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Specificity of human anti-carbohydrate IgG antibodies as probed with polyacrylamide-based glycoconjugates

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The TF, Tn, and SiaTn glycotopes are frequently expressed in cancer-associated mucins. Antibodies to these glycotopes were found in human serum. A set of polyacrylamide (PAA)—based glycoconjugates was applied to the direct and competitive enzyme-linked immunosorbent assays (ELISA) to characterize the specificity of serum IgG antibodies. The anti-TF, -Tn and -SiaTn IgG were affinity purified from serum of cancer patients and characterized using PAA-conjugates and free saccharides. The anti-TF and -Tn antibodies were shown to be specific. The anti-TF IgG bound both Galβ1-3GalNAcα- and Galβ1-3GalNAcβ-PAA, the latter was three-four times more effective inhibitor of antibody binding. The anti-Tn IgG reacted only with GalNAcα-PAA. The anti-SiaTn IgG cross-reacted with Tn-PAA but SiaTn-PAA was five-six times more effective inhibitor in a competitive assay. The IC_{50} values for PAA-conjugates with the corresponding antibodies typically ranged from 2 to 5 $\times$ 10^{-8} M. The antibodies display a low specificity to mucin-type glycoconjugates in comparison with PAA-conjugates as was shown for mucins isolated from human malignant tumor tissues, ovine submaxillary mucin (OSM) and asialo-OSM. The unusual IgG-antibody specificity to GalNAcβ and GalNAcβ1-3GalNAcβ ligands was found in human serum.
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Abbreviations: **TF, Thomsen-Friedenreich antigen, Galβ1-3GalNAcα-; Tn, GalNAcα-; SiaTn, sialyl-Tn; PAA, polyacrylamide; ELISA, enzyme-linked immunosorbent assays; OSM, ovine submaxillary mucin; AOSM, asialo-ovine submaxillary mucin; sp, spacer arm; HSA, human serum albumin; BSA, bovine serum albumin; TBS, Tris buffered saline; A, absorbance.**

Introduction

The role of natural antibodies in the regulation of immune response and maintenance of the immune homeostasis, as well as the distinction between natural autoreactivity and pathological autoimmunity are still poorly understood. It is considered that natural antibodies are present in the serum of healthy individuals without any deliberate immunization [1]. The natural anti-carbohydrate antibodies directed to Galβ1-3GalNAcα– (Thomsen-Friedenreich antigen, TF) and GalNAcα- (Tn) in humans appear to be produced as a result of the antigenic stimulation by intestinal microflora [2]. However autoimmune diseases can induce the elevation of antibody levels as well, for example, against gangliosides [3].

The TF, Tn, and SiaTn are widely known as tumor-associated antigens due to their overexpression in human carcinomas [4,5] because of an aberrant glycosylation. The binding of human anti-TF and anti-Tn antibodies to human tumor cell lines was documented [6,7]. In comparison with healthy donors, significantly lower levels of anti-TF IgM [8–10], anti-TF and anti-Tn IgG [11] were demonstrated in the serum of patients with cancer. However in some patients, high level of anti-TF, anti-Tn and anti-SiaTn IgG antibodies was observed [11].

The detailed research of the specificity of antibodies is required to identify antibody subpopulations and to clarify their role in different diseases. Natural antibodies, including those mentioned above, are difficult to study due to their heterogeneity and polyspecificity. The purified natural antigens are not sufficiently selective due to the presence of different epitopes

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and it makes them mostly unsuitable for such investigation. We applied the synthetic PAA-glycoconjugates [12,13] that are homogenous antigens with a single reiterative epitope with the aim to characterize the specificity of anti-carbohydrate antibodies.

