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ORIGINAL ARTICLE

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Native and sialic acid masked Lewis^a antigen reactivity in medullary thyroid carcinoma.

Distinct tumour-associated and prognostic relevant antigens

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Abstract Forty-six medullary thyroid carcinomas (MTC) were subjected to a qualitative and quantitative characterization of native and sialic acid masked Lewis^a (Le^a) antigens. Immunohistochemical investigations included monoclonal antibodies (MABs) directed against alpha(2,3)-sialyl-Le^a, i.e. CA19-9 (MAB 19-9), native Le^a (MAB anti Le^a) and alpha(2,3) sialyl type 1 structure, i.e. CA 50 (MAB C50). To detect sialic acid masked Le^a reactivity, MAB anti-Le^a was also applied to native and enzymatically desialylated tissue sections with and without masking of sialic acid residues by sialic acid and sequence specific lectins. Only 7 MTC (15%) displayed a weak expression of CA19-9, while 16 (33%) showed moderate positive staining for native Le^a. Twenty-seven tumours exhibited a strong staining by the N'ase MAB anti Le^a staining sequence. The latter could most effectively be inhibited by the simultaneous masking of alpha(2,3)-and alpha-(2,6)-linked sialic acid residues due to the comptetitive binding of sialic acid and sequence specific lectins: *Maackia amurensis* agglutinin (specific alpha(2,3)-linked sialic acid) and *Sambucus nigra* agglutinin (specific alpha(2,6)-linked sialic acid). Thus, in MTC the major portion of sialic acid masked Le^a antigen reactivity is different from that detected by the MAB 19-9. The antigen reactivity is probably due to Le^a structures containing both alpha(2,3) and alpha(2,6)-linked sialic acid residues. A highly significant correlation between the expression of CA50 and that detected by the N'ase MAB anti-Le^a staining sequence indicates that the alpha(2,3)-sialyl type 1 chain represents a common intermediate structure within the pathway of the biosynthesis of sialylated Le^a antigens, excluding the formation of CA19-9 via the formation of the disialyl type 1 structure. This is subsequently fucosylated to the corresponding sialic acid masked Le^a. Preliminary clinicopathological studies indicate that the sialic acid masked Le^a antigens detected by the N'ase MAB anti-Le^a staining sequence are related to biologically aggressive MTC.

Key words Lewis^a blood group antigens
Carbohydrate histochemistry
Medullary thyroid carcinoma
Sialic acid specific lectins
Tumour associated antigens

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Introduction

Analysis of the occurrence of type 1 chain derived antigens has shown that these carbohydrate sequences are expressed in thyroid cancer but not in normal thyroid tissue (Vierbuchen et al. 1989, 1992). Using a panel of monoclonal antibodies (MABs) that can distinguish the structural differences between type 1 chain derived antigens, we have reported that medullary thyroid carcinomas (MTC) most frequently expressed the Le^b, the Le^d, and the CA50 (alpha(2,3)-sialyl type 1 chain, Vierbuchen et al. 1992). Although most MTC displayed the biosynthesis of large amounts of CA50 which represents the immediate precursor of the alpha(2,3)-sialyl-Le^a antigen (CA19-9) only a few tumours demonstrated a weak staining for this fucosylated derivative of CA50 (Vierbuchen et al. 1992) a change which could not be attributed to a deficiency of the precursor nor to a lack of alpha(1,4)-fucosyltransferase activity (Vierbuchen et al. 1992). Using different MABs on native and desialylated tissue sections (Orntoft et al. 1987), the major task in studying the occurrence and nature of Le^a and espe-

cially that of sialic acid masked Le^a has been to determine two alternate pathways: whether MTC possess only a very limited capacity for the biosynthesis of sialylated-Le^a antigens, or alternatively, whether MTC possess the ability to biosynthesize sialic acid masked Le^a antigens which are different from those detected by the MAB 19-9.

Materials and methods

Tissue specimens of normal thyroid glands ($n=25$) obtained from operation samples due to head or neck malignancies and tissue specimens of 46 MTC formed the basis of the study.

