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Immunogenicity of synthetic TF-KLH (keyhole limpet hemocyanin) and sTn-KLH conjugates in colorectal carcinoma patients

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Abstract Mucins of colorectal carcinomas overexpress the cancer-associated disaccharides Thomsen-Friedenreich antigen (TF) and sialyl-Tn antigen (sTn), making these antigens suitable for active specific immunotherapy. Patients at high risk for recurrent colon cancer, but free from disease after surgical resection, were immunized with synthetic TF and sTn covalently attached by a two-carbon crotyl linker to keyhole limpet hemocyanin (KLH). Four groups of patients were treated with TF-KLH without adjuvant, TF-KLH plus the immunological adjuvant Detox, sTn-KLH plus Detox, or sTn-KLH plus the immunological adjuvant QS-21, and the serological response was monitored. Enzyme-linked immunosorbent assay (ELISA), dot-blot immunostains, and inhibition assays were used to identify antibody responses against synthetic TF and sTn epitopes and against natural antigens, including asialoglycophorin expressing TF antigen, and ovine submaxillary mucin and the human colon cancer line LS-C expressing sTn antigen. Our results demonstrate that vaccines containing TF or sTn-KLH conjugates plus immunological adjuvants Detox and especially QS-21 induced high IgM and IgG antibody titers against the respective synthetic disaccharide epitopes. However, when tested against natural antigens expressing these disaccharide epitopes, IgM antibodies showed weak to moderate reactivity, while IgG antibodies were almost totally unreactive. On the basis of these results we are continuing to test modifications of synthetic TF and sTn epitopes to identify those that induce IgM and IgG antibodies that are more reactive with these antigens as they are expressed on tumor mucins.

Key words Active specific immunotherapy · Adjuvants · Thomsen-Friedenreich (TF) · Sialyl-Tn (sTn) · Keyhole limpet hemocyanin (KLH) · Tumor vaccine

Introduction

Thomsen-Friedenreich (TF), and sialyl-Tn (sTn) antigens are blood-group-related disaccharides that are O-linked to serine and threonine residues of mucins on epithelial cancers and some normal tissues [7, 31, 33]. In normal tissues, TF antigen (Galβ1-3GalNAcα-*O*-Ser/Thr) and sTn (NeuAcα2-6GalNAcα-*O*-Ser/Thr) have been identified by immunohistology to be restricted to expression on the luminal surface of secretory cells, a site largely inaccesible to the immune system [11, 24, 32]. Altered glycosylation leads to exposure of these core structures in malignant tissues and increased expression has been correlated with poor prognosis in a number of epithelial cancers, including colon cancer [4, 12, 14, 30].

While all normal individuals have IgM antibody to TF, antibody titers may become reduced in patients with mucin-secreting cancers [2, 22, 29]. This suggests that, although these cancers are unable to induce an immune response against TF, natural antibodies are able to recognize TF on tumors or shed mucins and are removed from the circulation. TF and sTn may be poor immunogens because they are carbohydrates and because they are autoantigens. Augmentation of the immunogenicity of poor immunogens, including bacterial carbohydrate antigens, has been achieved by conjugation to immunogenic carrier proteins [5, 6]. Keyhole limpet hemocyanin (KLH) is perhaps the most potent of these carrier proteins and has been used to augment IgM and IgG antibodies against the TF epitope in mice [17]. The purpose of this study was to confirm the

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increased immunogenicity of KLH glycoconjugates in man, to analyze the specificity of the antibodies induced, and to study the effect of additional potent immunological adjuvants. We describe the immunization of four groups of colorectal carcinoma patients with TF-KLH and sTn-KLH glycoconjugates utilizing Detox and QS-21 adjuvants.

Materials and methods

Patients

A group of 22 patients with Dukes' B, C or D colorectal carcinoma, who were free from disease 2 weeks to 8 months after resection of all known disease, were treated. Blood was drawn before each vaccination and 2 weeks and 6 weeks after the fourth, fifth, and sixth vaccinations for serological testing. Six patients received TF-KLH alone, 6 patients received TF-KLH plus Detox, 5 patients received sTn-KLH plus Detox and 5 patients were administered sTn-KLH plus QS-21.

Antigens and adjuvants

A series of synthetic constructs were provided by Biomira Inc. (Edmonton, Alberta). Synthetic TF (Galβ1-3GalNAc-OR) and sTn (NeuAcα2-6GalNAc-OR) disaccharides were O-linked directly to ceramide or N-linked by a two-carbon crotyl linker arm (R) to human serum albumin (HSA) or KLH. The ratio of TF and sTn epitopes per KLH was 2823 and 3000 molecules respectively. TF and sTn disaccharides O-linked to serine were also provided. TF-crotyl-lysine and sTn-crotyl-lysine were prepared by ozonolysis of crotyl-TF and crotyl-sTn with conjugation to lysine achieved by reductive amination [1].