Materials and methods

Glycoconjugates and saccharides

The soluble PAA-glycoconjugates (30 kDa) containing 20 mol% of saccharides and affinity sorbents were obtained from Syntesome (Munich, Germany), PAA is poly(N-hydroxyethyl acrylamide). The following PAA-glycoocnjugates were used: TF, Galβ1-3GalNAcα; $T_{\beta\beta}$, Galβ1-3GalNAcβ; asialo-GM1, Galβ1-3GalNAcβ1-4Galβ1-4Glc; $T_{\alpha\alpha}$, Galα1-3GalNAcα; Tn, GalNAcα; SiaTn, Neu5Acα2-6GalNAcα; SiaLe^a, Neu5Acα2-3Galβ1-3(Fucα1-4)GlcNAcβ; Forssman disaccharide (Fs), GalNAcα1-3GalNAcβ; GalNAcβ; GalNAcβ1-3GalNAcβ; Lac-di-NAc, GalNAcβ1-4GlcNAcβ; A_{tri}, GalNAcα1-3(Fucα1-2)Galβ. Tris-PAA, tris(hydroxymethyl)aminomethane-PAA, was used as a negative control. The conjugates TF-, $T_{\beta\beta}$- and asialo-GM1-PAA were a substituted polyacrylamide with an epitope density of 10 mol% because of an elevated binding to human IgG antibodies.

In a competitive ELISA the following spacered saccharides were used: TF-sp, TF-O$(CH_2)_3NH_2$; Tn-sp, Tn-O$(CH_2)_3$ NHCOCF$_3$;GalNAcβ-sp, GalNAcβ-O$(CH_2)_3$NHCOCF$_3$; (SiaTn-sp-SiaTn), SiaGalNAcα-O$(CH_2)_3$NHCO$(CH_2)_4$ CONH-$(CH_2)_3$Oα-GalNAcSia (Syntesome), as well as free TF-disaccharide Galβ1-3GalNAc from the Laboratory of Dr. K. L. Matta (Rosewell Park Cancer Institute, Buffalo, NY).

Purification of human antibodies

Serum samples with high antibody levels were taken from cancer patients and used in a competitive ELISA as well as for isolation of antibodies. The anti-TF and anti-Tn antibodies were purified using PAA-saccharides covalently bound to macroporous glass as described in [14]. The anti-SiaTn IgG were purified on SiaTn-PAA-Sepharose FF (0.5 μmol of SiaTn per ml) in the same way.

Extraction of mucins from tumors

The tumor tissue obtained from patients with breast cancer was frozen immediately after surgery at -60°C. The extraction was performed using 8 M urea in 0.1 M glycine/0.01 M EDTA/0.02 M Tris HCl/0.1 M NaCl/0.1% BSA/1% Nonidet P-40, pH 7.4 as described in [14]. Alternatively, the precipitation with ethanol was included as well. The proteins were precipitated in 50% of ethanol at pH 5–6. The mixture was centrifuged under cooling. Mucins were precipitated in 85% ethanol/1% CH_3COOK. The mixture was kept overnight at -20°C and centrifuged in the cold. The precipitate of mucins was dissolved in TBS.

The mucin subunits were isolated in the presence of proteinase inhibitors (20 mM EDTA/100 mM ε-aminocaproic acid/5 mM benzamidine HCl/10 μM pepstatin A/0.033% phenylmethylsulfonylfluoride), (Sigma). Tumor tissue samples were taken from two patients with gastric cancer. After precipitation of proteins in 50% ethanol as described, mucins were precipitated in 90% acetone. The mixture was kept overnight at -20°C and centrifuged in the cold. The product was dried in vacuum and then dissolved in 7 M urea/0.1 M Tris HCl/0.32 M NaCl/1.5% betain/0.02% Chaps, pH 7.5. Reduction and alkylation of mucins was performed with 0.1 M dithiothreitol (5 h, 37°C) and then with 0.25 M iodoacetamide in the dark overnight at room temperature. The product was dialyzed and dissolved in 7 M urea/buffer. Mucin subunits were isolated by gel-filtration under denaturing conditions (7 M urea/buffer) on column (1 $\times$ 45 cm) with TSK-Gel HW-60 (Toyo Soda, Japan). The fractions eluted from void volume up to Mr 100 kD were collected, dialyzed and investigated in competitive assay. Mucins were determined by colorimetric assay in microtiter plates [15].

Desialylation of ovine submaxillary mucin (OSM)

The OSM was purchased from IsoSep AB (Sweden). Hydrolysis was performed as described in [14].

Direct and competitive ELISA

Both assays were performed as described in [10,14].

Statistics

Sigma Plot (version 4) and Curve Expert (version 1.34) were used. The linear regression analysis was conducted by Statgraphics Plus for Windows 3.0.

