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REVIEW

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Carbohydrate vaccines that induce antibodies against cancer. 1. Rationale

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Introduction

Thousands of patients have received a variety of cancer vaccines over the last 25 years (reviewed in [1–3]). Occasional significant tumor responses have been seen and the frequency of these responses is too high to be attributed to spontaneous regressions. While these results demonstrate that treatment of patients with tumor vaccines can occasionally induce tumor regression, they have failed to provide a solid foundation for the construction of increasingly effective vaccines. This is because the majority of these patients received vaccines of limited or unknown antigenicity and it is unclear whether the patients responded immunologically to the vaccines. In this setting it has not been possible to determine why individual patients responded or how to improve the efficacy of a vaccine that might have induced clinical responses in 10% of treated patients. What are required for the development of increasingly effective vaccines are methods to assess relevant immunogenicity that can be used to guide the process of vaccine construction and testing. Such methods have been available for serological responses against cell surface tumor antigens for 20 years (reviewed in [4, 5]). Consequently much has been learned about serologically defined tumor antigens and methods for augmenting their immunogenicity. More recently, assays capable of detecting augmented T lymphocyte responses against tumor antigens after vaccination have also been described, raising the possibility that comparable progress may be forthcoming with antigens defined by T lymphocytes over the next several years. In both cases the results are more definitive and build a sounder foundation when defined tumor antigens, the structure and expression of which on tumors and normal tissues in vivo are known, are used.

The treatment of cancer with tumor vaccines has been a dream of physicians since the first vaccines against infectious diseases were developed. Though vaccines against infectious diseases have generally been used to protect from future infections, some have been shown to be effective when administered after exposure [6, 7]. Cancer vaccines can induce protection against, and in some cases mediate regression of, established syngeneic cancers in mice [8, 9]. The human immune system has also been shown to have the power to destroy a considerable burden of growing allogeneic tumor transplanted accidentally [10, 11]. The problem with most human cancers is that the tumor antigens expressed are not so immunogenic. Nevertheless, antibodies and helper and cytotoxic T lymphocytes from cancer patients have now been used to define a number of human tumor antigens. In some cases these patients have had a particularly favorable clinical course [12–14]. Recent technological advances permit a variety of molecular biological and synthetic approaches to the identification and production of these antigens. In addition, there have been striking advances in our understanding of antigen-processing, presentation and the subsequent immune activation, and ways to enhance these processes. All of this provides a solid basis for optimism that cancer vaccines may one day play a role in the treatment of cancer in humans.

We focus here on defined cell-surface antigens recognized by antibodies and, in particular carbohydrate antigens, such as those shown in Fig. 1. The great majority of cell-surface antigens recognized by antibodies are carbohydrate antigens and the immune response against carbohydrates is largely restricted to antibodies. Consequently it is possible to focus on determining the benefit of inducing an antibody response in this setting without addressing the confounding and more complex issue of T cell immunity. The first part of this review will provide the rationale for tumor vaccines aimed at augmenting antibody responses against carbohydrate antigens. The second part will present the results of completed and ongoing trials with carbohydrate tumor antigen vaccines, and will discuss trials planned for the immediate future.

