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Correlation of Expression of Binding Sites for Synthetic Blood Group A-, B- and H-Trisaccharides and for Sarcolectin With Survival of Patients with Bronchial Carcinoma

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Carrier-immobilised carbohydrates were used to monitor the presence of specific carbohydrate-binding sites in tissue sections. Sarcolectin, an interferon-α/β antagonist and growth regulator, had been shown to bind the lymphokine macrophage migration inhibitory factor (MIF). The lectin is thus a MIF-specific probe. Biotinylated sarcolectin, neoglycoproteins with lactose or *N*-acetylglucosamine residues, and polyacrylamide-attached trisaccharides, that represent the ABH histo-blood group antigens, were applied to sections of 187 primary lung carcinomas. The panel of cases consisted of 57 epidermoid carcinomas, 55 adenocarcinomas, 43 large cell anaplastic carcinomas and 32 small cell anaplastic carcinomas. 47 cases with intrapulmonary metastatic tumours were also included. Expression of binding sites of both sarcolectin and trisaccharides of histo-blood group antigens A and H correlated with patient survival in lung cancer. In view of the widely performed analysis of the presence of histo-blood group antigens, concomitant profiling of binding sites for these sugar components is suggested to be of potential benefit.

Key words: blood group trisaccharides, macrophage migration inhibitory factor, lung cancer, neoglycoconjugate, interferon antogomat, prognosis, lectin

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INTRODUCTION

MONITORING OF carbohydrate epitopes is often performed to detect correlations with cellular transformation, biological behaviour and prognosis. Special attention has been focussed on the expression of histo-blood group antigens because their presence may detect patients prone to early relapse [1–5]. With respect to bronchial carcinoma, expression of histo-blood group A antigen is considered a good prognostic factor [6]. Interestingly, binding of *Dolichos biflorus* agglutinin, which recognises *N*-acetylgalactosamine terminal structures, like the histo-blood group A determinant, was also correlated with a favourable prognosis and reduced incidence of metastases [7]. Moreover, a monoclonal antibody to Fuc-α1,2-Gal-β1-R, occurring in H/Le^y/Le^b epitopes, revealed that expression of this structure is an indicator of poor prognosis [8]. This antibody reduced tumour cell motility and metastasis formation [9]. Thus, it is tempting to speculate that distinct carbohydrate structures are not only potentially valuable phenomenological characteristics, but also functionally relevant sites with ligand properties [10, 11].

To test this hypothesis, carrier-immobilised carbohydrate structures were employed as probes to assess the presence of respective binding sites [12]. With respect to lung cancer, certain probes have already shown that both small cell and non-small cell carcinomas can be distinguished on the basis of this glycohistochemical property [13–15]. Due to the documented relevance of expression of certain ABH histo-blood group antigens in lung cancer [6, 8], we used the terminal trisaccharide parts of the histo-blood group ABH antigens, shown in Figure 1, and fragments thereof, namely *N*-acetylglucosamine and lactose, as markers to detect specific binding sites. Besides neoglycoconjugates, we tested the potential of labelled sarcolectin, a human interferon α/β antagonist and growth regulator [16], to serve as a prognostic indicator. Sarcolectin's major binding protein in human placenta or lung is the macrophage migration inhibitory factor, as demonstrated biochemically and histochemically [17, 18]. This recently cloned factor activates human macrophages to kill tumour cells [19]. In the present study, we demonstrate that binding of human sarcolectin and of the A- and H-trisaccharides, respectively, is correlated with survival among the 234 tested patients with lung cancer.

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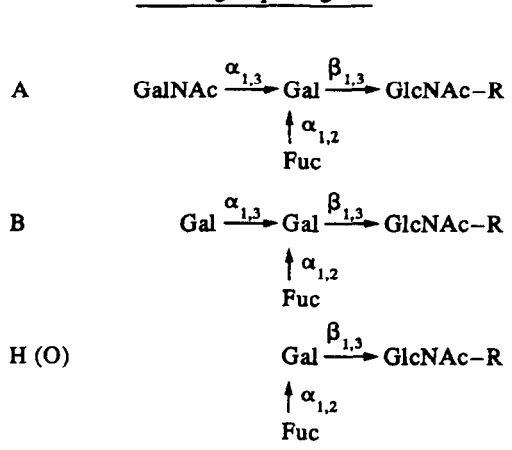

Figure 1. Carbohydrate determinants of blood group antigens (type 1).

MATERIALS AND METHODS

Patients

Our study included 187 patients undergoing surgery for primary lung cancer at the Thoraxklinik in Heidelberg, and 47 patients with pulmonary metastases of various primary sites. These included 21 sarcomas, 19 germ cell tumours and 7 carcinomas. The characteristics of the patients and their median survival are given in Tables 1 and 2. Histological diagnosis, using the commonly accepted WHO classification, was based on sections with a characteristic tumour area devoid of necrotic parts. All patients underwent a thorough clinical staging to exclude a generalised malignancy. Patients without potential curative resection were excluded from this study.