The sources and the working solutions of the mouse MABs (anti Le^a, anti Le^b, anti A, anti B, anti H type 1, anti H type 2, N-19-9, and C 50) have been described previously (Vierbuchen et al. 1989, 1992). The lectins *Maackia amurensis* agglutinin (MAA), *Sambucus nigra* agglutinin (SNA); (Boehringer Mannheim, Germany); peanut agglutinin (PNA), *Erythrina cristagalli* agglutinin (ECA), *Dolichos biflorus* agglutinin (DBA), *Ulex europaeus* agglutinin I (UEA I), and *Limax flavus* agglutinin (LFA); (Medac, Hamburg, Germany) were diluted in PBS containing up to 200 µg lectin/ml.

Table 1 summarizes the antibodies, sialic acid specific lectins, the carbohydrate determinants recognized, and the staining sequences used to characterize the different types of Le^a antigen reactivities investigated in this study.

The immunohistochemical demonstration of the different antigens was performed by a conventional 4 step peroxidase anti peroxidase technique as described previously (Vierbuchen et al. 1989, 1992). Desialylation was accomplished by the incubation of deparaffinized and rehydrated tissue sections with neuraminidase (*Vibrio cholerae*, Behringwerke Marburg, Germany 0.01 U enzyme/ml 0.05 M acetate buffer, pH 5.0 containing 9 mM calcium chloride) for 60 min at 37° C.

Quantitation of the antigen reactivity by computerized image analysis was performed using the Vids V (AI Tektron) in combination with the AMS Insight (Analytical measuring systems, London Road, Cambridge, United Kingdom). The system comprises a central processor with display and computer. The analysis is based on the television scanner converting the histological image into 500 000 pixels. The system can distinguish between 256 grey levels or grades of density between white and black, and the grey level of each picture can be recorded. Features at or above a preset threshold can be quantified by the central processor, under visual control by the video monitor. The grey level thresholds were used to determine the area of tumour cells stained by the antibodies, the total area occupied by tumour cells, and the stroma respectively. The extent of each component was determined in every single microscopic field (total magnification 80 ×). Electronic editing was used to eliminate section and staining artefacts. The amount of antigen staining was expressed by the labelled tumour cell percentage area (area of stained tumour cells/total tumour cell area × 100%). The occurrence of sialic acid masked Le^a antigen reactivity detected by the neuraminidase MAB anti Le^a staining sequence (N'ase MAB anti Le^a staining sequence) was determined by the difference: labelled tumour cell percentage area quantified on the desialylated tissue section – labelled tumour cell percentage area quantified on the adjacent native tissue section (Table 1, staining protocol 6). To analyse the nature of the Le^a reactivity detected by the N'ase MAB anti Le^a staining protocol enzymatic desialylation of tissue sections was performed in the presence sialic acid specific lectins (Table 1, staining protocol 7, 8, and 9) and lectins which lack sialic acid specificity (200 µ g lectin/ml solution). After several washings in PBS, tissue sections were stained for Le^a antigen reactivity. In addition, we tested the inhibitory effect of sialic acid and sequence specific lectins on the kinetics of the neuraminidase induced liberation of Le^a reactivity using tissue samples which contained only sialic acid masked Le^a antigens. The time course of neuraminidase induced Le^a reactivity was followed for incubations containing enzyme and lectins with different sugar specificities (the lectins used are given in the Figure). The reaction was terminated by 6% formalin. After several washings in PBS, tissue sections were stained for Le^a (see above) and the amount of Le^a reactivity liberated was determined by image analysis. Blanks were obtained using boiled enzyme (10 min 100° C) or PBS under conditions described above. Cytochemical controls of the immunohistochemical reaction sequences included: replacement of the specific MAB by MABs with irrelevant antigenic specificity and absorption of the MAB with blood group active glycoproteins (Vierbuchen et al. 1992).

Statistical analysis included the Spearman rank correlation- and Student's t test; univariate survival analysis was performed by the Kaplan-Meyer method using the Mantel-Cox and generalized Wilcoxon test statistics; multivariate survival analysis including all morphological, immunohistochemical, and clinical data was performed according to the proportional hazard model of Cox.