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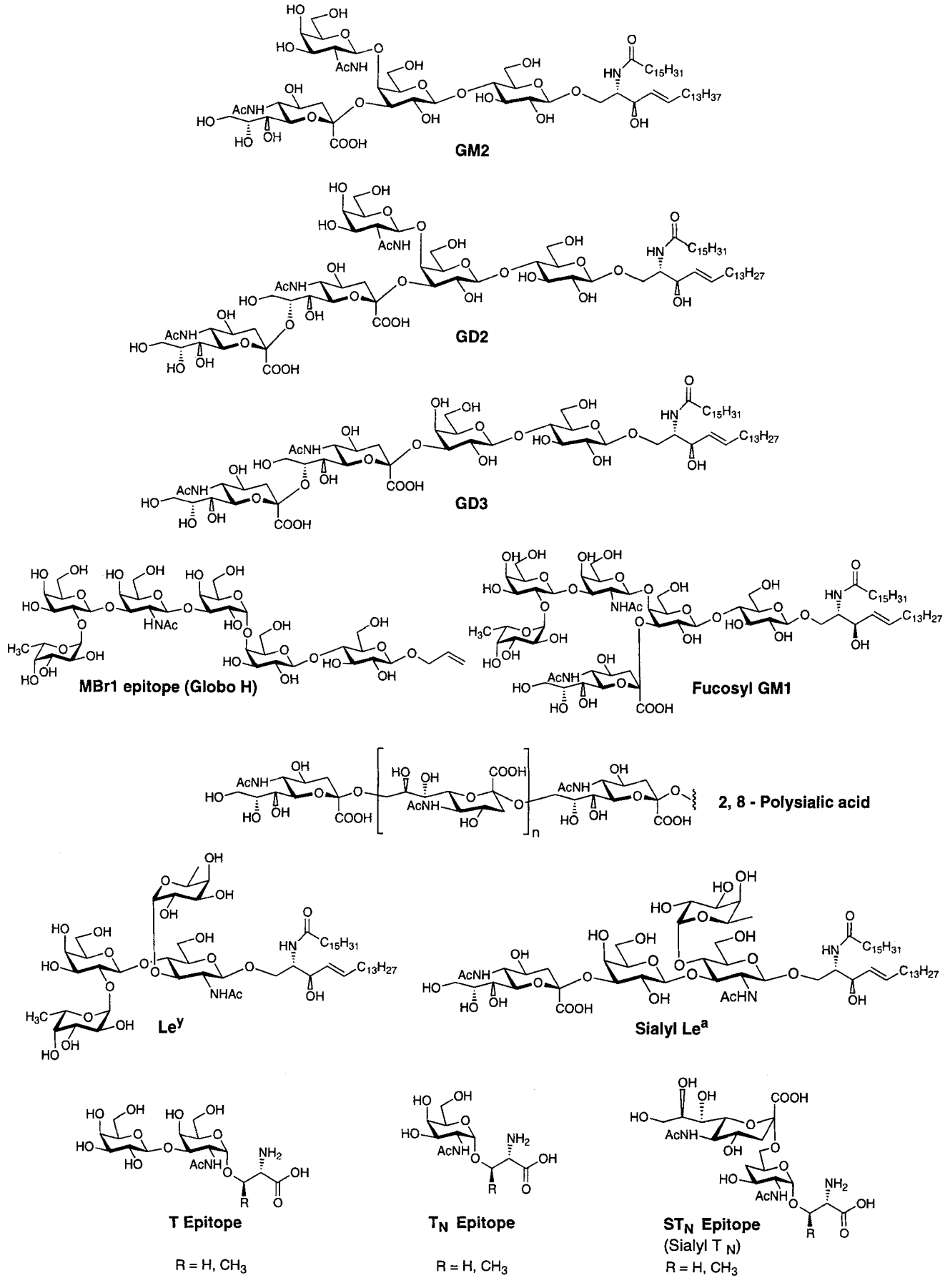

Fig. 1 Structures of well-characterized carbohydrate tumor antigens used or considered for use in human tumor vaccines