Results and discussion

The specificity of the whole sera

The binding of serum IgG to the tested or structurally related saccharides was analyzed in direct ELISA and verified by the inhibition assay at a concentration of PAA-conjugates of 20 and/or 200 μg/ml. The inhibition of the antibody binding to the adsorbed PAA-conjugate by the same (tested) PAA-conjugate in solution (20 μg/ml) was specific, typically within 76–100%, being higher than the inhibition by another (related) PAA-conjugate at a concentration of 200 μg/ml (Table 1). The mutual and complete inhibition of anti-TF antibodies was demonstrated for TF-PAA and structurally similar $T_{\beta\beta}$-PAA and it was typical of TF-positive sera (Table 1, entry 1). A similar effect for SiaTn-positive sera for the pair SiaTn/Tn (entry 6) and, to a lesser extent, for $T_{\beta\beta}$-PAA/asialo-GM1-PAA (entry 3) was observed. The TF-positive sera showed one-sided inhibition by Tn-PAA mainly, TF-PAA was weak inhibitor of anti-Tn IgG for Tn-positive sera (entry 2). The cross-reactivity for the pair

Table 1. The specificity of the whole sera as tested by inhibition assay[a]

Entry	*Adsorbed PAA-conjugate*	*PAA-conjugate as inhibitor, (concentration, μg/ml)*	*% of inhibition, index*
1	TF	TF (20)	100 *PL*
	TF	$T_{\beta\beta}$ (200)	100 *PL*
	$T_{\beta\beta}$	TF (200)	100 *PL*
2	TF	Tn (200)	99 *GT*
	Tn	TF (200)	37 *GT*
3	Asialo-GM1	Asialo-GM1 (20)	78 *PL*
	$T_{\beta\beta}$	Asialo-GM1 (200)	86 *PL*
	Asialo-GM1	$T_{\beta\beta}$ (200)	69 *PL*
4	$T_{\beta\beta}$	GalNAcβ (200)	11 *PL*
	GalNAcβ	$T_{\beta\beta}$ (200)	10 *VN*
5	Tn	Tn (20)	94 *PM*
	GalNAcβ	GalNAcβ (20)	93 *VN*
	Tn	GalNAcβ (200)	18 *VN*
	GalNAcβ	Tn (200)	12 *VN*
	Tn	$T_{\alpha\alpha}$ (200)	21 *FA*
6	SiaTn	SiaTn (20)	100 *KA*
	SiaTn	Tn (200)	100 *KA*
	Tn	SiaTn (200)	100 *KA*
7	A_{tri}	A_{tri} (20)	96 *VN*
	A_{tri}	Tn (200)	3 *VN*
	Tn	A_{tri} (200)	2 *VN*
	Fs	Fs (20)	88 *PM*
	Fs	Tn (200)	44 *IJ*
	Tn	Fs (200)	16 *VN*
8	GalNAcβ1-3GalNAcβ	GalNAcβ1-3GalNAcβ (20)	76 *FA*
	GalNAcβ1-3GalNAcβ	GalNAcβ (200)	12 *VN*
	GalNAcβ	GalNAcβ1-3GalNAcβ (200)	14 *VN*
	GalNAcβ	Lac-di-NAc (200)	10 *VN*
9	Fs	GalNAcβ1-3GalNAcβ (200)	0 *IJ*
	GalNAcβ1-3GalNAcβ	Fs (200)	0 *VN*
10	SiaTn	OSM (200)	1 *KA*
11	Tn	AOSM (200)	6 *FA*
	AOSM	Tn (200)	0 *PM*

[a]The binding activity of antibodies was calculated as (A_{test} minus $A_{control}$). A_{test} is absorbance with PAA-conjugate; $A_{control}$ is absorbance with Tris-PAA.

Tn/GalNAcβ as a rule was low (entry 5). The Fs (GalNAcα1-3GalNAcβ) and GalNAcβ1-3GalNAcβ differing in configuration of the terminal monosaccharide did not inhibit antibodies to each other (entry 9). The very low cross-reactivity for pair $T_{\beta\beta}$/GalNAcβ (entry 4) and A_{tri}/Tn (entry 7) was observed as well.