Table 1 Specificities of monoclonal antibodies (MABs), sialic acid specific lectins, and staining protocols used for the detection and characterization of native and sialic acid masked Le^a antigens in medullary thyroid carcinoma (MTC)

MABs/Lectins/ Staining protocol	Structure identified
1 MAB N-19-9	CA19-9; alpha(2,3)-sialyl-Le^a
2 MAB C 50	CA 50; alpha(2,3)-sialyl typeI
3 MAB anti Le^a	Le^a-antigen
4 MAA	Neu5Ac-alpha(2,3)Gal/GalNAc
5 SNA	Neu5Ac-alpha(2,6)Gal/GalNAc
5 Staining of desialylated tissue sections by MAB anti Le^a	Total amount of native and sialic acid masked Le^a antigens
6 Difference between (5) and (3)	sialic masked Le^a antigen reactivity determined by the N'ase MAB anti Le^a staining sequence
7 inhibition of (6) by SNA	indicates the presence of alpha(2,6)-sialyl-Le^a detected by protocol (6)
8 inhibition of (6) by MAA	indicates the presence of alpha(2,3)-sialyl-Le^a detected by protocol (6)
9 enhanced inhibition of (6) by SNA and MAA	indicates the presence of alpha(2,3), alpha(2,6)-disialyl-Le^a detected by protocol 6

Results

Normal thyroid tissue demonstrated neither the formation of Le^a nor that of the corresponding sialylated derivatives, as detected by the MAB 19-9 and the N'ase MAB anti Le^a staining sequence (Fig. 1). In contrast, MTC exhibited the formation of native and sialic acid masked Le^a antigens (Table 2). However, the tumours displayed pronounced differences of sialic acid masked Le^a antigen reactivity as detected by the different staining methods (Table 2). Only some MTC showed a weak staining for alpha(2,3)-sialyl-Le^a detected by the MAB 19-9, contrasting with the strong staining of most tumours by the N'ase MAB anti Le^a staining sequence (Figs. 1, 2, Table 2). We were surprised that in most MTC sialic acid masked Le^a could be detected after en-