Selection of particular carbohydrates for vaccine construction

Tumor antigens differentially expressed at the cancer cell surface have generally been identified first by antibodies, usually murine monoclonal antibodies (mAb). On the other hand, ganglioside expression can be quantified in cancers by extraction and thin-layer chromatography (TLC) [28, 29, 66]. This is possible because glycolipids can be extracted from tumor biopsies using chloroform and methanol and because biopsy specimens of neuroectoderm-derived tumors contain predominantly tumor cells. The identity of these gangliosides can be confirmed by TLC immunostaining. GM2, GD2 and GD3 ganglioside expression on melanomas, sarcomas and neuroblastomas has been defined in this way [28, 29, 66]. Glycoprotein tumor antigens, which are more characteristic of epithelial cancers, are less suitable for this sort of quantification because glycoproteins are more difficult to isolate and to fractionate and because tumor cells frequently constitute only 10%–20% of epithelial cancer biopsy specimens (the remainder being normal connective tissue or stroma). For these reasons immunohistochemistry using mAb of known specificity has been widely used to define the distribution of cancer antigens, including carbohydrate cancer antigens. This is performed on frozen sections when glycolipids are the target, so as to prevent glycolipid loss during paraffin imbedding.

We have used panels of mAb against carbohydrate antigens to screen a variety of malignant and normal tissues by immunohistochemistry [45, 67]. In general, ganglioside antigens and blood-group-related antigens had very different distributions on various malignancies. The antigens strongly expressed on 60% or more of biopsy specimens are listed in Table 1.

Expression of gangliosides on normal tissues was also very different from that of the blood-group-related antigens (see Table 2), but consistent with the expression on tumors summarized in Table 1. GM2, GD2 and GD3 were all expressed on brain cells, especially GD2, which is also expressed on some peripheral nerves [72]. Unexpectedly, GD2 was found to be expressed on B lymphocytes in the spleen and lymph nodes and GM2 was expressed at the secretory borders of most epithelial tissues. GD2 and GD3 were also expressed, though at lower levels, in connective tissues of multiple organs and GD3 is known to be expressed on a subset of human T lymphocytes [73]. Fucosyl GM1 was expressed only on occasional cells in the islets of Langerhans and in some sensory neurons in the dorsal root ganglia. Polysialic acid was expressed significantly in brain and some bronchial epithelial cells. GloboH and the blood-group-related antigens were expressed exclusively at the secretory borders of a variety of epithelial tissues, except that Le^x and sialyl Le^x were expressed in addition on polymorphonuclear leukocytes and sialyl Tn (sTn) was found on Leydig cells of the testis.

There is now sufficient experience from clinical trials with vaccine-induced antibody responses against GM2, GD2, TF and sTn antigens, and passive administration of mAb against GD2, GD3, Le^x and sTn to draw conclusions about the consequences of antigen distribution on various normal tissues. GM2, GD2 and GD3 exposure on cells in the brain [13, 15, 28, 66] and GM2, sTn and TF antigen expression in cells at the secretory borders of epithelial tissues [43, 45, 67–69] induce neither immunological tolerance nor autoimmunity once antibodies are present, suggesting they are sequestered from the immune system. Treatment with IgG mAb against GD2 (3F8) and GD3 (R24) has not induced central nervous system toxicity in children or adults [34, 36, 37]. Against this background, GM2, GD3, polysialic acid, T, Tn, sTn, GloboH and sialyl Le^a all appear to be good targets for active immunotherapy with vaccines. The known expression of Le^x and sialyl Le^x on polymorphonucleocytes, and the granulocytopenia seen after treatment of patients with mAb FC-2.15, later found to recognize Le^x [70, 71], may exclude these two carbohydrates as candidates for vaccine construction. Though the neutropenia following each mAb administration was short-lived and did not interfere with treatment, induction of Le^x antibodies lasting 1 year or more by vaccine administration would be more worrying. In addition, the consequences of antibodies against antigens expressed on B cells, islets of Langerhans and sensory neurons are of concern and largely unknown. Moderate titers of IgM (natural or vaccine-induced) against GD2 have not been associated with toxicity [4, 31, 37], but administration of high doses of some (but not other) IgG mAb against GD2 have been associated with peripheral neuropathy in melanoma patients [38]. Ongoing trials with vaccines against GD2 and fucosyl GM1 at our center will address these questions more directly.

