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# Immunoreactivity of Thomsen-Friedenreich (TF) antigen in human neoplasms: The importance of carrier-specific glycotope expression on MUC1

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**Summary.** On the basis of their known fine specificities we evaluated the immunohistochemical marker qualities of two monoclonal antibodies (mabs) defining the tumor-associated TF disaccharide Galß1-3GalNAc. This antigen is expressed in certain tumors in correlation with prognosis and metastasis. The reactivity of one of these mabs (A78-G/A7) depends on clustered TF disaccharides (glycosylation at vicinal Ser/Thr positions) while the other - mab BW835 - has been characterized to bind specifically to TF disaccharide linked to a motif within the MUC1 repeat. Therefore, mab BW835 represents an interesting tool for the identification of tumor-associated glycoforms of MUC1, which are involved in tumor progression and metastasis, but also in the recognition of tumor cells by cytotoxic T cells.

As references the TF-binding lectins from peanut (PNA) and *Artocarpus integrifolia* (jacalin) were applied. The binding patterns of these immunoreagents were strikingly distinct. Mab BW835 showed a significantly stronger reactivity than mab A78-G/A7, especially in gastric, mammary, pancreatic, thyreoideal, renal and bladder carcinomas. PNA and jacalin receptors exhibited an expression in the majority of all cancer types, with the exception of seminoma and glioblastoma/sarcoma. These results can be explained by the broader fine specificities of the lectins. Furthermore, a strong expression of MUC1-bound TF antigen is indicated by the staining pattern of mab BW835. The marker qualities of both antigens, TF and MUC1, are combined in the binding specificity of BW835, and hence this antibody may have a high impact for the immunodetection of these tumor-associated antigens.

**Key words:** Thomsen-Friedenreich (TF) antigen, Lectin(s), Monoclonal antibody (mab), Immunohistology, Tumor-associated antigen(s)

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## Introduction

During the last two decades, the Thomsen-Friedenreich (TF) antigen which is identical to the protein-linked disaccharide Galß1-3GalNAcα has gained increasing interest as a carcinoma-associated antigen (Springer et al., 1983; Springer, 1984; Hanisch and Baldus, 1997). Some studies revealed correlations of TF expression and clinico-pathological parameters as well as a prognostic impact in mammary (Wolf et al., 1988), gastric (Chung et al., 1996) and colorectal cancer (Cao et al., 1995, 1997). In addition to the great number of histopathological studies documenting the marker qualities of this antigen, its possible functional involvement in the process of metastasis could be demonstrated. In this context, the asialoglycoprotein receptor of the liver (Ashwell and Morell, 1974; Kolb-Bachofen et al., 1982) has been postulated to mediate metastasis. Moreover, TF antigen may be involved in the regulation of cell proliferation, since PNA and TF-specific antibodies exerted proliferative effects in colorectal cancer cell lines (Ryder et al., 1992; Yu et al., 1997). According to immunohistochemical investigations, various "TF-specific" reagents with different binding specificities were identified in the class of plant lectins or generated as monoclonal antibodies to natural or synthetic TF antigens. Initially, the TF expression in normal and neoplastic tissues was characterized by the immunohistochemical reaction with peanut agglutinin (PNA), which is well-known to bind TF antigen (Uhlenbruck et al., 1969, Lotan et al., 1975), but to cross-react with related ß-galactosides (Neurohr et al., 1982; Wu et al., 1994). Another lectin derived from *Artocarpus integrifolia* (jacalin) shares the ability to bind to TFα antigen (Sastry et al., 1986; Mahanta et al., 1990), but it also recognizes Tn antigen (Wu et al., 1994). Meanwhile, a large number of monoclonal antibodies (mabs) directed against TF antigen have been generated, which were, however, only partially characterized with respect to their binding patterns in human neoplastic tissues (Hanisch and Baldus, 1997). In this study, we applied mab A78-G/A7 (Karsten et al.,

1995) which binds to clustered α- and β-anomers of TF antigen irrespective of their carrier molecules. In contrast to PNA and jacalin, this antibody does not cross-react with related oligosaccharide structures. On the other hand, we evaluated the marker qualities of mab BW835, which reacts with Galß1-3GalNAc only if it is bound α-anomerically to the tandem repeat peptide of MUC1. This epitope seems to be of special interest, since MUC1 is also well known to represent a tumor-associated antigen which is involved in tumor biology. Its expression was described as a negative prognostic factor in gastric cancer (Baldus et al., 1998a). Additionally, in the human colorectal adenoma-carcinoma sequence expression of MUC1 and TF antigen is correlated to the process of malignant transformation and a tubular/papillary histological pattern of carcinomas (Baldus et al., 1998b).