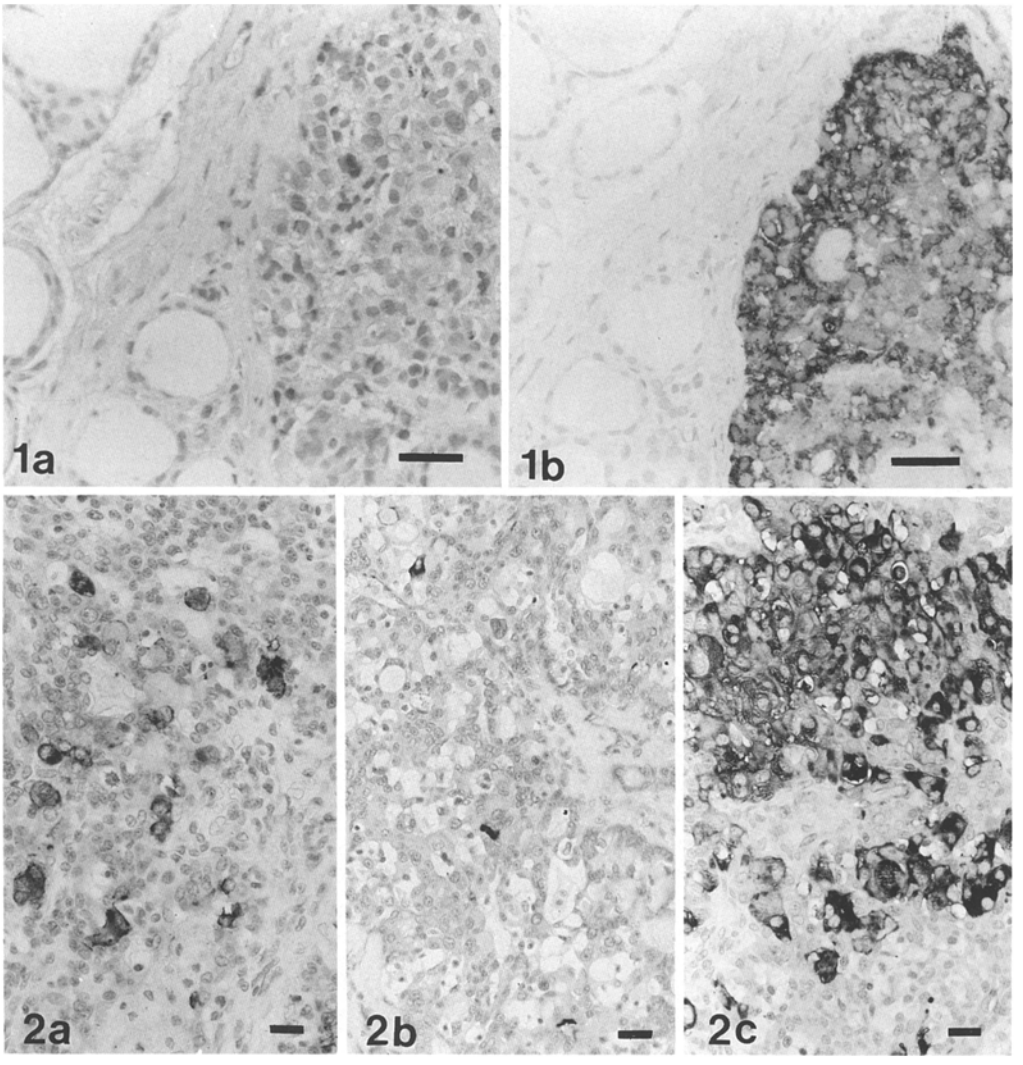

Fig. 1a, b, 2 Effect of enzymatic desialylation on the Le^a antigen reactivity in non-tumorous thyroid tissue and MTC. **a** Tissue section stained for the Le^a antigen. Both, the non-tumorous thyroid tissue and the carcinoma lack Le^a antigen reactivity. **b** Adjacent tissue section. Enzymatic desialylation results in a strong and uniform staining of the carcinomatous tissue (*right side*), contrasting with the lack of Le^a antigen reactivity of the non-tumorous thyroid tissue (*left side*). Original magnification a, b $\times 220$. *Bar* 50 μm

Fig. 2a–c Comparison of the staining patterns for native and sialic acid masked Le^a antigens detected by the various staining procedures on replicate sections from a MTC. **a** A moderate number of tumour cells display an immunoreactivity for the Le^a antigen. **b** Adjacent tissue section stained for CA 19-9. Only few tumour cells show alpha(2,3)-sialyl Le^a antigen reactivity. **c** Following the removal of sialic acid, most tumour cells are intensely stained by MAB anti Le^a. Original magnification $\times 220$. *Bar* = 50 μm

Table 2 Occurrence of native and sialic acid masked Le^a antigen reactivity in 46 MTC as detected by the MAB anti Le^a, MAB 19-9, and the N'ase MAB anti Le^a staining sequence

Staining protocol	Staining			
	Negative	Positive	Range	Mean ± sdm**
	n(%)	(n%)	(%pos.cells)*	
MAB anti Le^a	30(67)	16(33)	0.45–22.0	4.8 ± 6.4
MAB N-19-9	39(85)	7(15)	0.16–3.12	0.8 ± 1.1
N'ase MAB anti Le^a	19(41)	27(59)	1.28–70.0	11.8 ± 16

* Labelled tumour cell percentage area, ** standard deviation

zymatic desialylation, whereas only a few tumours displayed the formation of alpha(2,3)sialyl-Le^a as detected by the MAB 19-9. Therefore, we tested the effect of enzymatic desialylation on the binding of antibodies directed against other type 1 chain derived carbohydrate sequences (Table 3). Enzymatic desialylation had only affected the staining of type 1 chain derived structures which were not substituted on the terminal galactose or which contained a terminal sialic acid residue. However, staining of carbohydrate sequences containing fucosylated terminal galactose was not affected by enzymatic desialylation (Table 3). Staining by the MAB 19-9 indi-