Table 1. Synopsis of material in relation to sex, tumour classification and blood group status

Feature	Blood group A	B	H	Total
Sex				
Male	67	28	70	165
Female	34	10	25	69
Cell type				
Epidermoid	24	10	23	57
Adenocarcinoma	23	11	21	55
Large cell anaplastic carcinoma	18	6	19	43
Small cell anaplastic carcinoma	13	6	13	32
Metastases	23	5	19	47
T stage				
T-1	20	6	18	44
T-2	39	19	43	101
T-3	14	6	13	33
T-4	5	2	2	9
T-X	23	5	19	47
N stage				
N-0	31	10	30	71
N-1	16	8	26	50
N-2	19	8	21	48
N-3	8	3	7	18
N-X	27	9	11	47

Table 2. Survival rates (median survival) in relation to sex, tumour classification and blood group status

Feature	(*n*)	Deaths	Survival (months)
Sex			
Male	(165)	82	13.2
Female	(69)	44	11.7
Cell type			
Epidermoid	(57)	25	16.8
Adenocarcinoma	(55)	26	14.8
Large cell	(43)	23	12.5
Small cell	(32)	27	11.7
Metastases	(47)	25	10.4
T-stage			
T-1	(44)	28	17.8
T-2	(101)	42	13.6
T-3	(33)	23	12.7
T-4	(9)	8	6.2
N-stage			
N-0	(71)	22	15.8
N-1	(50)	30	13.2
N-2	(48)	36	11.6
N-3	(18)	13	9.4
Blood group			
A	(101)	58	13.1
B	(38)	16	13.5
H	(95)	52	13.3

Tissue preparation

Tumour specimens were fixed in buffered formalin, embedded in paraffin, then cut into 4–5 μm sections, soaked in xylene to remove paraffin, and rehydrated in graded alcohol solutions, as previously described [13]. The endogenous peroxidase activity was blocked by incubation with 0.1% methanolic hydrogen peroxide for 30 min. Protein binding sites were saturated by a further incubation step with 1% carbohydrate-free bovine serum albumin in Tris-buffered saline for 30 min.

Synthesis of markers

p-Aminophenyl glycosides of lactose and *N*-acetylglucosamine were used to attach the carbohydrate moieties to the carrier bovine serum albumin, and the neoglycoproteins were biotinylated with biotinyl-*N*-hydroxysuccinimide ester, as described previously [20]. Histo-blood group trisaccharides, prepared as 3-aminopropylglycosides, were attached to poly(4-nitrophenylacrylate), which was labelled with a spacered trifluoroacetate derivative of biotin, as described previously [21, 22]. Residual active sites on the polymer were blocked by treatment with ethanolamine. Sarcolectin was purified from human placenta by pepsin treatment of extract, gel filtration on Sephacryl S-200 and ion exchange chromatography on DEAE cellulose [16, 17]. It was biotinylated with biotinamidocaproyl hydrazide without impairment of its binding activity to macrophage migration inhibitory factor, as described previously [17, 18].

Histological staining

Biotinylated markers were applied to serial sections for 60 min at room temperature at a concentration of 10 μg/ml. After thorough washes to remove unbound probe, ABC kit reagents and the chromogenic substrates diaminobenzidine/hydrogen peroxide were successively added, as described previously

[13, 18]. Following development of the chromogenic product, the sections were washed, counterstained and mounted. Control experiments included competitive inhibitions, omissions of the step using the biotinylated probe to exclude any binding of kit reagents and performance of binding on placenta for sarcolectin, the source of the lymphokine in biochemical purification, as described previously [17]. Cases were judged to be positive if a dark brown staining was present at least in clusters of tumour cells.

Statistical evaluation

Survival was calculated from the day of surgery. Survival curves were computed by the method of Kaplan and Meier [23], and Mantel's log-rank test was applied to compare differences in the periods of survival between the two subgroups [24].

RESULTS

The number of positive cases with each type of marker in relation to features of the patients is given in Table 3. Notably, in addition to *N*-acetylglucosamine and lactose, histo-blood trisaccharides bound to 38–47% of the primary lung cancer cases with no notable preference to any cell type. No correlation of blood group status to any computed characteristic of the patients was apparent (Tables 1, 2). The percentage of cases with detectable binding sites for each of the analysed ligands was similar in primary bronchial carcinomas and intrapulmonary metastases. However, when the survival of patients was related to expression of binding sites for different markers, statistically significant differences between the subgroups were discerned.