Porcine submaxillary mucin (PSM) and ovine submaxillary mucin (OSM) were purchased from BioCarb (Lund, Sweden). PSM was desialylated with 0.1 M trifluoroacetic acid at 100 °C for 1 h. Asialoglycophorin (3.8 mg/ml) was produced from glycophorin by hydrolysis in 0.1 M HCl at 80 °C for 1 h. HSA and GM3 were purchased from Sigma Chemicals (St. Louis, Mo.). Anti-sTn monoclonal antibodies B72.3 and TKH-2 were supplied by Dr. K. O. Lloyd and anti-TF monoclonal antibody 49-H was provided by Biomira Inc. [3, 13, 19].

Detox containing monophosphoryl lipid A and bacillus Calmette-Guérin (BCG) cell wall skeleton was provided by Ribi Immunochem (Hamilton, Mont.). QS-21 adjuvant was provided by Cambridge Biotech Corp. (Worcester, Mass.). QS-21 is a saponin fraction purified from the *Quillaja saponaria* Molina bark [10, 18]. Cyclophosphamide (Cytoxan, Mead Johnson and Co., Evansville, Ind.) 200 mg/m^2 was administered i. v. to patients 4–6 days prior to the first vaccination.

Vaccine preparation and administration

On the day of vaccination, 0.25 ml Detox (250 μg BCG cell wall skeleton + 25 μg monophosphoryl lipid A) was mixed with 100 μg sTn-KLH or TF-KLH, or 100 μg QS-21 was mixed with 100 μg sTn-KLH, and brought to a final volume of 0.50 ml with phosphate-buffered saline (PBS). The vaccine was vortexed for 2–3 min before administration. Four vaccinations were administered subcutaneously at 2-week intervals followed by two more at 8-week intervals.

Serological assays

ELISA were performed using alkaline-phosphatase-conjugated goat anti-(human IgM) or anti-(human IgG) (Kierkegaard and Perry Labs, Gaithersburg, Md.) as previously described [16]. sTn-HSA or TF-HSA at 0.5 μg/well was dried onto Nunc microwell plates (Nunc, Denmark). Absorbance was measured at 414 nm and the highest serum dilution with an absorbance at least 0.100 was defined as the antibody titer. Plates were incubated, before measuring absorbance, at 37 °C for approximately 20 min. Inhibition ELISA were performed to determine more sensitively the specificity of antibodies. For these assays, the designated quantity of antigen was incubated with patient sera for 1 h and the mixture then tested on the synthetic hapten-HSA conjugates by ELISA. Percentage inhibition was calculated on the basis of the difference in absorbance from the uninhibited serum. ELISA were also performed on the sTn-positive LS-C and sTn-negative LS-B clones of the human colon cancer cell line LS174T [26]. Supernatants of murine monoclonal antibodies B72.3 and TKH-2, which recognize sTn, were used as positive controls. Dot-blot immune stains were performed on nitrocellulose membrane strips as described previously [8, 18] at a serum dilution of 1:150. Peroxidase-conjugated goat anti-(human IgM) or anti-(human IgG), purchased from Tago (Burlingame, Calif.), was used.

Delayed-type hypersensitivity

Patients vaccinated with TF-KLH were skin tested with Tn-HSA, TF-HSA, and HSA; patients receiving TF-KLH plus Detox were skin tested with TF-HSA, sTN-HSA, HSA; patients receiving sTn-KLH plus Detox and sTn-KLH plus QS-21 were skin tested with sTn-HSA. Samples of 25 μg of each were diluted in 0.05 ml PBS and administered intradermally. Skin tests for delayed-type hypersensitivity against mumps were also performed in all patients; results were interpreted as described previously [15].

Results

Vaccine toxicity was adjuvant-induced

Of 6 patients receiving TF-KLH alone, 5 had neither systemic nor local reactions at vaccination sites; the 6th patient experienced flu-like symptoms and 4 cm erythema and induration at injection sites after the third vaccination only.

All patients receiving Detox developed local erythema, induration and pruritis at vaccination sites, lasting 1–3 days. This was most prominent in patients receiving the sTn-KLH vaccine. Most patients in the Detox groups also developed nontender nodules at some vaccination sites, which resolved after 2–12 weeks.

Patients receiving sTn-KLH plus QS-21 developed 2–15 cm erythema and induration and tenderness at vaccination sites, which subsided within 2–4 days. These local reactions required no treatment and did not interfere with the patients' daily activities. Systemic side-effects were minor, occasional patients developing low-grade fever to 38 °C or mild flu-like symptoms lasting 4–24 h, with either adjuvant.