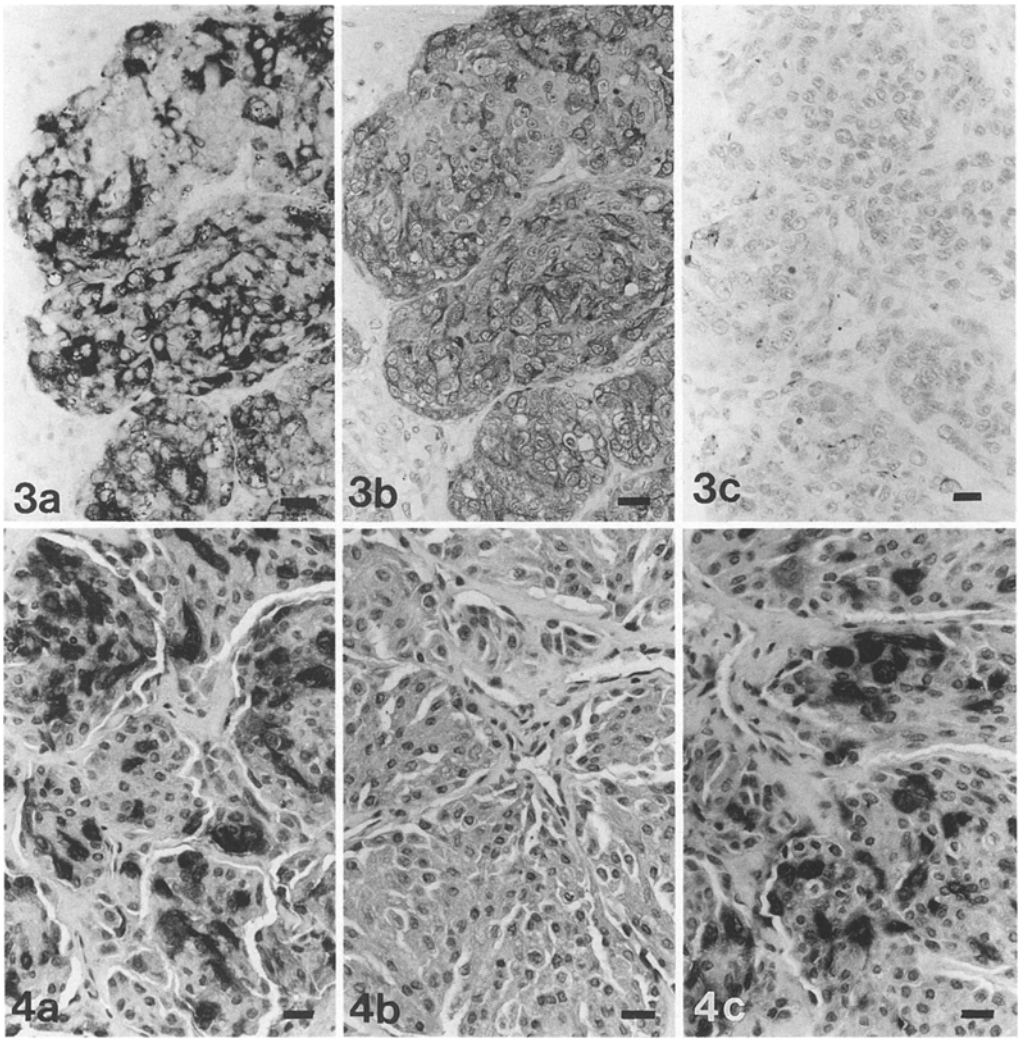

Fig. 3a–c Inhibitory capacity of MAA and SNA on the neuraminidase induced liberation of Le^a reactivity in MTC. **a** Tumour tissue showing strong Le^a antigen reactivity after enzymatic desialylation. **b** Pre- and co-incubation of the tissue section with MAA reduced the Le^a antigen reactivity detected by the N'ase MAB anti Le^a staining sequence. **c** Simultaneous pre-incubation and co-incubation of tissue sections with MAA and SNA nearly completely inhibited the staining obtained by the N'ase MAB anti Le^a staining sequence. Original magnification a–c × 200. *Bar* = 25 μm

Fig. 4a–c Different inhibitory effects of lectins specific for particular sialic acid sequences on the N'ase induced liberation of Le^a antigen reactivity. **a** After enzymatic removal of sialic acid residues most tumour cells display intense Le^a antigen reactivity. **b** Pre-incubation of tissue section with SNA almost totally blocks the subsequent N'ase MAB anti Le^a staining sequence. **c** Preincubation of tissue sections with MAA did not affect the neuraminidase induced liberation of Le^a antigen reactivity. Original magnification a–c ×200. *Bar* = 25 μm

Table 3 Effect of enzymatic desialylation on the binding of MABs directed against blood group antigens derived from type 1 chain precursors (↑ enzymatic desialylation increased the binding [staining intensity] of the MABs; ↓ enzymatic desialylation decreased the binding (staining intensity) of the MABs; → enzymatic desialylation did not affect the binding [staining intensity] of the MABs)