## Material and methods

### *Monoclonal antibodies and lectins*

Monoclonal antibody A78-G/A7 was generated as described and characterized as TF specific (Karsten et al., 1995). The fine specificity of this antibody was analyzed in binding studies on synthetic MUC1 glycopeptides comprising one repeat unit. According to these analyses, A78-G/A7 binding is strictly dependent on clustered TF disaccharides (glycosylation at vicinal Ser/Thr positions). No reactivity was found on glycopeptides with single (isolated) TF disaccharide substitution (Karsten et al., unpublished results). Mab BW835 (Behringwerke, Marburg, Germany) was generated as described and characterized to recognize specifically the TF disaccharide within MUC1 repeat peptide (Hanisch et al., 1995). In addition to this, it has been shown that a cross-reactivity exists to sialylated TF (NeuAcα2-6(Galß1-3)GalNAcα-Ser) in linkage to serine (Hanisch et al., unpublished results). A78-G/A7 was used as an undiluted culture supernatant, whereas BW835 was diluted to 10 μg/ml in Tris-buffered saline (TBS), pH 7.2, containing 2.5 % bovine serum albumin (BSA; Sigma, Munich, Germany). Biotinylated peanut agglutinin (PNA) from *Arachis hypogaea* and jacalin were purchased from Sigma (Munich, Germany). Both were applied at a concentration of 50 μg/ml in TBS/2.5% BSA.

### *Tissues*

Routinely fixed (5 % phosphate-buffered formalin) and paraffin-embedded normal and neoplastic human tissues were derived from the files of the Institute of Pathology of the University of Cologne.

### *Immunohistochemistry*

After deparaffinization of 5 μm-thick specimens according to standard histological techniques, an avidin-biotin-complex(ABC)-peroxidase assay was performed in order to visualize mab epitopes or lectin binding sites according to the following protocol: 1) Blocking of endogeneous peroxidase by 1% $H_2O_2$/methanol, 30 min, 20 °C. 2) Normal swine serum X901 (DAKO, Copenhagen, Denmark), 1:20, 30 min, 20 °C. 3) Primary (non-biotinylated) mabs or (biotinylated) lectins (diluted as indicated above), 30 min, 20 °C. 4) (only after application of non-biotinylated primary mabs) Biotinylated rabbit-anti-mouse-immunoglobulin E413 (DAKO), 1:300, 30 min, 20 °C. 5.) Streptavidin-peroxidase conjugate P397 (DAKO), 1:400, 30 min, 20 °C. 6) 200 μg/ml 3-amino-9-ethyl-carbazol (Sigma, Munich, Germany) in 50 mM sodium acetate/5% dimethylformamide/0.01% $H_2O_2$, 30 min, 20 °C. 7) Counterstaining with haematoxylin (5 min) and mounting in glycerol jelly.

In steps 2, 4 and 5, TBS/2.5% BSA was used as dilution buffer. After every step - with the exception of steps 2 and 7 - specimens were threefold-washed with TBS. As negative control, mabs and lectins were replaced by normal mouse serum or washing buffer.

Specimens were regarded as positively stained, if more than 5% of the normal tissue or tumor area contained reaction products at a magnification of x400. Reactivity within neoplasms was scored as indicated: 0, 0-5%; +, 5-35%; ++, 35-100%.

## Results

### *Normal human tissues*

All lectins and antibodies under study exhibited very similar binding patterns in normal human tissues. A constant staining was observed in the lung (respiratory and alveolar epithelium as well as mucous glands), acini of the mammary gland, distal tubules and collecting ducts of the kidneys, endometrium and glandular prostatic epithelium.