The specificity of isolated anti-TF antibodies

Sera with high IgG-binding activity to both TF-PAA and $T_{\beta\beta}$-PAA as well as some activity to bind Tn-PAA (Table 2) were used. A strong binding of isolated antibodies to TF and $T_{\beta\beta}$ disaccharides was demonstrated; the binding to $T_{\beta\beta}$ was twice higher (Table 3). The similar higher $T_{\beta\beta}$-binding activity was also observed for all TF-positive sera ($n = 36$). The $T_{\beta\beta}$-PAA was more potent inhibitor of antibody binding than TF-PAA (Figure 1). Although the antibodies were purified by

Table 2. The binding activity of sera used for purification of antibodies. Direct ELISA

Serum	*Dilution*	*PAA-conjugate*	*IgG-binding activity, A_{test} - $A_{control}$*
AM, anti-TF IgG	1:50	TF	1.72
	1:50	Tn	0.27
	1:600	$T_{\beta\beta}$	0.82
	1:25	Asialo-GM1	0.42
Pool, anti-Tn IgG	1:50	Tn	0.99
	1:50	TF	0.03
	1:50	GalNAcβ	0.12
	1:50	A_{tri}	0.55
	1:50	Fs	1.39
KA, anti-SiaTn IgG	1:100	SiaTn	0.52
	1:50	Tn	0.47

Table 3. The specificity of affinity-purified IgG to PAA-conjugates and IC_{50} values

Adsorbed PAA-conjugate	*Anti-TF*		*Anti-Tn*		*Anti-SiaTn*	
	% [a]	*IC_{50}, nM*	*%*	*IC_{50}, nM*	*%*	*IC_{50}, nM*
TF	100	125; (47)	6; (3)		0; (0)	
Tn	11; (5)		100	23; (28)	26; (33)	256; (266)
SiaTn	0; (0)		7; (1)		100	44; (51)
$T_{\beta\beta}$	207; (197)	31; (15)	0; (1)			
Asialo-GM1	3; (5)					
$T_{\alpha\alpha}$	0; (0)		0; (0)			
GalNAcβ	1; (4)		2; (3)		1; (0)	
SiaLe[a]					0; (0)	

[a]The binding activity of antibodies (percentage) in the direct ELISA was calculated as (A_{test} minus $A_{control}$). The activity of IgG antibodies to the corresponding ligand was taken as 100%. The values for antibodies purified from other sera are shown in brackets.

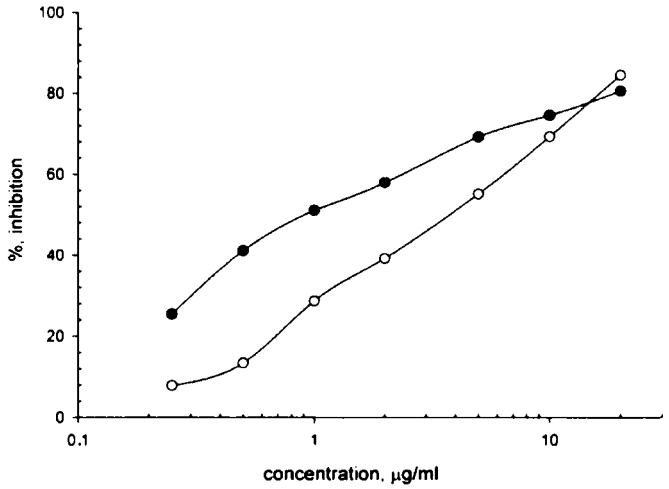

Figure 1. The inhibition of purified anti-TF IgG binding with adsorbed PAA-conjugates by soluble conjugates. ○—adsorbed TF-PAA, inhibitor—TF-PAA. ●—adsorbed $T_{\beta\beta}$-PAA, inhibitor—$T_{\beta\beta}$-PAA.

chromatography on the TF-adsorbent, the affinity to $T_{\beta\beta}$-PAA was three-four times higher as compared by IC_{50} (Table 3).

The IgG-binding activity of whole sera to asialo-GM1-PAA, which also contains $T_{\beta\beta}$ residue, was found to be 3–50 times lower compared to TF-PAA. The anti-TF IgG also only slightly bound asialo-GM1-PAA (Table 3) that may be explained by the low affinity of isolated antibodies to this ligand. Despite some individual differences, the IgG-binding activity with conjugates for all tested TF-positive sera ($n = 36$) was as follows: $T_{\beta\beta}$-PAA > TF-PAA > asialo-GM1-PAA (the direct ELISA). The monovalent TF-sp (not shown) and TF-disaccharide were weak inhibitors for anti-TF IgG in comparison with other antibodies and the corresponding saccharides (Table 5, Figure 3). As shown by other authors, the TF-sp as an inhibitor was 5000 times weaker than TF-PAA for human anti-TF IgM [16]. Taken as a whole, these data show that the specificity of anti-TF IgG differs from that to free TF-saccharide and directed to the disaccharide site (possibly to glycopeptide site) rather than to the oligosaccharide fragment of glycolipid asialo-GM1.