Antibody responses

Antibody titers against the synthetic TF and sTn epitopes conjugated via crotyl linker to HSA were measured by ELISA. Preimmunization sera showed IgM reactivity with TF (median titers 1:80) and sTn (median titer 1:30) as shown in Table 1 and Fig. 1. TF-HSA IgM titer were enhanced slightly by immunization with TF-KLH (median titer 1:640). Patients receiving TF-KLH plus Detox and

Table 1 Peak enzyme-linked immunoassay (ELISA) antibody titer against synthetic TF, sTn, and porcine and ovine submaxillary mucins. Prevaccination and postvaccination sera for all patients were tested by ELISA to detect antibodies against the respective synthetic and natural antigens. Sera from patients receiving TF-KLH or TF-KLH plus Detox were tested on TF-HSA and PSM (porcine submaxillary mucin); sera from patients receiving sTn-KLH plus Detox and sTn-KLH plus QS-21 were tested on sTn-HSA and OSM (ovine submaxillary mucin). Prevaccination and peak postvaccination sera on each patient are presente here. *KLH* keyhole limpet hemocyanin, *HSA* human serum albumin

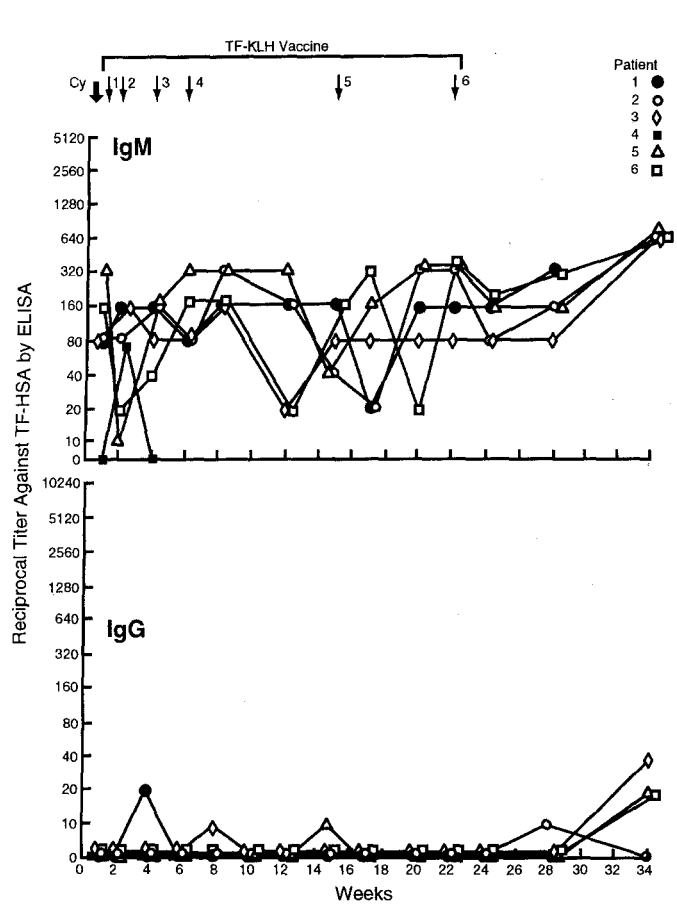
Vaccine	Serum	No. of patients	TF-HSA		Desialylated PSM	
			IgM	IgG	IgM	IgG
TF-KLH	Prevaccine	6	0(1), 80(3), 160(1), 320(1)	0(6)	0(2), 10(3), 20(1)	0(6)
	Postvaccine	6	80(1), 320(1), 640(4)	0(1), 10(1), 20(3), 40(1)	0(1), 10(2), 20(2), 40(1)	0(6)
TF-KLH + Detox	Prevaccine	6	20(1), 80(1), 40(1), 160(3)	0(5), 10(1)	0(1), 10(2), 20(2), 320(1)	0(6)
	Postvaccine	6	640(1), 1280(2), 2560(2), 10240(1)	160(2), 320(2), 1280(2)	0(1), 20(2), 80(1), 320(1), 1280(1)	0(6)
			sTn-HSA		OSM	
			IgM	IgG	IgM	IgG
sTn-KLH + Detox	Prevaccine	5	20(2), 40(1), 80(1), 320(1)	0(5)	0(4), 80(1)	0(5)
	Postvaccine	5	320(1), 1280(3), 10240(1)	40(2), 320(2), 640(1)	0(1), 20(2), 40(1), 80(1)	0(4), 10(1)
sTn-KLH + QS-21	Prevaccine	5	10(2), 20(1), 40(2)	0(3), 10(1), 40(1)	0(4), 20(1)	0(4), 40(1)
	Postvaccine	5	1280(2), 5120(3)	2560(4), 5120(1)	20(1), 40(1), 80(2), 160(1)	20(2), 40(2), 80(1)

sTn-KLH plus Detox showed a higher-titer IgM response (median titers 1:1920 and 1:1280 respectively). Patients in the sTn-KLH plus QS-21 trial responded with the highest IgM titer (median titer 1:5120). None of the patients had preexisting IgG antibodies against TF-HSA and only 1 patient had detectable preexisting IgG antibodies against sTn-HSA (Table 1, Fig. 1). Moderate levels of IgG antibodies were induced against TF-HSA and sTn-HSA in the Detox groups (median titer 1:320), but no IgG antibodies were detected in patients treated with TF-KLH alone. The highest-titer IgG antibodies against sTn were induced by vaccination with sTn-KLH plus QS-21 (median titer of 1:2560).