An inconsistent reactivity (only in a part of the specimens) of all the reagents was observed in pancreatic acini and ducts, mammary ducts, thyreoid gland epithelium and colloid, transitional epithelium of the bladder and cervical glands. On the other hand, negative staining was encountered in parotis glands, glomeruli, Bowman's capsules and proximal tubules of the kidneys, ovaries, squamous epithelium of the cervix and cerebral tissue.

Staining was mainly located at the luminal surfaces or in secretory products. However, in some organs cytoplasmic structures probably of the Golgi region (perinuclear areas) were also reactive (respiratory epithelium, mucous bronchial gland vacuoles, pancreatic acini, transitional epithelium of the bladder, distal tubules and collecting ducts in the kidneys, endometrium).

### *Neoplastic human tissues*

The binding patterns of human neoplasms showed striking differences between the lectins and mabs (Table

1). If mab A78-G/A7 was compared to mab BW835, the latter bound to a greater number of neoplasms especially in gastric, mammary and pancreatic adenocarcinomas, thyreoideal carcinomas, clear cell carcinomas of the kidney, transitional cell carcinomas of kidney and bladder as well as acinus cell carcinomas of the parotid. Additionally, mab BW835 showed an expression of MUC1-bound TF in a greater percentage of tumor area in gastric, pancreatic, mammary, renal, bladder and ovarian carcinoma.

In well- and moderately-differentiated adenocarcinomas, mostly luminal membranes and intratubular secretions were stained. Besides this, golgi-associated and other cytoplasmic reactivities also occurred in most of the tumors under study (Fig. 1). It was generally predominant in squamous cell and low differentiated tumors, as, for example in giant cell carcinomas of the lung, or poorly differentiated carcinomas of pancreas and mammary gland.

Generally, PNA and jacalin reacted with a larger number of tumor specimens than the monoclonal antibodies. Additionally, they exhibited a more homogeneous positivity within the individual tumors. On the other hand, between the antibodies (A78-G/A7,

