochemistry of complex carbohydrates in human cervix. Histochem. J. 17, 627-654.

Schulte B.A., Rao K.P.P., Kreutner A., Thomopoulos G.N. and Spicer S.S. (1985b). Histochemical examination of glycoconjugates of epithelial cells in the human fallopian tube. Lab. Invest. 52, 207-219.

Schumacher U., Horny H.-P., Welsch U. and Kaiserling E. (1991). Lectin histochemistry of human bone marrow: investigation of trephine biopsy specimens in normal and reactive states and neoplastic disorders. Histochem. J. 23, 215-220.

Seitz R.C., Fischer K., Stegner H.E. and Poschmann A. (1984).

Detection of metastatic breast carcinoma cells by immunofluorescent demonstration of Thomsen-Friedenreich antigen. Cancer 54, 830-836.

Shankar V., Gilmore M.S., Elkins R.C. and Sachdev G.P. (1994). A novel human airway mucin cDNA encodes a protein with unique tandem-repeat organization. Biochem. J. 300, 295-298.

Shimizu M. and Yamauchi K. (1982). Isolation and characterization of mucin-like glycoprotein in human milk fat globule membrane. J. Biochem. 91, 515-524.

Shue G.L., Kawa S., Kato M., Oguchi H., Kobayashi T., Koiwai T., Tokoo M., Furuta S., Kanai M. and Homma T. (1993). Expression of glycoconjugates in pancreatic, gastric, and colonic tissue by *Bauhinia purpurea*, *Vicia villosa*, and peanut lectins. Scand. J. Gastroenterol. 28, 599-604.

Skutelsky E., Hoenig S., Griffel B. and Alroy J. (1988). The distribution of lectin receptor sites in human breast lesions. Pathol. Res. Pract. 183, 469-475.

Slupsky J.R., Duggan-Keen M., Booth L.A., Karpas A., Rhodes E.G.H., Cawley J.C. and Zuzel M. (1993). The peanut-agglutinin (PNA)-binding surface components of malignant plasma cells. Br. J. Haematol. 83, 567-573.

Sobrinho-Simoes M. and Damjanov I. (1986). Lectin histochemistry of papillary and follicular carcinoma of the thyroid gland. Arch. Pathol. Lab. Med. 110, 722-729.

Sorensen T., White T., Wandall H.H., Kristensen A.K., Roepstorff P. and Clausen H. (1995). UDP-N-acetyl-alpha-D-galactosamine: polypeptide N-acetylgalactosaminyltransferase. Identification and separation of two distinct transferase activities. J. Biol. Chem. 270, 24166-24173.

Sotozono M.-A., Okada Y. and Tsuji T. (1994). The Thomsen-Friedenreich antigen-related carbohydrate antigens in human gastric intestinal metaplasia and cancer. J. Histochem. Cytochem. 42, 1575-1584.

Springer G.F. (1984). T and Tn, general carcinoma antigens. Science 224, 1198-1206.

Springer G.F. and Ansell N.J. (1958). Inactivation of human erythrocyte agglutinogens M and N by influenza viruses and receptor-destroying enzyme. Proc. Natl. Acad. Sci. 44, 182-189.

Springer G.F. and Desai P.R. (1977). Cross-reacting carcinoma-associated antigens with blood group and precursor specificities. Transplant. Proc. 9, 1105-1111.

Springer G.F., Desai P.R. and Banatwala I. (1974). Blood group MN specific substances and precursors in normal and malignant human breast tissues. Naturwissenschaften 61, 457-458.

Springer G.F., Desai P.R. and Banatwalay I. (1975). Blood group MN antigens and precursors in normal and malignant human breast glandular tissue. J. Natl. Cancer Inst. 54, 335-339.

Springer G.F., Cheingsong-Popov R., Schirrmacher V., Desai P.R. and Tegtmeyer H. (1983). Proposed molecular basis of murine tumor cell-hepatocyte interaction. J. Biol. Chem. 258, 5702-5706.

Stadie T., Chai W., Lawson A.M., Byfield P. and Hanisch F.-G. (1995). Studies on the order and site-specificity of GalNAc-transfer to MUC1 tandem repeats by UDP-GalNAc: polypeptide N-acetyl-galactosaminyl transferase from milk or mammary carcinoma cells. Eur. J. Biochem. 229, 140-147.

Stanley M.W., Kiang D.T. and Sibley R.K. (1986). Peanut lectin binding in breast carcinoma. Cancer 58, 2046-2051.