Specificity analysis of TF and sTn antisera

Dot-blot immunostains were performed on prevaccination sera and on peak titer sera of all 22 patients (Fig. 2). With regard to IgM antibodies, several patients in the TF-KLH alone and TF-KLH plus Detox groups showed increased reactivity against asialoglycophorin (TF) after immunization, indicating that the synthetic TF disaccharide vaccine enhances preexisting IgM antibody levels against natural TF antigen. None of the sera showed reactivity with TF-disaccharide O-linked to ceramide. Three out of 5 patients in the sTn-KLH plus Detox group and all 5 patients in the sTn-KLH plus QS-21 group showed an increase in IgM reactivity with OSM, demonstrating that these IgM antibodies could react with natural sTn. The IgG antibodies however, failed to react (Detox groups) or reacted weakly (QS-21 group) with the natural TF or sTn epitopes expressed by asialoglycophorin or OSM. This was confirmed by ELISA assays (see Table 1). The IgG antibodies appear to recognize epitopes on synthetic TF and sTn conjugates that are different from those present on TF and sTn epitopes expressed by natural mucins. Serum from a patient immunized with OSM plus BCG was included in the assay to demonstrate the difference in specificity of the induced antibodies [25]. Most sera from the OSM trial had antibodies that were low-titer, but which reacted equally with the synthetic and natural sTn epitopes, in contrast to the antibodies induced by the synthetic constructs tested here.

Inhibition of ELISA reactivity against TF-HSA or sTn-HSA was assayed to analyze the specificity of vaccine-induced IgM and IgG antibody with greater precision. Sera from immunized patients were incubated with various amounts of TF-HSA, TF-ceramide, asialoglycophorin, TF-serine, lactose, TF-crotyl, and TF-crotyl-lysine (data not shown), or sTn-HSA, sTn-crotyl-lysine, OSM, GM3 ganglioside, sTn-serine, and HSA. Results of a representative experiment are demonstrated in Fig. 3 for a patient immunized with sTn-KLH plus QS-21. Both IgM and IgG reactivities of serum from this patient were strongly inhibited by synthetic sTn-HSA. IgM reactivity was more effectively inhibited by OSM than IgG reactivity, but in both cases a 100- to 1000-fold excess of OSM sTn epitopes compared to synthetic sTn-HSA epitopes was required. In