The clear-cut correlation for the highly cross-reactive TF and $T_{\beta\beta}$ as well as for $T_{\beta\beta}$ and asialo-GM1 were demonstrated (Table 4). High values of $r = 0.8$–0.9 are due to the mutual reactivity of antibodies to epitopes. The correlation for TF vs. Tn was found as well, but only for TF-positive sera. It may be explained by prevalent unilateral reactivity of anti-TF antibodies to Tn (Table 1, entry 2). Although the high reactivity of anti-TF IgG to the Tn ligand in the whole sera were observed, the purified anti-TF IgG antibodies bound Tn-PAA weakly (Table 3). Hence, antibodies were separated for the most part from the serum IgG fraction with the Tn-binding activity. An excess of blood sera was taken for purification, therefore antibodies with a high affinity to TF-ligand were obtained mainly. Thus, the purified anti-TF antibodies exhibited TF- and $T_{\beta\beta}$-restricted specificity.

The specificity of isolated anti-Tn antibodies

The pooled sera that were used for isolation of anti-Tn IgG contained IgG antibodies against other Tn-related ligands (Table 2). The purified antibodies were highly active to Tn-PAA (Figure 2, Table 3) but only a very low binding to GalNAcβ-PAA was

Table 4. The correlation between sera by IgG-binding to the pairs of structurally similar saccharides. Direct ELISA

PAA-conjugate	*r*	*P*	*n*
TF vs. $T_{\beta\beta}$	0.89	<0.0001	36
$T_{\beta\beta}$ *vs.* asialo-GM1	0.85	0.0001	36
TF vs. Tn (TF-positive sera)[a]	0.57	0.0001	36
TF vs. Tn (combined group)[b]	−0.10	0.494	46
Tn vs. GalNAcβ	0.35	0.034	36
GalNAcβ vs. GalNAcβ1-3GalNAcβ	0.22	0.201	48

[a]TF-positive sera, ratio Atest/Acontrol ≥ 2, dilution 1:50.
[b]TF-positive, negative and Tn-positive sera.

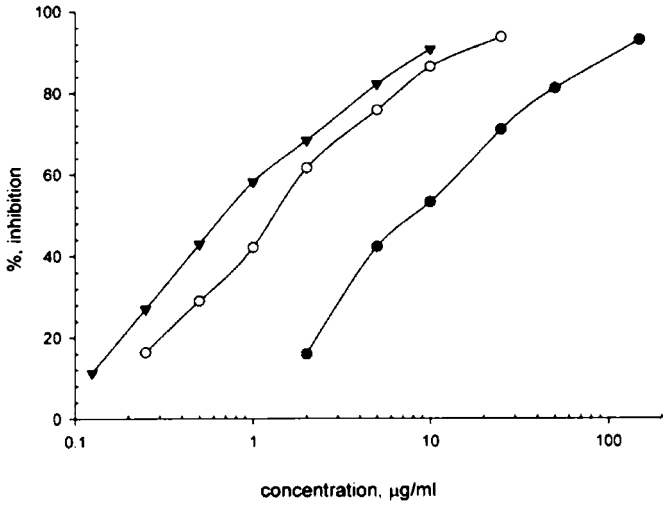

Figure 2. The inhibition of purified anti-Tn IgG and anti-SiaTn IgG binding with adsorbed PAA-conjugates by soluble conjugates. ▼—adsorbed Tn-PAA + anti—Tn IgG, inhibitor—Tn-PAA. ○—adsorbed SiaTn-PAA + anti-SiaTn IgG, inhibitor—SiaTn-PAA. ●—adsorbed SiaTn-PAA + anti-SiaTn IgG, inhibitor—Tn-PAA.