Table 3. Binding capacity of applied markers (number of cases)

Feature	(*n*)	Applied marker SL	A	B	H	GlcNAc	Lac
Sex							
Male	(165)	146	64	85	67	58	43
Female	(69)	43	22	24	26	18	12
Cell type							
Epidermoid	(57)	52	22	34	30	20	12
Adenocarcinoma	(55)	42	16	23	15	16	6
Large cell	(43)	35	20	20	26	19	12
Small cell	(32)	24	14	12	10	10	16
Metastases	(47)	36	14	20	12	11	9
T-stage							
T-1	(44)	35	13	16	17	11	9
T-2	(101)	82	46	53	50	41	28
T-3	(33)	31	11	15	10	9	5
T-4	(9)	5	2	5	4	4	4
N-stage							
N-0	(71)	62	27	32	30	23	19
N-1	(50)	36	18	23	21	19	13
N-2	(48)	41	23	26	25	21	13
N-3	(18)	14	4	8	5	2	1
Blood group							
A	(101)	85	35	47	40	36	28
B	(38)	32	15	21	14	12	7
H	(95)	72	36	41	39	28	20

SL, sarcolectin. The blood group trisaccharides, immobilised to the carrier, are referred to as A, B, H, as illustrated in Figure 1. The two glycoproteins are referred to by their carbohydrate part: glcNAc, *N*-acetyl-D-glucosamine; lac, lactose.

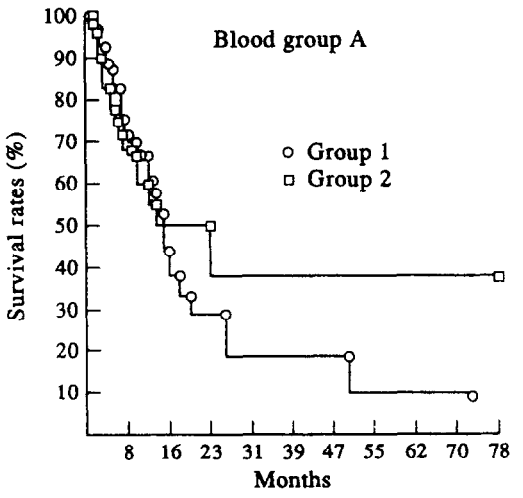

Figure 2. Representation of survival of the blood group A trisaccharide binding cases (□) and the negative cases (○); $P < 0.05$.

Among the neoglycoconjugates, binding of the histo-blood group trisaccharides A and H, but not B, was associated with a relatively more favourable prognosis (Figures 2–4). Neither binding of lactose nor *N*-acetylglucosamine showed marked correlation to survival, when the respective curves were computed (not shown). Binding of sarcolectin was also correlated with survival (Figure 5). Thus, absence or presence of binding sites for sarcolectin as well as for a galactose moiety, carrying a fucose residue in α-1,2 position exclusively or extended by a *N*-acetylgalactosamine residue, but not a further galactose moiety in α1,3-linkage, can distinguish subgroups of patients with significantly different survival curves.

DISCUSSION

Although expression of histo-blood group antigens is extensively monitored in histopathology, with potentially important correlations to survival being detected, the actual functional implication of their presence on tumour cells is still enigmatic. Interestingly, the carbohydrate structure fuc-α1,2-gal-β1-R can influence cell motility [9], and a tetrasaccharide that is related to histo-blood groups A and Le^x has been demonstrated to reduce proliferation of transformed neural cells at micromolar concen-

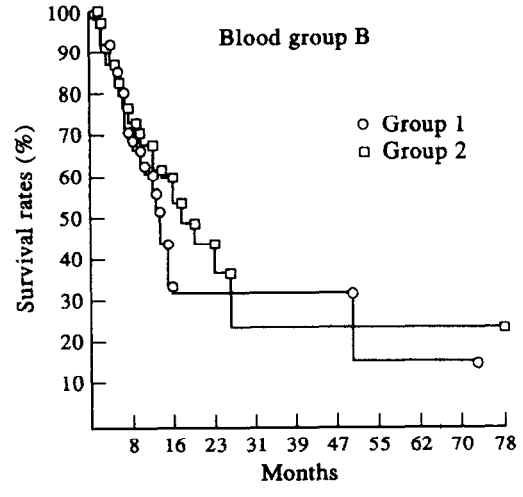

Figure 3. Representation of survival of the blood group B trisaccharide-binding cases (□) and the negative cases (○).