TF-KLH Vaccine
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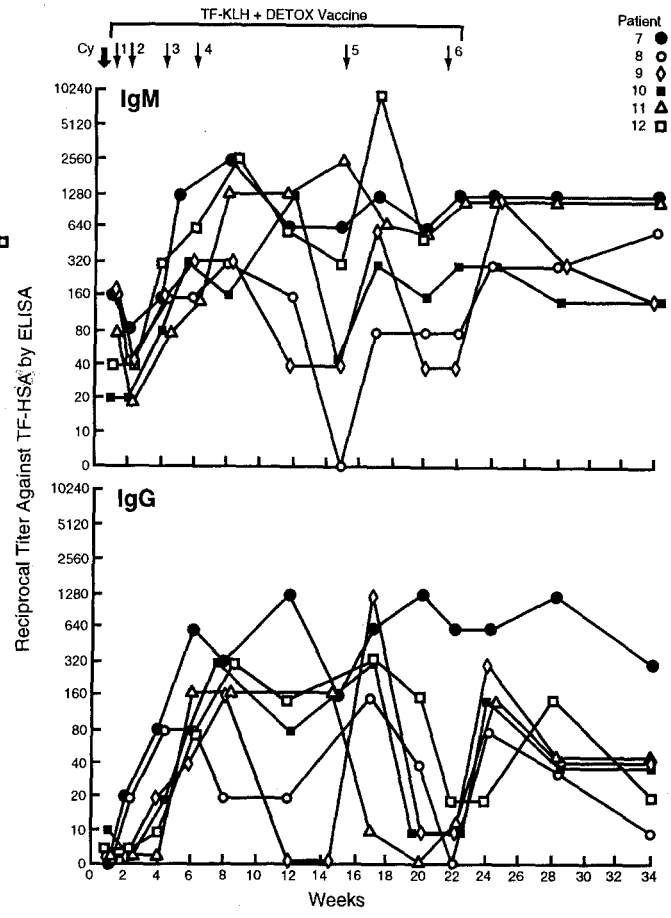
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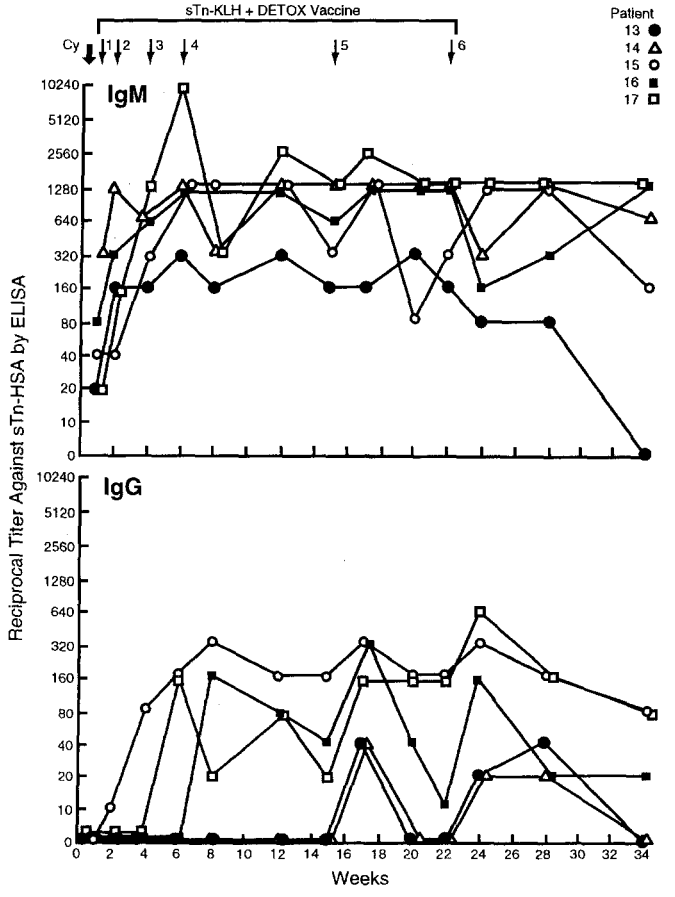
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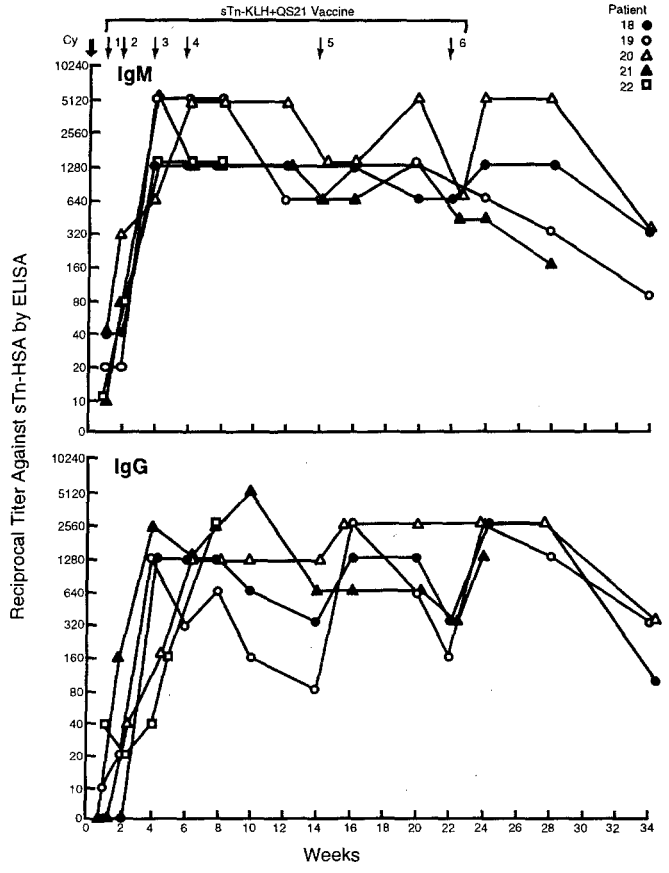
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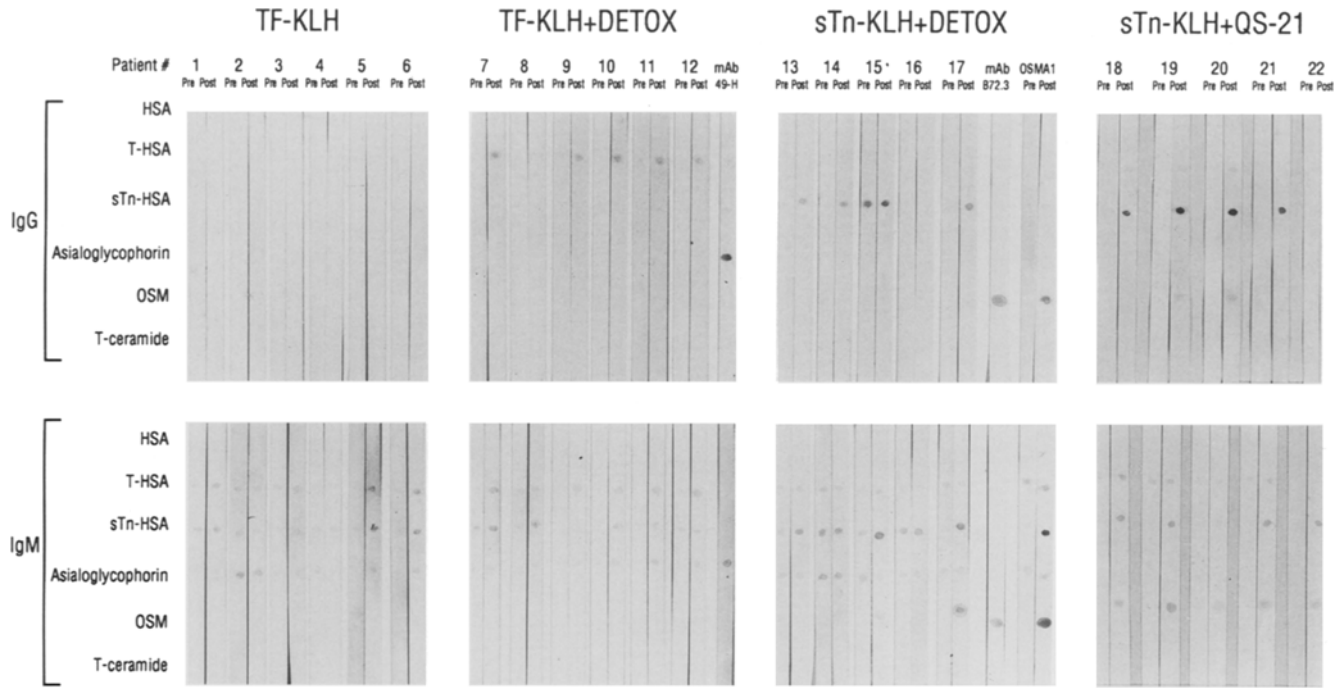