**Table 1.** Reactivity of human neoplasms with PNA, jacalin, mabs A78-G/A7 and BW835

| | PNA | | | | Jacalin | | | A78-G/A7 | | | BW835 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | 0 | + | ++ | 0 | + | ++ | 0 | + | ++ | 0 | + | ++ |
| *Gastric carcinoma* | | | | | | | | | | | | | |
| Intestinal-type | 8 | 0 | 1 | 7 | 0 | 2 | 6 | 0 | 6 | 2 | 0 | 2 | 6 |
| Diffuse-type | 3 | 0 | 0 | 3 | 0 | 1 | 2 | 0 | 1 | 2 | 0 | 1 | 2 |
| *Pancreatic carcinoma* | | | | | | | | | | | | | |
| Adenocarcinoma | 10 | 3 | 6 | 1 | 3 | 3 | 4 | 7 | 2 | 1 | 3 | 4 | 3 |
| *Parotid carcinoma* | | | | | | | | | | | | | |
| Adenocarcinoma | 3 | 2 | 1 | 0 | 1 | 1 | 1 | 1 | 2 | 0 | 1 | 1 | 1 |
| Acinus cell | 5 | 5 | 0 | 0 | 4 | 1 | 0 | 5 | 0 | 0 | 3 | 1 | 1 |
| Clear cell | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| *Lung carcinoma* | | | | | | | | | | | | | |
| Giant cell | 8 | 4 | 2 | 2 | 4 | 3 | 1 | 5 | 3 | 0 | 3 | 4 | 1 |
| Squamous cell | 6 | 3 | 1 | 2 | 2 | 1 | 3 | 4 | 2 | 0 | 2 | 2 | 2 |
| Adenocarcinoma | 6 | 0 | 1 | 5 | 0 | 4 | 2 | 0 | 5 | 1 | 0 | 3 | 3 |
| Bronchoalveolar | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 |
| Mucoepidermoid | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 |
| *Mammary carcinoma* | | | | | | | | | | | | | |
| Intraductal/ductal | 10 | 1 | 3 | 6 | 0 | 3 | 7 | 2 | 6 | 2 | 0 | 0 | 10 |
| Poorly differentiated | 5 | 3 | 2 | 0 | 0 | 1 | 4 | 3 | 2 | 0 | 0 | 3 | 2 |
| Mucinous | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 |
| *Thyroideal carcinoma* | | | | | | | | | | | | | |
| Follicular | 4 | 3 | 1 | 0 | 3 | 1 | 0 | 4 | 0 | 0 | 3 | 1 | 0 |
| Papillary | 6 | 4 | 2 | 0 | 5 | 1 | 0 | 6 | 0 | 0 | 2 | 3 | 1 |
| Medullary | 2 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 |
| Anaplastic | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| *Renal carcinoma* | | | | | | | | | | | | | |
| Clear cell | 9 | 2 | 4 | 3 | 3 | 2 | 4 | 5 | 4 | 0 | 2 | 4 | 3 |
| Transitional cell | 5 | 2 | 3 | 0 | 1 | 3 | 1 | 3 | 2 | 0 | 1 | 2 | 2 |
| *Bladder carcinoma* | | | | | | | | | | | | | |
| Transitional cell | 9 | 3 | 3 | 3 | 2 | 4 | 3 | 4 | 4 | 1 | 0 | 3 | 6 |
| *Ovarian neoplasias* | | | | | | | | | | | | | |
| Serous papillary carcinoma | 6 | 0 | 2 | 4 | 0 | 2 | 4 | 0 | 6 | 0 | 0 | 1 | 5 |
| Mucinous adenocarcinoma | 2 | 0 | 1 | 1 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 2 | 0 |
| Endometrioid carcinoma | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 2 | 0 |
| Teratoma | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 1 | 1 | 0 | 0 | 2 |
| *Cervical carcinoma* | | | | | | | | | | | | | |
| Squamous cell carcinoma | 2 | 0 | 2 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 |
| *Uterine carcinoma* | | | | | | | | | | | | | |
| Endometrial carcinoma | 5 | 0 | 0 | 5 | 0 | 3 | 2 | 1 | 3 | 1 | 0 | 3 | 2 |
| *Prostatic carcinoma* | | | | | | | | | | | | | |
| Adenocarcinoma | 9 | 0 | 5 | 4 | 0 | 4 | 5 | 0 | 6 | 3 | 1 | 8 | 0 |
| *Testis* | | | | | | | | | | | | | |
| Seminoma | 8 | 6 | 2 | 0 | 7 | 1 | 0 | 8 | 0 | 0 | 8 | 0 | 0 |
| *Brain* | | | | | | | | | | | | | |
| Glioblastoma | 3 | 2 | 1 | 0 | 2 | 1 | 0 | 3 | 0 | 0 | 3 | 0 | 0 |
| Gliosarcoma | 4 | 4 | 0 | 0 | 4 | 0 | 0 | 4 | 0 | 0 | 4 | 0 | 0 |