Monoclonal antibody used in the N'ase MAB staining sequence	Effect of enzymatic desialylation on the staining intensity
MAB anti Le^a	↑
MAB N-19-9	↓
MAB C 50	↓
MABs anti Le^d, anti ALe^b anti Le^b	→

cates the presence of alpha(2,3)-sialyl Le^a. In contrast to the well defined nature of this antigen, the N'ase MAB anti Le^a staining sequence indicates the presence of sialic acid masked Le^a antigen reactivity but reveals no informations on the exact nature of the sialic acid sequences stained. Therefore, we used sialic acid and sequence specific lectins to mask distinct sialic acid moieties and tested this effect on the neuraminidase induced liberation of Le^a antigens. Desialylation of tissue sections in the presence of SNA (specific for alpha(2,6)-

Table 4 Correlation between the occurrence of CA 50, native-, and sialic acid masked Le^a antigens in 46 MTC (Spearman rank correlation test)

Antigen/ staining sequence	Le^{a1}	N-19-9^2	N'ase MAB anti Le^{a3}	CA50^4
Le^a	1.000 $p=0.000$	0.2191 $p=0.049$	0.2792 $p=0.043$	0.2888 $p=0.030$
N-19-9		1.000 $p=0.000$	0.2290 $p=0.063$	0.2719 $p=0.034$
N'ase MAB anti Le^a			1.000 $p=0.0000$	0.542 $p=0.000$
CA 50				1.000 $p=0.000$

(coefficient/cases (46)/ 1-tailed significances
[1] Le^a antigen detected by MAB anti Le^a
[2] Alpha(2,3)-sialyl Le^a antigen detected by the MAB 19-9
[3] Sialic acid masked Le^a antigen reactivity detected by the N'ase MAB anti Le^a staining sequence
[4] Alpha(2,3)-sialyl-type 1 structure detected by the MAB C50

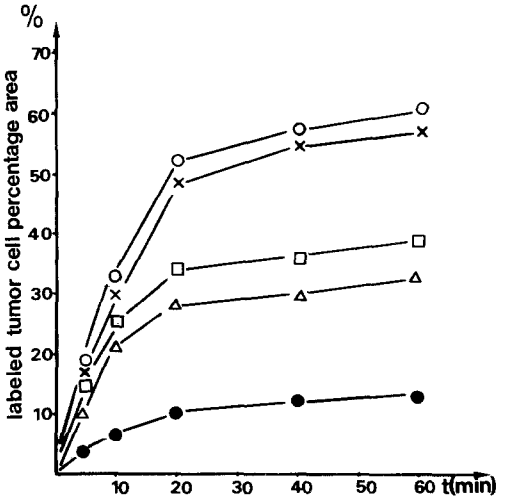

Fig. 5 Time course and inhibition studies on the effect of different lectins on the neuraminidase induced liberation of Le^a in a section from MTC; ○ Le^a reactivity (incubation containing no lectin); □ Le^a reactivity (incubation containing 200 µg SNA), △ Lea reactivity (incubation containing 200 µg MAA), ● Le^a reactivity (incubation containing 200 µg SNA and 200 µg MAA), × Le^a (incubation containing 200 µg ECA)

linked sialic acid residues) or MAA (specific for alpha(2,3)-linked sialic acid residues) both inhibited the N'ase MAB anti Le^a staining sequence (Fig. 3). However, the staining sequence was most effectively inhibited by the simultaneous presence of MAA and SNA (Fig. 3). In few cases the N'ase induced liberation of Le^a was completely inhibited by SNA, whereas MAA had no effect on the N'ase MAB anti Le^a staining sequence (Fig. 4). Furthermore, time course studies performed in the presence and absence of lectins with different sugar specificities clearly demonstrated the inhibition of the N'ase MAB anti Le^a staining sequence by sialic acid specific lectins (Fig. 5). Lectins which lacked sialic acid specificity did not block the enzymatic release of Le^a antigen reactivity (Fig. 5). LFA which possesses a broad sialic acid and linkage specificity (Schulte et al. 1984) showed an inhibitory effect on the N'ase MAB anti Le^a staining sequence similar to that observed by the simultaneous action of MAA and SNA. The analysis of the different tumour associated antigen reactivities obtained from the computerized image analysis revealed that all antigens detected displayed a significant relation to Le^a. The sialic acid masked Le^a antigen reactivity detected by the N'ase MAB anti Le^a sequence displayed