Fig. 2 Dot-blot immunostains for IgM and IgG antibodies in sera of 22 patients immunized with TF-KLH alone, TF-KLH plus Detox, sTn-KLH plus Detox or sTn-KLH plus QS-21. Pre- and post-sera of a patient immunized with ovine submaxillary mucin (*OSM*) plus BCG vaccine was used from our previous trial (OSMA1). Antigens were spotted on nitrocellulose strips (indicated on the *vertical axis*), and incubated with prevaccination serum and peak titer postvaccine serum of each patient. The immunostains were developed using anti-(human IgM) or anti-(human IgG) antibody linked to peroxidase. mAb B72.3 was used as positive control for natural sTn in OSM, mAb 49-H was used as a control for natural TF in asialogylcophorin [7, 28, 33], in both cases with the appropriate anti-(mouse Ig) secondary antibody

sTn-serine, sTn is O-linked to serine, making this construct similar to the natural sTn epitope. sTn-lysine is N-linked, via a crotyl linker to lysine. This is similar to the vaccine sTn-KLH with sTn N-linked via a crotyl arm to the lysine of KLH. Inhibition experiments show that the IgG antibodies generated against the synthetic sTn epitope are inhibited preferentially by sTn-HSA. sTn-crotyl-lysine also inhibits but to a lesser extent. sTn-serine inhibits reactivity poorly. The results from dot-blot and inhibition studies confirm that increased-titer IgM antibodies from all patients react strongly with the respective synthetic epitopes (TF-HSA, sTn-HSA) and to a lower extent with natural epitopes (asialoglycophorin, OSM) while the IgG antibodies react almost exclusively with the synthetic disaccharides.

Antibody titers against natural sTn epitopes on the cell surface of sTn-positive (LS-C) and sTn-negative (LS-B) subclones of the human colon cancer cell line LS174T were also measured. Pre- and post-treatment sera from the 10 patients vaccinated with sTn-KLH were tested on both cell lines and the results on the negative control, LS-B, were subtracted from results obtained with LS-C (see Fig. 4). Comparing pre-treatment and post-treatment IgM ELISA titers against LS-C, 8 of 10 patients had positive post-treatment ELISA results (A $\geq$0.1) and 9 out of 10 patients had positive post-treatment IgM ELISA results compared to the pre-treatment values. On the other hand, only 2 of 10 patients had positive post-treatment IgG ELISA results and no patients had post-treatment ELISA results that were positive compared to the pre-treatment values. Though positive, the IgM reactivity against LS-C was significantly lower than the reactivity with anti-sTn monoclonal antibodies (B72.3 and TKH-2) and significantly lower than reactivity of the same post vaccination sera on the synthetic epitopes.

Fig. 1 IgM and IgG enzyme-linked immunosorbent assay (*ELISA*) antibody titers against TF-human-serum-albumin (HSA) or sTn-HSA in sera obtained from 22 patients immunized with vaccines containing TF or sTn-keyhole-limpet-hemocyanin(KLH) conjugates plus immunological adjuvants Detox or QS-21

Delayed-type hypersensitivity responses after vaccination

No DTH responses against TF-HSA or sTn-HSA were detected. Reactivity to the standard mumps skin tests was seen in 10 of 19 patients (three patients were not skin-tested) with a median measurement of 5 × 5 mm.