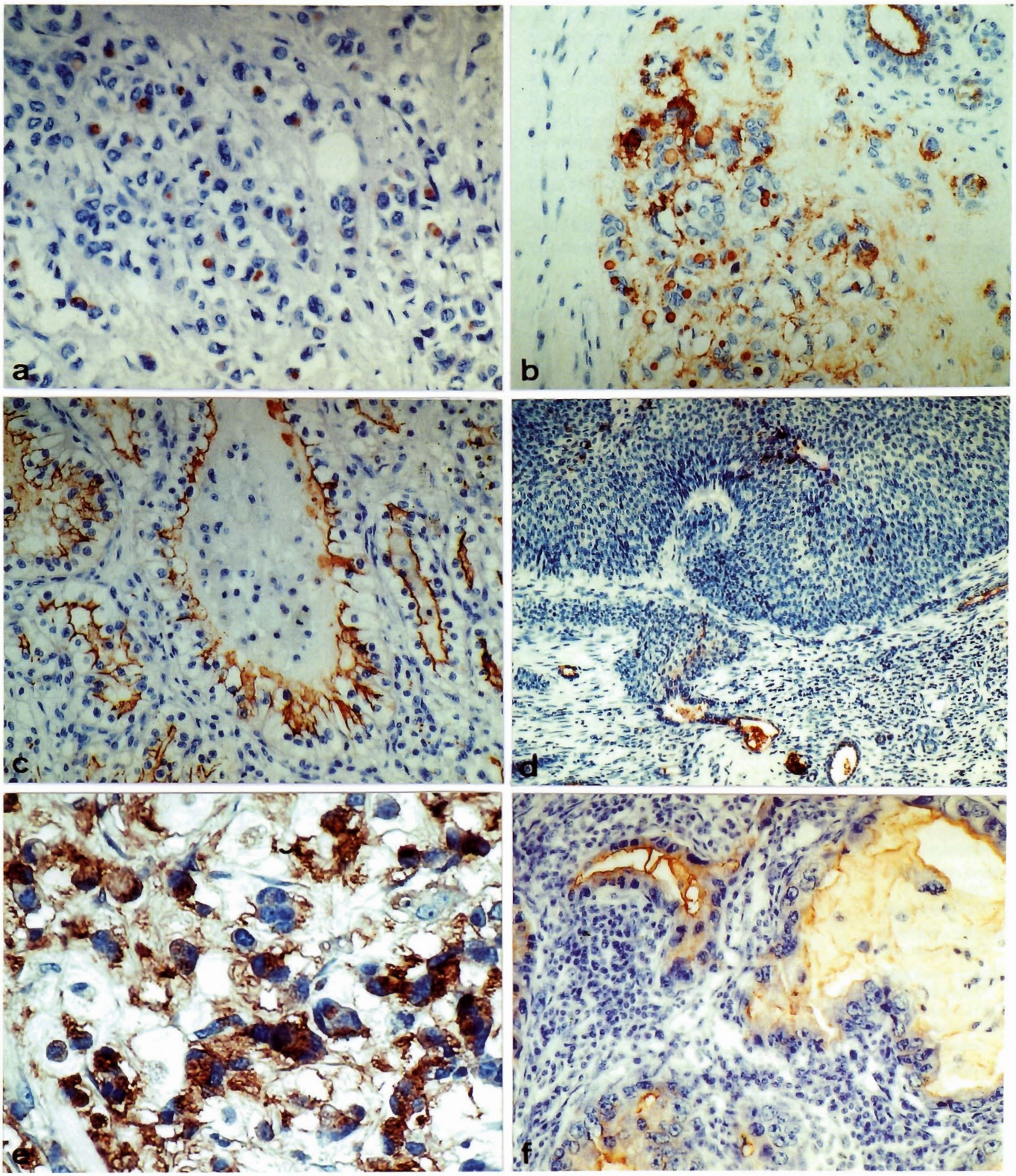


**Fig. 1.** The figures show representative patterns of reactivity: Mammary carcinomas containing vacuoles positively stained by PNA **(a)** as well as mab A78-G/A7 **(b)**. Clear cell carcinoma of the kidney **(c)** showing a reaction with (PNA) and transitional cell carcinomas of the bladder **(d)** exhibiting some scattered BW835 reactive cells. A signet-ring cell carcinoma of the stomach **(e)** expressing BW835 epitope and an adenopapillary carcinoma of the lung **(f)** which reveals jacalin binding sites. a-c, e, x 580; d, f, x 350

BW835), differences could be observed regarding the presence/absence as well as the intensity of staining.

## Discussion

The comparative immunohistochemical evaluation of TF-recognizing lectins and mabs revealed strikingly different binding patterns. Generally, both PNA and jacalin reacted with the majority of normal as well as neoplastic tissue samples. This is in agreement with numerous previous studies which were based on the peanut lectin and suffered from the poor specificity of the reagent (Hanisch and Baldus, 1997). The lectin has been shown to cross-react to α-lactose, to Galß1-4GlcNAcα and Gal ß1-3GlcNAcß (Neurohr et al., 1982; Wu et al., 1994). In a similar way, jacalin, a lectin from *Artocarpus integrifolia*, recognizes TFα (Sastry et al., 1986; Mahanta et al., 1990) but also Tn antigen (Wu et al., 1994).