Table 5 Clinicopathological features of MTC in relationship to the expression of CA 50, Le^a-, and sialic acid masked Le^a antigen reactivity

	Clinicopathological features			
	Lymph node metastases		Survival	
	Absent $n=23$	Present $n=13$	Alive $n=13$	Dead $n=10$
Antigen reactivity[a] Le^a	0.78 ± 1.95	1.45 ± 1.85	1.14 ± 2.53	1.32 ± 1.65
	$p=0.160$		$p=0.423$	
N'ase MAB anti Le^a	3.79 ± 7.46	10.57 ± 18.84	2.34 ± 6.89	20.25 ± 6.40
	$p=0.117$		$p=0.038$	
CA19-9	0.031 ± 0.022	0.11 ± 0.31	0.14 ± 0.4	0.14 ± 0.4
	$p=0.112$		$p=0.215$	
CA 50	8.52 ± 13.59	15.93 ± 18.51	9.15 ± 16.36	20.61 ± 19.63
	$p=0.12$		$p=0.10$	

[a] Amount of antigen determined by image analysis (tumour cell percentage area) ± sdm; p = one tailed significance level (Student's t test)

Table 6 Univariate test for the association between the occurrence of CA 50, native-, and sialic acid masked Le^a antigens in MTC ($n=23$) and survival

Antigen	Test	Statistics	p values
Le^a	Mantel Cox	1.508	0.2194
	Generalized Wilcoxon-(Breslow)	0.725	0.3011
N'ase MAB anti Le^a	Mantel Cox	18.780	0.0000
	Generalized Wilcoxon-(Breslow)	15.993	0.0001
CA 19-9	Mantel Cox	0.642	0.4231
	Generalized Wilcoxon-(Breslow)	0.447	0.5036
CA50	Mantel Cox	2.414	0.1202
	Generalized Wilcoxon-(Breslow)	1.363	0.2430

a highly significant correlation to the tumour associated expression of CA 50; i.e. Neu5Ac-alpha(2,3)Gal-beta-(1,3)GlcNAc (Table 4). Preliminary studies on clinicopathological features related to Le^a and sialic acid masked Le^a antigen reactivity revealed no significant association between the tumour associated antigen expression and the lymph node status (Table 5). However, there was a significant association with the sialic acid masked Le^a (detected by the N'ase MAB anti Le^a staining sequence) and the survival (Table 5). Univariate analysis of survival (Table 6) confirmed that this variable presented a significant influence on the age corrected survival time. In addition, all the factors (age, quantified antigen reactivity, pTNM stage) were tested in a Cox forward stepping proportional hazard model. The results revealed the significant impact of sialic acid masked antigens (detected by the N'ase anti Le^a staining sequence) on the prognosis in comparison with the other factors ($p=0.001$).