Discussion

Despite the widespread occurrence of natural IgM antibodies against the TF and sTn antigens, some level of immunological tolerance against them might be expected from their known expression on some normal fetal and adult

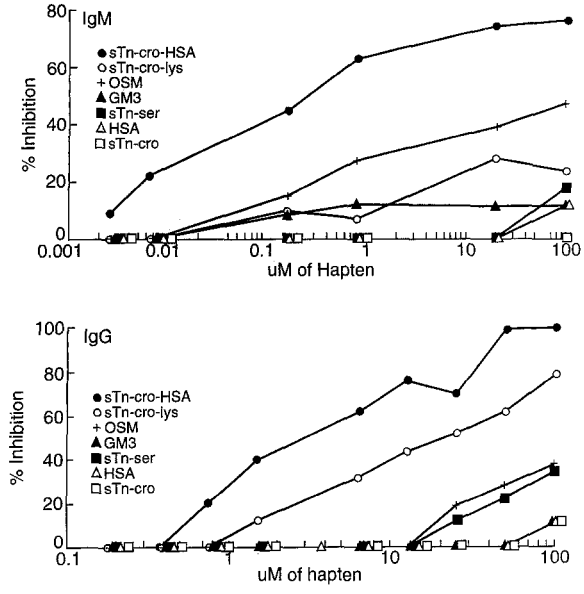

Fig. 3 Inhibition of IgM and IgG ELISA reactivity against sTn-HSA by various natural and synthetic sTn molecules. High-titer postvaccination serum from patient 17, who had received sTn-KLH plus Detox, was incubated at a serum dilution of 1:80 with natural and synthetic antigens at various concentrations, and then reacted with sTn-HSA by ELISA. *sTn-cro-HSA* sTn-crotyl-HSA, *OSM* ovine submaxillary mucin, *sTn-cro-lys* sTn-crotyl-lysine, *sTn-ser* sTn-serine, *sTn-cro* sTn-crotyl, *HSA* human serum albumin, *GM3* monosialoganglioside; ganglioside nomenclature designated by Svennerholm (1963). ELISA were processed with alkaline-phosphatase-labeled anti-(human IgM) or anti-(human IgG) antibody as indicated

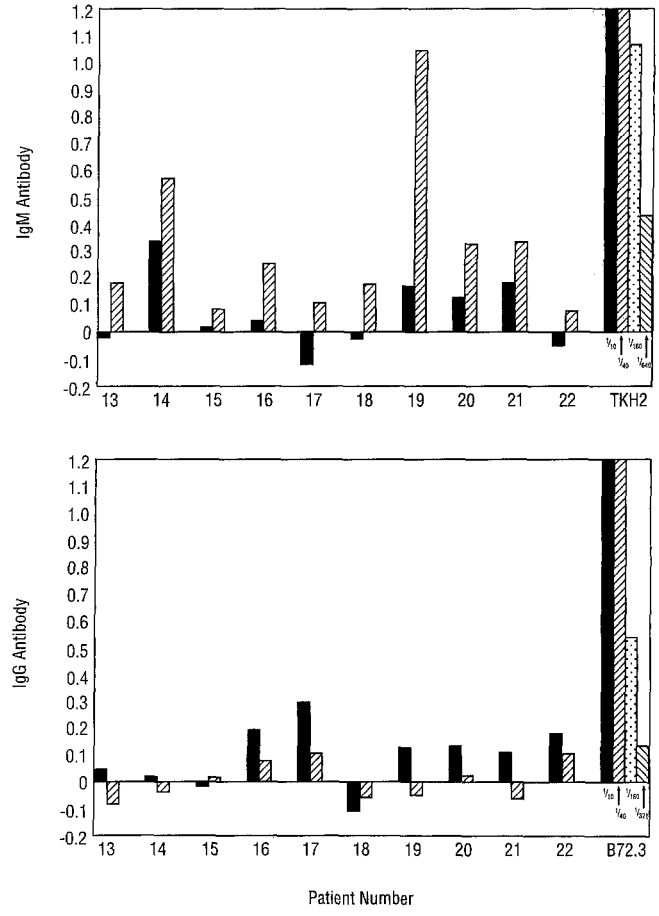

Fig. 4 IgM and IgG ELISA reactivity against sTn-positive human colon carcinoma cell line LS-C. Values represent absorbances at 405 nm indicating the response against the sTn-positive LS-C minus the response against the sTn-negative subclone LS-B. Pretreatment (*solid bars*) and posttreatment (*striped bars*) values for patients 13–22 at a serum dilution of 1/80 are shown. Values with murine mAb B72.3 and TKH-2 are shown as positive controls

tissues. We have previously immunized groups of patients with partially desialylated ovine submaxillary mucin (OSM) expressing Tn and sTn, plus BCG and Detox, and seen augmentation of preexisting IgM antibody against both OSM and synthetic sTn to a moderate level, but only occasional IgG antibodies [25]. We assumed that this was a consequence of the low immunogenicity of the mucin protein backbone, resulting in inefficient T cell help for a T-cell-dependent antibody response. KLH was selected here as protein carrier to provide this help with TF and sTn disaccharides covalently attached via a crotyl linker arm to available KLH lysine groups. Induction of long-lasting high-titer IgG antibodies against TF and sTn, in addition to IgM antibodies, was our goal. IgM antibodies are known to be highly effective at mediating complement lysis and opsonization (resulting in phagocytosis by the reticulo-endothelial system). IgG antibodies have a larger distribution pool in the body and may mediate antibody-dependent cell-mediated cytotoxicity. Consequently, the combination of IgM and IgG antibodies may be more productive than either class of antibodies alone.