observed (0.5 mg/ml results in a 34% inhibition in a competitive ELISA). The correlation between serum antibodies to these ligands was low (Table 4). A comparison of the monovalent Tn-sp and GalNAcβ-sp also showed a significantly higher inhibiting capability for Tn-sp (Table 5). The binding of anti-Tn antibodies to TF-PAA and SiaTn-PAA was low as well (Table 3) and it is in accordance with the data for whole sera: a majority of Tn-positive sera were TF- and SiaTn-negative. Like TF-PAA, SiaTn-PAA was weak inhibitor of anti-Tn IgG for Tn-positive sera (not shown). The affinity purification of anti-Tn IgG eliminated anti-GalNAcβ, anti-A_{tri} and anti-Fs components, because antibodies reacted with neither one of them. Interestingly, the purified anti-Tn antibodies were specific in spite of the heterogeneous mixture of eight sera used for purification as well as anti-Tn IgG isolated from the other serum taken separately. Thus, unlike anti-TF IgG, the anti-Tn antibodies demonstrated a high specificity to the α-anomer of GalNAc.

The reactivity of sera to other GalNAc-related ligands

Two unusual populations of IgG antibodies reacting with GalNAcβ and GalNAcβ1-3GalNAcβ ligands were revealed in direct ELISA ($n = 65$). It was confirmed in inhibition assay. As discussed above, the reactivity of antibodies to GalNAcβ differs from that to Tn. To determine whether IgG antibodies with a distinct reactivity to GalNAcβ and GalNAcβ1-3GalNAcβ are present, sera with IgG-binding activity to ligands were compared. In fact, only a partial cross-reactivity for both ligands was observed (Table 1, entry 8). Strong differences between antibody levels to each of the ligands as well as a lack of correlation were found (Table 4). Besides, the specificity of antibodies to each of the ligands also differed from that of antibodies to other known structurally related ligands: Lac-di-NAc and Fs (Table 1, entry 8, 9). The activity of IgG to bind (Lac-di-NAc)-PAA was not revealed in the sera with high anti-GalNAcβ or anti-GalNAcβ1-3GalNAcβ IgG level. Taken together, the data show that IgG antibodies with a distinct specificity to GalNAcβ and GalNAcβ1-3GalNAcβ are present in human serum. A ratio Atest/Acontrol > 2 at dilution of sera 1:25 was found in 15% of sera for the first and in 29% for the second ligand.

The specificity of purified anti-SiaTn antibodies

High Anti-SiaTn antibodies were revealed in sera rarely [11]. The SiaTn-positive sera showed complete mutual cross-reactivity to Tn-PAA (*KA, MA*, Table 1, entry 6). The purified anti-SiaTn IgG showed high binding to SiaTn and reactivity to Tn epitope. In the inhibition assay, Tn-PAA, like SiaTn-PAA, brought about a total inhibition of anti-SiaTn IgG binding but the affinity was five-six times lower (Figure 2, Table 3). A similar effect observed for SiaTn-sp-SiaTn compared to Tn-sp (Figure 3, Table 5). Hence, the subpopulation of these antibodies exhibits cross-reactivity to Tn, which is an internal fragment of the epitope. The subpopulation of anti-Tn IgG antibodies with a partial SiaTn-binding capability may be present in the sample as well, however as a rule, Tn-positive sera showed a lack of reactivity to SiaTn.

The purified anti-TF and anti-SiaTn IgG contained a minute amount of the corresponding IgM. The anti-Tn IgG antibodies

Table 5. The inhibition of binding the purified antibodies to conjugates by free saccharides

Antibodies	*Adsorbed PAA-conjugate*	*Inhibitor*	*Concentration of inhibitor, μM*	*Inhibition, %*
Anti-TF	TF-PAA	TF-disaccharide	400	17; (12)
			800	13; (9)
Anti-Tn	Tn-PAA	Tn-sp	16	50
	Tn-PAA	GalNAcβ-sp	250 (max.)[a]	35 (max.)
Anti-SiaTn	SiaTn-PAA	SiaTn-sp-SiaTn	8	50
	SiaTn-PAA	Tn-sp	56	50

[a]For GalNAcβ-sp maximum tested concentrations are shown.