Discussion

Immunohistochemical studies on type 1 chain derived blood group antigens and related carbohydrate sequences revealed that most MTC displayed a strong staining for Le^b, Le^d, and especially for the MAB C 50 defined epitope (alpha(2,3)-sialyl type 1 structure) contrasting with the lack of antigen expression in normal thyroid tissue. Although the CA 50 represents the immediate precursor of CA 19-9, only a few MTC showed a weak staining for this antigen. Using the MAB 19-9 and an alternative method based on the staining of enzymatically desialylated tissue sections by the MAB anti Le^a (N'ase MAB anti Le^a staining sequence), we examined the question, wether MTC possess sialic acid masked Le^a antigen reactivity which is different from that defined by the MAB 19-9. These studies revealed that enzymatic desialylation liberates strong Le^a reactivity in many tumours and tumour areas which were negative for CA 19-9. From these results it is reasonable to conclude that the major portion of sialylated Le^a antigens formed in MTC are different from CA 19-9. Sialic acid residues have been found to differ in the linkage to other sugars. Sialic acid is linked to either terminal beta-D-Gal by a alpha 2-> 3(6) glycosidic linkage or by a alpha2->6 glycosidic bond to subterminal GalNAc or GlcNAc moieties, respectively (Schauer 1982). During the past years SNA specific for alpha(2,6)-linked- and the MAA specific for alpha (2,3)-linked sialic acid moieties have been reported (Shibuya et al. 1987; Wang and Cummings 1988; Sata et al. 1989). In the present study we used these lectins to mask sialic acid sequences and examined their effect on the neuraminidase induced liberation of Le^a reactivity. Although in situ inhibition by lectin binding is not without problems (Hennigar et al. 1987), the inhibitory effect of the sialic acid and sequence specific lectins on the N'ase MAB anti Le^a staining sequence support the concept that the carbohydrate structure detected by this staining protocol is most probably due to the presence of two distinct sialic acid moieties on the Le^a molecule; alpha(2,3),alpha(2,6) disialyl Le^a. This conclusion is supported by the results with the sialic acid specific lectin LFA which possesses no sequence specificity and displayed and inhibitory capacity similar to that observed for the combined action of MAA and SNA. O-linked oligosaccharides have been reported to contain the disialylated structure Neu5Ac-alpha(2,3)-Gal-beta(1,3)-[Neu5Ac-alpha(2,6)]-GlcNAc sequence (De-Heij et al. 1986; Weinstein et al. 1982). Starting from Lacto-N-tetraose as substrate, in vitro studies on the biosynthesis of disialyl-type 1 chain derivatives indicated that the Neu5Ac-alpha(2,3)Gal-beta(1,3)[Neu5Ac-alpha(2,6)]-GlcNAC tetrasaccharide is synthesized by the sequential actions of two sialyltransferases: by a alpha(2,3)-sialyltransferase resulting in the formation of Neu5Ac-alpha(2,3)Gal-beta(1,3)-GlcNAc (sialyl type 1 chain recognized by the MAB C 50) and by an alpha(2,6)-sialyltransferase leading to the formation of greater than 95% of the tetrasaccharide Neu5Ac-alpha(2,3)Gal-beta(1,3)-[Neu5Ac-alpha(2,6)]GlcNAc (De-Heij et al. 1986). There was a significant relation between the CA 50 and the sialic acid masked Le^a reactivity detected by the N'ase MAB anti Le^a staining sequence. This supports the concept that the alpha-(2,3) sialyl type 1 structure is involved in the tumour associated formation of the sialic acid masked Le^a antigens. CA 50 is probably sialylated to the Neu5Ac-alpha(2,3)Gal-beta(1,3)-[Neu5Ac-alpha(2,6)GlcNAc sequence which is subsequently fucosylated to the corresponding disialylated Le^a antigen (Sell 1990). This pathway can explain the rare expression of alpha(2,3)- or alpha(2,6) monosialylated Le^a in MTC. Recently a ganglioside has been isolated from human adenocarcinomas carrying the disialyl Le^a. The epitope is recognized by the MAB FH7 (Nudelman et al. 1986). In the present study, sialic acid masked Le^a antigens detected in conventional histological sections were resistant to extensive lipid extraction indicating that oligosaccha-

ride sequences of glycoproteins represent the carrier of the antigen rather than the oligosaccharide chains of glycolipids. Normal thyroid tissue did not contain Le^a-, C 50-, C 19-9 and sialic acid masked Le^a antigen reactivity as detected by the N'ase MAB anti Le^a staining sequence. The evolution of MTC must thus be associated with a strict and sequential expression of sialyl- and fucosyltransferases. Quantitative and qualitative changes of cell surface glycoproteins are thought to be associated with altered cell adhesion and the development of invasiveness, metastatic properties and resistance against proteolytic digestion, immunogenicity, and receptor activity (Velican et al. 1970; Jeanloz and Condington 1976; Steck and Nicolson 1983; Altevogt et al. 1984). The results indicate that the sialic acid masked Le^a determined by the N'ase MAB anti Le^a staining sequence is associated with an aggressive tumour type. According to findings on the prognostic significance of the different sialylated Le^a antigens it is reasonable to conclude that the biological effect probably depends on the sialic acid moieties, especially on the alpha(2,6)-linked sialic acid. Additional studies on sialoglycoconjugates with different patterns of terminal sialylation and different subterminal carbohydrate sequences (lacto type 1 versus lacto type 2-chain derived antigens) will reveal further information on the significance of terminal glycosylation and that of the subterminal carrier sequences for the tumour biology.

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