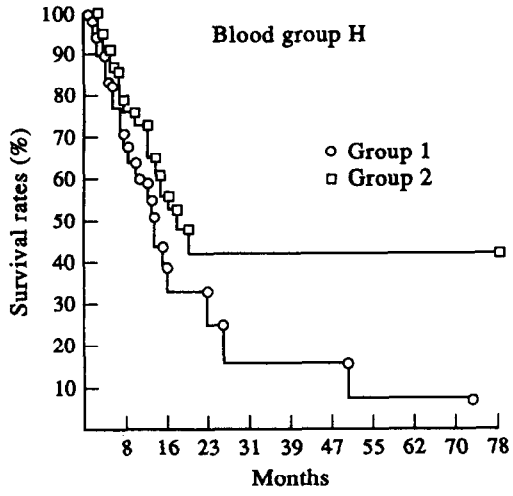

Figure 4. Representation of survival of the blood group H trisaccharide binding cases (□) and the negative cases (○); $P < 0.05$.

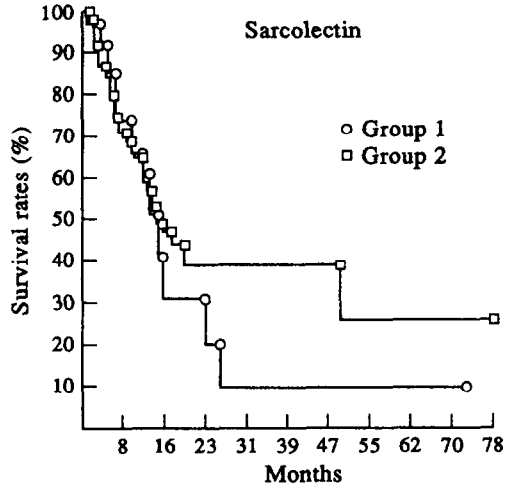

Figure 5. Representation of survival of the sarcolectin-binding cases (□) and the negative cases (○); $P < 0.05$.

trations [25]. Lacto-*N*-fucopentaose I, presenting a H-like terminal structure, inhibits attachment of mouse blastocysts on endometrial monolayers and trophoblast outgrowth at concentrations of 0.01–1 mM [26]. Similar to our approach, a neoglycoprotein was employed to visualise the fuc-α1,2-gal-β1,3-glcNAc-β1,3-gal-β1,4-glc-specific binding sites on trophoectoderm [27], and histo-blood group carbohydrate-specific binding was likewise ascertained for leukaemic cells [22].

Mammalian lectins with binding capacity for histo-blood group antigens have been described that may account for the specific binding reported herein [28–30]. The analysis of their functions may be helpful to turn statistical correlations into physiological explanations. Notably, histochemical application of such tissue lectins has recently been initiated [12, 31]. Whether lectins serve as growth or motility control elements, or as biological response modifiers, as described for a galactoside-specific plant lectin [32, 33], is thus amenable to further experiments. This line of research is also pursued with sarcolectin, whose major binding protein in human placenta is a macrophage migration inhibitory factor [17]. Overall, our results suggest that the A/H trisaccharide structures, attached to a labelled carrier, and human sarcolectin deserve further attention in attempts to accurately define markers of reliable clinical value.

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Colour Doppler Demonstrates Venous Flow Abnormalities in Breast Cancer Patients with Chronic Arm Swelling

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Chronic arm oedema following breast cancer treatment is traditionally attributed to lymphatic obstruction, with venous obstruction as an infrequent complicating factor. The axillo-subclavian venous systems of 81 patients with arm swelling following breast cancer treatment were examined with colour Doppler, duplex Doppler and grey scale ultrasound. Over half (57%) had evidence of venous outflow obstruction and a further 14% had signs of venous "congestion". Only 30% of the swollen arms had normal venous outflow. The venous systems of the contralateral non-swollen arms were all normal as were both arms in 28 control patients who had similar treatment but had not developed arm swelling. These findings suggest that venous outflow obstruction is an important contributory factor in the pathophysiology of arm swelling following breast cancer treatment.
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INTRODUCTION

CHRONIC ARM swelling is one of the commonest complications of breast cancer treatment occurring in 25% of 200 consecutive patients in one series [1]. The reported incidence ranges from 7 to 63% [2]. The cause is attributed to lymphatic obstruction, but this may be an oversimplification of the pathophysiology, which is poorly understood.

The contribution of venous obstruction has long been controversial. Veal in 1938 demonstrated abnormal venous anatomy by venography in 46 cases of arm oedema, but not in 28 control patients [3]. Other studies [4, 5] have also supported the view that venous obstruction is a major cause of the swelling. Conversely, MacDonald [6] and Lobb and Harkins [7] demonstrated that axillary vein resection did not increase the incidence or extent of arm swelling.

Clinical observation of compromised venous drainage (dilated skin venules, dilated collaterals around the shoulder or a palpable brachial vein with the arm elevated) prompted a re-evaluation of venous outflow in breast cancer patients with arm swelling.

The recent ultrasound development of colour Doppler imag-

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