The basis for vaccines that induce antibodies

Antibodies are the primary mechanism for elimination of pathogens from the blood stream. They are ideally suited for elimination of circulating tumor cells and micrometastases. The importance of antibodies in mediating protection from tumor recurrence is well documented in experimental animals (reviewed in [15]). Experiments involving administration of monoclonal antibodies (mAb) against melanoma glycolipid antigens, such as the ganglioside GD2, have been particularly informative (Zhang H., Zhang S. et al., manuscript in preparation). When 250 μg mAb 3F8 (which recognizes GD2) is administered prior to intravenous tumor challenge with the GD2-expressing syngeneic lymphoma EL4, no tumors result and most mice remain disease-free. Significant protection is also seen when the antibody is administered for treatment of micrometastases as long as 2–4 days after tumor challenge. This timing may be comparable to antibody induction in the adjuvant setting (after surgical resection of primary malignancies or lymph node metastases) in humans, since in both cases the targets are circulating tumor cells and micrometastases. Administration of any dose of 3F8 after the first week has little impact on tumor progression.

Table 1 Carbohydrate antigens expressed on tumor biopsies as detected by immunohistochemistry. *Le* Lewis antigen, *sTn* sialyl-Tn

Tumor	Antigens (mAb)
Melanoma	GM2 (696), GD2 (3F8), GD3 (R24)
Neuroblastoma	GM2 (696), GD2 (3F8), GD3 (R24), polysialic acid (735)
Sarcoma	GM2 (696), GD2 (3F8)
B cell lymphoma	GM2 (696), GD2 (3F8)
Small-cell lung cancer	GM2 (696), fucosyl GM1 (F12), polysialic acid (735), globoH (MBr1), sialyl Le^a (19.9)
Breast	GM2 (969), GloboH (MBr1), TF (49H.8)
Prostate	GM2 (696), Tn (1E3), sTn (CC49), TF (49H.8), Le^y (S193)
Lung	GM2 (696), GloboH (MBr1), Le^y (S193), sialyl Le^x (Cslex-1)
Colon	GM2 (696), sTn (CC49, B72.3), TF (49H.8), sialyl Le^a (19.9), Le^y (S193), sialyl Le^x (Cslex-1)
Ovary	GM2 (696), GloboH (MBr1), sTn (CC49, B72.3), TF (49H.8), Le^y (S193)
Stomach	GM2 (696), Le^x (SH1), Le^y (S193), Le^a (T-174), sialyl Le^a (19.9)

There is also evidence in cancer patients that natural or passively administered antibodies are associated with a more favorable prognosis. (i) Paraneoplastic syndromes have been associated with high titers of natural (not induced by vaccine or passive administration) antibodies against onconeural antigens expressed on neurons and certain malignant cells. The antibodies are apparently induced by tumor growth and have been associated with autoimmune neurological disorders and also with delayed tumor progression and prolonged survival [16, 17]. (ii) Patients with AJCC stage III melanoma and natural antibodies against GM2 ganglioside, treated at two different medical centers, have an 80%–90% 5-year survival compared to the expected 40% rate, as shown in Fig. 2 [12, 18]. (iii) Patients with small-cell lung cancer and natural antibodies against small-cell lung cancer had prolonged survival compared to antibody negative patients [19]. (iv) Patients with Dukes C colon cancer, treated with monoclonal antibody 17-1A in the adjuvant setting, had a significantly prolonged disease-free and overall survival compared to randomized controls [20].