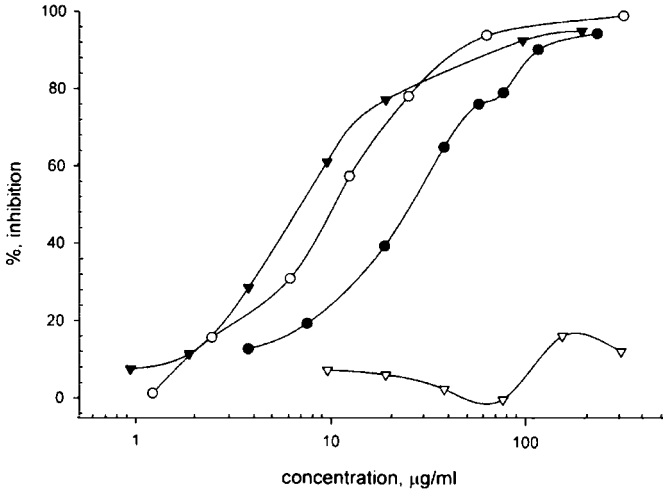

Figure 3. The inhibition of purified antibody binding with adsorbed PAA-conjugates by free saccharides. ▼—adsorbed Tn-PAA + anti-Tn IgG, inhibitor—Tn-sp. ○—adsorbed SiaTn-PAA + anti-SiaTn IgG, inhibitor—SiaTn-sp-SiaTn. ●—adsorbed SiaTn-PAA + anti-SiaTn IgG, inhibitor—Tn-sp. ▽—adsorbed TF-PAA + anti-TF IgG, inhibitor—TF-disaccharide.

contained 17% of anti-Tn IgM as revealed by an isotype-specific ELISA.

On the whole, the specificity of serum antibodies as evaluated with PAA-conjugates and confirmed with purified antibodies may be summarized as follows: (i) high correlation and cross-reactivity between TF, $T_{\beta\beta}$ and asialo-GM1 was typical of TF-positive sera, the specificity of anti-TF IgG was directed to TF and $T_{\beta\beta}$; (ii) low correlation and as a rule low cross-reactivity between Tn and GalNAcβ, the specificity of anti-Tn IgG was directed to Tn only; (iii) the cross-reactivity between SiaTn and Tn was typical of SiaTn- positive sera; the specificity of anti-SiaTn IgG was directed to SiaTn and Tn.

Specificity of antibodies to mucins

Natural SiaTn and Tn epitopes are expressed in ovine submaxillary mucin (OSM) and its asialo-form (AOSM), respectively. A low activity of IgG to bind OSM and AOSM was registered in rare cases in direct and competitive ELISA (Table 1, entry 10, 11). The purified anti-SiaTn antibodies from sera were also weakly reactive to OSM: the inhibition of binding to SiaTn-PAA was about 10% at 0.1–0.5 mg/ml OSM. The binding of anti-Tn IgG to asialo-OSM was negligible as compared to control OSM. It is in agreement with the finding of other authors: IgG antibodies against synthetic conjugates (induced with SiaTn- or TF-KLH) failed to react or reacted weakly with natural epitopes (OSM or asialoglycophorin) [17]. Probably anti-SiaTn and anti-Tn antibodies have a specificity to the solitary epitopes that are present in PAA-glycoconjugates (saccharide density of 20 mol%) and therefore are weakly bound to the clustered epitopes that are typical of mucins [18,19].

Purified antibodies demonstrated a weak binding to mucins from malignant breast tumors in comparison with PAA-glycoconjugates as well. Only three of the twenty extracts inhibited the anti-TF IgG binding to the adsorbed TF-PAA completely and in a dose-dependent manner [14]. The inhibition of anti-Tn and anti-SiaTn IgG-binding to PAA-conjugates by mucins (0.05–0.2 mg/ml) did not exceed 14 and 24%, respectively. Mucin subunits were isolated by gel-filtration under denaturing conditions on column with TSK-Gel HW-60 after reduction and alkylation. But such treatment did not expose the reactivity with antibodies.

The weak interaction of purified antibodies with mucins isolated from tumors can be explained by the following reasons: (i) the purification on PAA-conjugates has resulted in the isolation of a restricted population of antibodies; (ii) inadequate conditions for the isolation of antibodies. Under competitive chromatographic conditions, antibodies with a high affinity to carbohydrates presented on PAA-conjugates appeared to have been obtained. But such antibodies may display a low affinity to mucins and thus be not adequate for competitive assay. (iii) other antigens (glycopeptides, glycolipids) may be natural ligands for antibodies. Further investigations will be required to elucidate all the possibilities.

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