Stein R., Chen S., Grossman W. and Goldenberg D.M. (1989). Human lung carcinoma monoclonal antibody specific for the Thomsen-Friedenreich antigen. Cancer Res. 49, 32-37.

Strauchen J.A. (1984). Lectin receptors as markers of lymphoid cells. II. Reed-Sternberg cells share lectin-binding properties of monocyte macrophages. Am. J. Pathol. 116, 370-376.

Takagi M., Takakuwa T., Ushigome S., Nakata K., Fujioka T. and Watanabe A. (1987). Sarcomatous variants of Willms tumor. Immunohistochemical and ultrastructural comparison with classical Wilms tumor. Cancer 59, 963-971.

Teshima S., Hirohashi S., Shimasato Y., Kishi K., Ino Y., Matsumoto K. and Yamada T. (1984). Histochemically demonstrable changes in cell surface carbohydrates of human germ cell tumors. Lab. Invest. 50, 271-277.

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

Therkildsen M.H., Mandel U., Christensen M. and Dabelsteen E. (1995). Thomsen-Friedenreich antigen as marker of myoepithelial and basal cells in the parotid gland, pleomorphic adenomas and adenoid cystic carcinomas. APMIS 103, 558-567.

Thiele J., Vierbuchen M., Arnold G., Walgenbach S. and Fischer R. (1986). Lectin-binding sites in human parathyroid tissue. J. Histochem. Cytochem. 34, 1201-1206.

Thiele J., Wickenhauser C., Baldus S.E., Kuemmel T., Zirbes T.K., Drebber U., Wirtz R., Thiel A., Hansmann M.-L. and Fischer R. (1995). Characterization of $CD34^+$ human hemopoietic progenitor cells from the peripheral blood: enzyme-, carbohydrate- and immunocytochemistry, morphometry and ultrastructure. Leuk. Lymph. 16, 483-491.

Thomas D.B. and Winzler R.J. (1969). Structural studies on human erythrocyte glycoproteins. Alkali-labile oligosaccharides. J. Biol. Chem. 244, 5943-5946.

Thomsen O. (1927). Ein vermehrungsfähiges Agens als Veränderer des isoagglutinatorischen Verhaltens der roten Blutkörperchen, eine bisher unbekannte Quelle der Fehlbestimmungen. Z. Immun-Forsch. 52, 85-107.

Tomita M., Furtmayr H. and Marchesi V.T. (1978). Primary structure of human erythrocyte glycophorin A. Isolation and characterization of peptides and complete amino acid sequence. Biochemistry 17, 4756-4770.

Toribara N.W., Roberton A.M., Ho S.B., Kuo W.L., Gum E., Hicks J.W., Gum J.R., Byrd J.C., Siddiki B. and Kim Y.S. (1993). Human gastric mucin. Identification of a unique species by expression cloning. J. Biol. Chem. 268, 5879-5885.

Uhlenbruck G., Pardoe G.I. and Bird G.W.G. (1969). On the specificity of lectins with a broad agglutination spectrum. II. Studies on the nature of the T-antigen and the specific receptors for the lectin from *Arachis hypogaea* (ground nut). Z. Immun.-Forsch. 138, 423-433.

Ulrich W., Horvat R. and Kirsch K. (1985). Lectin histochemistry of kidney tumors and its pathomorphological relevance. Histopathology 9, 1037-1050.

Urdiales-Viedma M., De Haro-Munoz T., Martos-Padilla S., Abad-Ortega J.M., Varela-Duran J. and Granda-Paez R. (1995). Jacalin, another marker for histiocytes in paraffin-embedded tissues. Histol. Histopathol. 10, 597-602.

Vaith P., Assmann G. and Uhlenbruck G. (1978). Characterization of the oligosaccharide side chain of apolipoprotein C-III from human plasma very low density lipoproteins. Biochem. Biophys. Acta 541, 234-240.

van den Doel L.J., Pieters R., Huismans D.R., van Zantwijk C.H.,

Loonen A.H., Broekema G.J., de Waal F.C. and Veerman A.J.P. (1988). Immunological phenotype of lymphoid cells in regenerating bone marrow of children after treatment for acute lymphoblastic leukemia. Eur. J. Haematol. 41, 170-175.

van der Linden J.C., Baak J.P.A., Lindeman J., Smeulders A.W.M. and Meyer C.J.L.M. (1985). Carcinoembryonic antigen expression and peanut agglutinin in primary breast cancer and lymph node metastases; lack of correlation with clinical, histopathological, biochemical and morphometric features. Histopathology 9, 1051-1059.