We found that in vaccines containing Detox or QS-21 as immunological adjuvants, TF and sTn disaccharides conjugated to KLH were able to augment natural IgM antibodies that cross-reacted (though at lower titers) with the natural epitopes on PSM and asialoglycophorin or OSM. However, the IgG antibodies induced by vaccination failed to react or reacted only weakly in ELISA and dot-blot immunostaining with the naturally expressed epitopes. This pattern of reactivity is different, however, from that seen with sera from patients vaccinated with OSM, where IgM and IgG antibodies reacted (though at low titers) with natural and synthetic epitopes equally [25]. Inhibition studies confirmed the direct assay results. IgM antibodies against synthetic epitopes were clearly inhibited by natural epitopes on asialoglycophorin or OSM but, like the weaker inhibition seen with IgG antibody, inhibition required 100- to 1000-fold excess of the natural epitopes. It is difficult to be certain from this whether the difference in IgM and IgG reactivity toward natural and synthetic epitopes is due to recognition of different epitopes or differences in antibody affinity. In the setting of low-affinity antibodies, IgM antibodies with their polyvalency are known to show higher reactivity than IgG antibodies [27]. This difference may be heightened if the epitopes recognized are found in clusters, as has been recently described for the expression of sTn on mucins [34]. MacLean et al. have immunized ovarian and breast cancer patients with these same synthetic TF-KLH and sTn-KLH conjugates plus Detox [20, 21]. They de-

tected moderate-titer IgM antibodies reactive with synthetic and natural epitopes and high-titer IgG antibodies against synthetic epitopes, which reacted more clearly than in our study, though at low titers, with the natural antigens.

The recent availability of LS-C (sTn-positive) and LS-B (sTn-negative), clones of the human colon cancer cell line LS174T [26], has made it possible to determine whether our findings concerning the reactivity of immune sera with the natural mucin (OSM) apply as well to sTn expressed on human colon cancer cells. The results were similar. IgM antibodies induced by vaccination with sTn generally reacted with LS-C but not LS-B, while IgG antibodies induced by vaccination reacted with neither. Overall, our results demonstrate that high-titer IgM and IgG antibodies against synthetic epitopes induced by disaccharide-KLH plus adjuvant vaccines result in moderate IgM titers and low or undetectable levels of IgG antibodies against natural sources of these same disaccharides.

We have recently screened a variety of carrier molecules and selected KLH as optimal for inducing IgG antibodies against another carbohydrate antigen, GD3 ganglioside [9]. However, the potent immunological adjuvant QS-21 was crucial for optimal IgG titers, even in the context of conjugation to KLH. In preclinical studies with the same TF-KLH preparation used here, immunological adjuvant QS-21 (an extract of *Quillaja saponaria* bark) resulted in significantly higher anti-TF IgG titers than seen with Detox or other adjuvants [17]. This vaccination approach, covalent attachment to KLH and the use of QS-21 as immunological adjuvant, has been optimal in our hands with a variety of carbohydrate antigens [18]. Patients immunized here with sTn-KLH plus QS-21 showed the highest-titer IgM and IgG antibodies. Unfortunately, the specificity of these high-titer antibodies was unchanged by this more potent adjuvant.

Clearly, the synthetic disaccharide epitopes that we used do not accurately imitate the epitopes recognized by the human immune system on natural mucins or cancer cells. Our previous studies with OSM vaccines demonstrated that it is possible to induce IgM and IgG antibodies of the appropriate specificity, albeit with low titer. We are currently testing the immunogenicity of two modifications of the synthetic epitopes discussed here. The first is TF and Tn disaccharide O-linked to serine, which is then conjugated to KLH to reflect more accurately the way these disaccharides are naturally expressed, O-linked to serine or threonine. In addition, Nakada et al. have shown that the natural epitope detected by anti-Tn monoclonal antibody MLS128 consists of at least three consecutive glycosylated Tn-Ser/Thr residues and that this may be essential for Tn antigenicity [23]. Based on Nakada's reports and findings such as those presented here, a vaccine containing trimeric O-linked sTn-Ser conjugated to KLH has been prepared at Biomira Inc. Preclinical studies with this cluster construct (linked to KLH) demonstrate that the murine immune system and murine monoclonal antibodies such as B72.3 and B195.3R11 recognize two distinct configurations of sTn on tumor cells, clustered and nonclustered, and that the clustered form may be most specific for cancer cells [34]. Perhaps the sTn cluster construct linked to KLH will induce IgM and IgG antibodies in humans that react more effectively with natural sources of sTn.

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