According to our results the binding spectrum of monoclonal antibodies seems to be more restricted. Up to now, only scattered data and anecdotal descriptions concerning a comparison between PNA and TFα/ß-specific mabs have been reported. In normal tissues, the epitopes of mabs HH8 and A78-G/A7 were co-expressed with PNA binding site in some tissues. Others (epidermal layers and hair follicles, sweat glands, gastrointestinal mucosae, mucous bronchial glands, glandular epithelium of the prostate) did not express TFα/ß which, on the other hand, were PNA reactive (Cao et al., 1996). With regard to neoplasms, only the colorectum (Yuan et al., 1986; Cao et al., 1995) and bladder (Langkilde et al., 1992a,b) have been investigated extensively. Anti-TF mab HH8, but not PNA was a predictor of recurrence in bladder carcinoma, without correlation of their binding patterns (Langkilde et al., 1992a). In colorectal neoplasia, PNA displayed the highest sensitivity in staining of hyperplastic polyps, adenomas and carcinomas, but the reactivity of mab AH8-258 correlated with polyp size, histopathological type and degree of dysplasia in adenomas (Yuan et al., 1986). In the latter study, PNA additionally stained about 50% of the normal colorectal mucosa, whereas mab binding was virtually absent. In a later study, a correlation of TF expression as detected by mab A78-G/A7 (Karsten et al., 1995) with the capacity to metastasize was observed in colorectal cancer (Cao et al., 1995). Additionally, a correlation of A78-G/A7 but also of BW835 reactivity with increasing dysplasia in adenomas was found (Baldus, 1998b). Furthermore, a strong correlation of both antigens, MUC1 and TF, could be shown in adenomas and moderately differentiated carcinomas, whereas mucinous carcinomas co-expressed MUC2 and TF antigen (Baldus et al., 1998b). The latter results have to be discussed in the context of the diversity of possible TF carrier proteins and again indicate the tumor-diagnostic relevance of MUC1. In normal epithelia, only breast tissue revealed TF antigen in high activity. As carrier protein, a mucin could be identified in lactating mammary tissue (Shimizu and Yamauchi, 1982; Fischer et al., 1984). The primary structure of its tandem repeats was analyzed (Gendler et al., 1988), and putative glycosylation sites of the peptide core, now named MUC1, could be localized (Müller et al., 1997). Mab BW835 is of particular interest, since it detects TF only when it is bound α-anomerically to a MUC1-specific peptide motif. In the present study, it is characterized by immunohistochemistry investigating a great number of human neoplasms for the first time.

Comparing the histochemical staining patterns obtained with mabs BW835 and A78-G/A7 there is a need to explain the obvious discrepancies revealed for the two TF-specific reagents. Possibly, the broader staining of BW835 could in part be assigned to a cross-reactivity with sialylated derivatives of the TF antigen as revealed by inhibition with NeuAcα2-6(Galß1-3)GalNAcα-Ser. On the other hand, the apparently more restricted binding pattern of A78-G/A7 should result from the fact that the antibody exhibits a strict dependency on clustered TF antigen. According to binding analyses on synthetic TF glycopeptides corresponding to the MUC1 repeat, the antibody failed to react to single or "isolated" TF disaccharide, but was strongly reactive on two TF disaccharides in vicinal positions. The latter observation indicates that A78-G/A7 should not be simply regarded as a TF reagent which substitutes for the peanut agglutinin, but instead is characterized by a distinct fine specificity. The failing reactivity of A78-G/A7, accordingly, cannot be interpreted by assuming that TF antigen is not expressed in the respective tissue specimen.

In conclusion, mab BW835 reveals important features regarding possible carriers of TF antigen in human neoplasms. As suggested by our results, immunoreactivity of MUC1 mucin core-bound Galß1-3GalNAc is strongly enhanced during carcinogenesis. This may be explained by a reduced chain-elongation of oligosaccharides which are obviously masking the TF and MUC1 immunoreactivity in most normal mucins. For example, MUC1 mucin seems to be involved in cell adhesion (Regimbald et al., 1996; Yamamoto et al., 1997) as well as T cell recognition and cytotoxicity (Magarian-Blander et al., 1996; Agrawal et al., 1998). In the latter context, MUC1-bound carbohydrates, like TF antigen, were reported to exert an enhancing effect (Böhm et al., 1997).

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