Vavasseur F., Dole K., Yang J., Matta K.L., Myerscough N., Corfield A., Paraskeva C. and Brockhausen I. (1994). O-glycan biosynthesis in human colorectal adenoma cells during progression to cancer. Eur. J. Biochem. 222, 415-424.

Vierbuchen M., Klein P.J., Uhlenbruck G., Klein P.J., Schäfer H.E. and Fischer R. (1980). The significance of lectin receptors in the kidney and in hypernephroma (renal adenocarcinoma). Rec. Res. Cancer Res. 75, 68-75.

Vigneswaran N., Haneke E. and Peters K.P. (1987). Peanut agglutinin immunohistochemistry of basal cell carcinoma. J. Cutan. Pathol. 14, 147-153.

Vigneswaran N., Haneke E. and Hornstein O.P. (1989). A comparative lectin histochemical study of major and minor salivary glands with special reference to the labial glands. Arch. Oral. Biol. 34, 739-748.

Vigneswaran N., Peters K.P., Horstein O.P. and Diepgen T.L. (1990). Alteration of cell surface carbohydrates associated with ordered and disordered proliferation of oral epithelia: a lectin histochemical study in oral leukoplakias, papillomas and carcinomas. Cell. Tissue Kinet. 23, 41-55.

Walker R.A. (1985). The binding of peroxidase-labelled lectins to human breast epithelium. IV. - The reactivity of breast carcinomas to peanut, soy bean and *Dolichus biflorus* agglutinins. J. Pathol. 145, 269-277.

Wang X.C., Kochi N., Tani E., Kaba K., Matsumoto T. and Shindo H. (1989). Lectin histochemistry of human gliomas. Acta Neuropathol. 79, 176-182.

Wolf M.F., Ludwig A., Fritz P. and Schumacher K. (1988). Increased expression of Thomsen-Friedenreich antigens during tumor progression in breast cancer patients. Tumor Biol. 9, 190-194.

Wu A.M. (1984). Differential binding characteristics and applications of DGalß1-3DGalNAc specific lectins. Mol. Cell. Biochem. 61, 131-141.

Yacyshyn M.B., Poppema S., Berg A., MacLean G.D., Reddish M.A., Meikle A. and Longenecker B.M. (1995). $CD69^+$ and $HLA\text{-}DR^+$ activation antigens on peripheral blood lymphocyte populations in metastatic breast and ovarian cancer patients: correlations with survival following active specific immunotherapy. Int. J. Cancer 61, 470-474.

Yamada T., Fukui I., Yokokawa M. and Oshima H. (1988). Changing expression of ABH blood group and cryptic T-antigens of non-invasive and superficially invasive papillary transitional cell carcinoma of the bladder from initial occurrence to malignant progression. Cancer 61, 721-726.

Yamashita K., Yonezawa S., Tanaka S., Shirahama H., Sakoda K., Imai K., Xing P.-X., McKenzie I.F.C., Hilkens J., Kim Y.S. and Sato E. (1993). Immunohistochemical study of mucin carbohydrates and core proteins in hepatolithiasis and cholangiocarcinoma. Int. J. Cancer 55, 82-91.

Yang J.-M., Byrd J.C., Siddiki B.B., Chung Y.-S., Okuno M., Sowa M., Kim Y.S., Matta K.L. and Brockhausen I. (1994). Alterations of O-glycan biosynthesis in human colon cancer tissues. Glycobiology 4, 873-884.

Yeatman T.J., Bland K.I., Copeland E.M. III and Kimura A.K. (1989). Tumor cell-surface galactose correlates with the degree of colorectal liver metastasis. J. Surg. Res. 46, 567-571.

Yen Y., Schmiemann C. and Damjanov I. (1988). Lectin histochemistry of uterine adenocarcinomas. Arch. Pathol. Lab. Med. 112, 791-793.

Yuan M., Itzkowitz S.H., Boland C.R., Kim Y.D., Tomita J.T., Palekar A., Bennington J.L., Trump B.F. and Kim Y.S. (1986). Comparison of T-antigen expression in normal premalignant, and malignant human colonic tissue using lectin and antibody immunohistochemistry. Cancer Res. 46, 4881-4847.

Zhang S.M., Wu M.C., Chen H., Zhang X.Z., Chong W.M. and Sho H.K. (1989). Characteristics of the distribution of lectin receptors in intrahepatic cholangiocellular caricnoma. Histochem. J. 21, 296-300.