Mechanisms of antibody action

On the basis of studies of bacterial infections, the mechanism of protection by antibodies is probably complement-mediated attack and lysis, and possibly antibody-dependent cell-mediated cytotoxicity of tumor cells, with cell-surface antigens as targets. In some cases, antibody may also have a direct effect, for example by inhibiting tumor cell attachment or growth hormone receptor. In general, however, the interaction of antibody and antigen is with out significance unless Fc-mediated secondary effector mechanisms are activated. Activation of the complement system is the most important of the effector mechanisms. Antigen-bound IgM is the most active complement activator in the intravascular space while IgG1 or -3 is the most important complement activator extravascularly. This complement activation at the cell surface mediates inflammatory reactions, opsonization for phagocytosis, clearance of antigen-antibody complexes from the circulation, and membrane-attack-complex-mediated lysis. Fc receptors on IgG1 and IgG3 are also the primary targets for effector cells mediating antibody-dependent cell-mediated cytotoxicity of tumor cells. FCδRI (CD64), FCδRII (CD32), and FCδRIII (CD16) receptors on a range of effector cells, including especially NK cells, but also T lymphocytes and cells of myeloid lineage, react with these tumor-cell-bound antibodies, resulting in activation of inherent cytotoxic mechanisms in the effector cells.

If antibodies of sufficient titer can be induced against one or several cell surface antigens to eliminate tumor cells from the blood and lymphatic systems and to eradicate micrometastases (as demonstrated in the mice), this would dramatically change our approach to treating cancer patients. With repeated showers of metastases no longer possible, as a consequence of high levels of circulating antibodies, aggressive local therapies of established metastases, including surgery and intralesional treatments, might result in long-term control of even metastatic cancers. It is also possible that complement-mediated inflammation, improved antigen presentation by specifically immune B lymphocytes, and decreased circulating tumor antigen may facilitate T lymphocyte immunity, as has been described in other systems [21, 22]. For instance, B lymphocyte tolerance against murine cytochrome *c* in the mouse can be broken by immunization with human cytochrome *c* (xenogenization). The resulting immune B lymphocytes can then induce cytochrome-*c*-reactive T lymphocytes when administered to tolerant mice with cytochrome *c* [21]. Similar results have been described with insulin-dependent diabetes mellitus in the mouse [22] and T lymphocyte autoreactivity in the rat [74], specifically immune B lymphocytes are able to be sufficiently potent antigen-presenting cells to induce T lymphocyte activation and the breaking of tolerance.

The basis for focusing on carbohydrate antigens

Of the many well-defined bacterial antigens studied as targets for vaccine therapy, carbohydrate antigens have proven the most clinically relevant. Antibodies against capsular polysaccharides on *Neisseria meningitidis*, *Streptococcus pneumonia* and *Haemophilus influenzae* type b

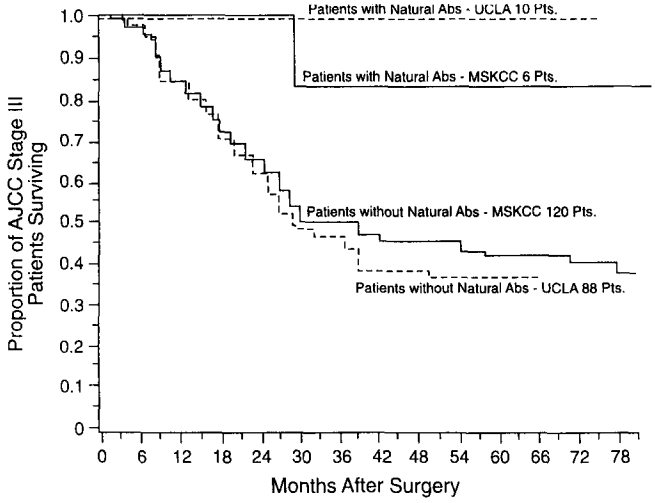

Fig. 2 Correlation between the presence of natural serum antibodies and survival in AJCC stage III melanoma patients subsequently treated with tumor vaccines at Memorial Sloan-Kettering Cancer Center (MSKCC) [18] or UCLA School of Medicine (UCLA) [12]

have been shown to correlate with protection from subsequent bacterial challenge [23–25] and vaccines containing these purified carbohydrate antigens have been shown to be protective. With regard to human cancer antigens, carbohydrate antigens have also proven to be unexpectedly potent targets for immune recognition and attack, because of both their abundance at the cell surface and their immunogenicity.

While occasional cancer cells express as many as 10^6 molecules of protein cancer antigens, such as epidermal growth factor receptor and HER2neu, at the surface of each cancer cell [26, 27], the median number of carbohydrate epitopes is often much greater. Thus the median number of molecules of GM2 or GD2 on melanoma cells in biopsy specimens is over 10^7 and for sarcomas or neuroblastomas it is over 5×10^7 [28–30]. The median number of GD3 molecules expressed on melanoma cells is also about 5×10^7.

In a series of studies, we have immunized 110 patients with a number of melanoma cell or melanoma cell lysate vaccines mixed with various adjuvants, and analyzed the serological responses obtained (reviewed in [1, 2, 4]). Eleven of these patients mounted serological responses against melanoma antigens on autologous and allogeneic melanoma cells as a consequence of the immunizations. The only antigens recognized by more than one patient were the gangliosides GM2 and GD2. Tai et al. [31] also found GM2 and GD2 to be particularly immunogenic. They showed that 10 of 26 patients vaccinated with a mix of irradiated allogeneic melanoma cell lines produced detectable IgM antibodies against GM2 and 2 patients produced antibodies against GD2. Gangliosides are acidic glycosphingolipids that are overexpressed at the cell surface of melanomas, sarcomas and other tumors of neuroectodermal origin [28, 30]. Gangliosides have also been shown to be effective targets for passive immunotherapy with monoclonal antibodies. Major clinical responses have been seen following treatment of patients with monoclonal antibodies against GM2, GD2 and GD3 [32–38].

Another class of carbohydrates that has received attention as tumor antigens are the Thomsen-Friedenreich antigen (TF), Tn and sialylated Tn (sTn) blood-group-related antigens that are expressed on mucins in a variety of epithelial cancers. sTn expression by various epithelial cancers correlates with a more aggressive phenotype and a more ominous prognosis [39]. The administration of radiolabelled B72.3 monoclonal antibody against sTn has resulted in good localization of even small tumors [40]. Immunization with Tn and sTn vaccines protects mice from subsequent challenge with syngeneic cancer cells expressing these antigens [41, 42]. Antibodies against TF and sTn are naturally present in some human sera and these titers have been increased by vaccination. The resulting antibodies correlate with a more favorable prognosis [43]. Hence, both active and passive immunotherapy trials have identified gangliosides and the blood-group-related carbohydrate antigens as uniquely effective targets for cancer immunotherapy.

There are a variety of other carbohydrate antigens which may be suitable tumor antigens for cancer immunotherapy as well. These include fucosyl-GM1, which is a ganglioside expressed on small-cell lung cancers, polysialic acid chains of eight or more sialic acids characteristic of "embryonal intercellular adhesion molecule (ICAM)" and expressed on small-cell lung cancers and neuroblastomas, and the Lewis Y and Globo H antigens, which are expressed on a variety of epithelial cancers [44–50]. The restricted distribution of these antigens on normal tissues and their extensive expression on some malignancies suggests that these carbohydrate antigens should also be good targets for immunotherapy.

There are advantages and disadvantages that result from the use of carbohydrate antigens as targets for active immunotherapy of cancer with vaccines. Carbohydrates are generally categorized as T-lymphocyte-independent antigens, meaning that they are not recognized by T lymphocytes, which consequently do not provide help (cytokines) to the B lymphocytes. Whether this is invariably true is a murky issue at the moment. Clearly T lymphocytes with $\gamma\delta$ receptors can recognize non-peptide antigens [51], and T lymphocytes with standard $\alpha\beta$ receptors can recognize carbohydrates on short peptides [52–54]. However, it has not yet been possible to use these findings to construct vaccines aimed at inducing T lymphocyte immunity against carbohydrate cancer antigens, because it is not known to which amino acids on which peptides any given carbohydrate cancer epitope is linked. In any case, immunization with carbohydrate cancer antigens does not usually result in detectable T lymphocyte help for the B lymphocyte response. Thus, relatively low-titer, low-affinity antibodies follow immunization with carbohydrate antigens in purified form or expressed on tumor cells, and these antibodies remain predominantly IgM despite repeated vaccinations. Delayed hypersensitivity responses, cytokine release and cytotoxic T lymphocyte responses do

Table 2 Carbohydrate antigens expressed on normal tissues as detected by immunohistology

Antigen (mAb)	Normal tissues
GM2 (696)	Brain grey matter, most epithelial tissues
GD2 (3F8)	Brain, splenic white pulp, lymph node germinal center, connective tissue stroma, uterus smooth muscle
GD3 (R24)	Brain, connective tissue stroma
Polysialic acid (735)	Brain grey matter, lung epithelia and pneumocytes, colon capillary endothelial cells and ganglions
Fucosyl GM1 (F12)	Islet cells of pancreas, dorsal root sensory neurons
Le^y (S193), Le^a (T-174), sialyl Le^a (19.9)	Many epithelial tissues
Le^x (SH1), sialyl Le^x (Cslex-1)	Many epithelial tissues, splenic red pulp polymorphonucleocytes
Tn (1E3)	Occasional epithelial cells of stomach and ovary
sTn (B72.3)	Occasional epithelial cells of stomach and ovary, Leydig cells of testis
TF (49H.8), Globo H (MBrl)	Many epithelial tissues

Table 3 Carbohydrates as cell surface targets: misuse by parasites. *GP* Glycoprotein, *GSL* glycosphingolipid

Target	Receptor	Target organ
NANA (α2-6) Gal: GP	Influenza type A virus	Respiratory
NANA (α2-3) Gal: GP	Influenza type B virus	Respiratory
Poly mannose complex: GP	*Escherichia coli* 1	Urinary tract
Gal (β1-4) Gal: GSL	*E. coli* P	Urinary tract
NANA (α2-3) Gal (β1-3) GalNAc: GSL	*E. coli* S	Neural
NANA (α2-8)-: GSL	*E.coli* CFA/1	Intestine
Gal (β1-4) Glcβ: GSL	*Neisseria gonorrhea*	Genital
GlcNAc (β1-3) Gal: GP	*Streptococcus pneumonia*	Respiratory
Gal (β1-4) Glc: GSL	*Candida albicans*	Skin, mucosa
Gal (β1-4) GlcNAc: GP	*Entamoeba histolytica*	Intestine
Man-6-P: GP	*Giardia lamblia*	Intestine
Le^b and NANA: GLS/GP	*Helicobacter pylori*	Stomach
GM1: GSL	Cholera toxin	Intestine

not occur. Conjugate vaccines, in which the carbohydrate antigen is covalently attached to an immunogenic carrier protein, are able to overcome the lack of T lymphocyte help and can induce higher-titer IgM antibodies and partial class-switching to IgG antibodies. This occurs when T lymphocytes are activated by the protein carrier and secrete a variety of cytokines which the bystander anti-carbohydrate B lymphocytes require for optimal activation. These conjugate vaccines are not able, however, to induce helper or cytotoxic T lymphocyte activation against the carbohydrate antigens. The general inability of carbohydrate-based vaccines to induce T cell immunity, and consequently their probable inability to alter the course of well-established cancers, is the main limitation in selecting carbohydrate antigens as targets for immunotherapy of cancer.

There are, however, a number of advantages to using cell-surface carbohydrate antigens as targets for immunotherapy of cancer with vaccines, especially in the adjuvant setting. These include: (i) their abundance at the tumor cell surface; (ii) their immunogenicity (at least in terms of antibody responses) with properly constructed conjugate vaccines; (iii) the fact that antibodies (especially antibodies mediating complement lysis) are ideally suited for tumor eradication in the adjuvant setting where the target is micrometastases and circulating tumor cells; (iv) carbohydrates having been found to be uniquely effective targets for active and passive immunotherapy of cancer and (v) our ability to isolate or synthesize these carbohydrate antigens, facilitating vaccine construction. Another advantage is that many carbohydrates play important roles in intracellular interactions as targets for selectins and adhesins, which may be crucial, not discretionary, to tumor cell survival and the metastatic process. It may be possible to construct vaccines against carbohydrate antigens that induce antibodies capable of interfering with these processes.

Biological roles of cell surface carbohydrates

Although the great majority of cell-surface proteins are glycosylated, the functional role of the carbohydrate moieties has, until recently, remained uncertain. Glycosylated lipids (glycosphingolipids) are also well represented in the plasma membrane of cells. Traditionally, the oligosaccharide components of glycoproteins have been considered to have structural, protective and stabilizing roles (reviewed in [56–58]). Glycoproteins and glycolipids have also been recognized as the receptors for noxious agents such as bacteria and their toxins and viruses (Table 3). More recently it has been demonstrated that carbohydrate structures play more physiological roles in the cell and in cell/cell and cell/matrix interactions (Table 4). The first of these functions to be discovered was the effect of desialylation on

Table 4 Carbohydrates as cell surface targets: physiologic uses

Target	Receptor	Purpose
Cell-Cell Interactions		
$3SO_4GlcA$-: GSL/GP	HNK-1 Receptor	Neural cell adhesion
SSEA-1 (Le^x) GSL/GP	Le^x	Embryogenesis, compaction
Sialylated or sulfated Le^x or Le^a: GP	E, L, or P selectins	Lymphocyte trafficking
NANA (2-6) Gal: GP	CD22	Lymphocyte adhesion
Hyaluronate: GP	CD44	Adhesion (many cell types)
Specific sugars: GSL/GP	Glycosyl transferases	Sperm adhesion
Clearance form the blood		
Desialylated serum GPs	Hepatocyte asialo GP receptor	Clearance by liver
Mannosyl glycoproteins (GPs)	Macrophage mannose receptor	Phagocytosis

the clearance of plasma glycoproteins from the blood via the hepatocyte asialoglycoprotein receptor [59]. More recently, with recognition of carbohydrate structures as ligands for selectins and sialoadhesions, appreciation of the biological function of carbohydrates has been extended to their roles in cell/cell and cell/matrix interactions. In the case of selectins, which are involved in leukocyte/endothelium or platelet/endothelium interactions, the minimal recognized structures are sialylated (or sulfated) Le^x and Le^a oligosaccharides [60]. Sialoadhesins, on the other hand, are more widely expressed and recognize 2,6- or 2,3-linked sialic-acid-containing structures [61]. The possible involvement of these and other carbohydrate structures in the metastatic process [62] provides a rationale for the enhanced expression of some carbohydrate epitopes on cancer cells [44]. In contrast to the emerging picture of the role of oligosaccharides on glycoproteins, there is much less information on the role of cell-surface glycolipids. Nevertheless, evidence is accumulating that glycolipids may also play important roles in cell/cell interactions, cell proliferation and metastasis [63–65]. Again, the altered expression of glycolipids on tumors may be a reflection of their role in tumor cell behavior.

In conclusion

Carbohydrate antigens are the most abundant antigens expressed at the cancer cell surface and have been shown to be uniquely effective targets for immune recognition and attack. The basis for cancer vaccines that primarily induce an antibody response, such as vaccines against these carbohydrate antigens, is now well established in both experimental models and the clinical setting. In both cases, antibody administration or induction has been especially effective in the adjuvant setting when the targets are circulating tumor cells and micrometastases. The patterns of carbohydrate antigens expressed by different tumor types has been established, paving the way for polyvalent-antibody-inducing vaccines. This then forms the rationale for the construction and testing of carbohydrate vaccines against cancer, the focus of the second part of this review.

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