Enhanced Immune Recognition of Cryptic Glycan Markers in Human Tumors

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Abstract

Abnormal glycosylation is one of the hallmarks of the cancer cell and is associated with tumor invasion and metastasis. The development of tumor-associated carbohydrate antigen (TACA) vaccines has been problematic due to poor immunogenicity. However, when appropriate targets can be identified, passive immunization with monoclonal antibodies (mAbs) directed against TACAs has been shown to have antitumor activity. Fas ligand (FasL) is a transmembrane protein that induces apoptosis in cells expressing its receptor, Fas. When grafted into mice, FasL-expressing tumor cells break immunologic tolerance to self-antigens and induce antibody-mediated tumor immunity. Here, five IgM mAbs were produced from mice vaccinated with FasL-expressing B16F10 mouse melanoma cells. They recognize various syngeneic and allogeneic murine tumor cell lines. One mAb, TM10, recognizes a range of human tumor cell lines, including melanoma, prostate, and ovarian cancer. It does not bind to untransformed cells. The epitopes recognized by all the mAbs were carbohydrates expressed on proteins. Using carbohydrate microarrays, the antigenic targets of TM10 were found to be high-mannose core structures of N-linked glycans. In normal cells, high-mannose clusters are hidden by extensive saccharide branching but they become exposed in cancer cells as a result of abnormal glycosylation pathways. Vaccination with FasL-expressing tumors therefore enables the immune system to break tolerance to self-antigens, allowing identification of novel TACAs that can form the basis of future humoral anticancer therapy.


Introduction

Fas ligand (FasL; CD95L) is a transmembrane protein that induces apoptosis in cells bearing its receptor, Fas (CD95; refs. 1, 2). Fas is expressed on almost all human tumor cells (3) and so the FasL/Fas pathway is an attractive target in cancer immunotherapy. However, the systemic administration of FasL is not feasible due to unacceptable hepatotoxicity (3), consequently so other approaches have been explored. Perhaps the most interesting finding among these is that when grafted into mice, tumor cells forced to overexpress FasL are rapidly rejected, and this is followed by the development of a protective, antibody-mediated tumor immunity (4–6).

Approximately 80% of cell surface proteins and 5% of lipids are glycosylated (7). Altered patterns of carbohydrate expression are one of the hallmarks of the cancer cell and changes include overexpression and underexpression of naturally occurring glycans, abnormal branching of glycoproteins and glycolipids, and neoexpression of sugars normally restricted to embryonic tissue (8, 9). Abnormal glycosylation is associated with tumor cell invasion, metastasis, and angiogenesis (10, 11). Over 50% of cancers are known to express the tumor-associated carbohydrate antigens (TACA) described thus far, which include glycolipids (e.g., GM1, GM2, GD2, and GD3), Lewis antigens (such as Lea, Leb, and Lex), and Thomsen-Friedenreich antigen (12).

In this article, we report on the generation of a panel of new monoclonal antibodies (mAb) from tumor-immune mice following vaccination with FasL-expressing mouse melanoma cells. One mAb, termed TM10, recognizes a broad range of both murine and human tumor cells. Its carbohydrate epitope, high-mannose core structures of N-linked glycans, is a novel tumor antigen and is described here for the first time.

Materials and Methods

Cells and mice. Murine tumor cell lines used include B16F10 melanoma (syngeneic with the C57BL/6 mouse strain), K1735 melanoma (C3H), NS1 myeloma (BALB/c), MC57 fibrosarcoma (C57BL/6), CT26 colon carcinoma (BALB/c), methylcholanthrene (MCA)-transformed L-cell fibroblasts (C3H), P815 mastocytoma (DBA/2), and GM95 (ceramide-specific glucosyltransferase-deficient cells derived from B16F10 melanoma). FasL-expressing B16F10 cells (B16FasL) were generated as described (6). Human cells used were primary prostate fibroblasts, dermal fibroblasts, 293T cells, melanoma (Trombelli and MM5), prostate (PC3, DU145, and LNCaP), breast (ZR75.1 and MDA-MB-468), and ovarian cancer cell lines (PEA-1, PEO-1, and SK-OV-3), kind gifts from Professor Jonathan Waxman, Dr. Tahereh Kalamati, Professor Charles Coombes, and Professor Hani Gabra (Imperial College, London, United Kingdom). Female C57BL/6 mice, aged 5 to 7 wk, were purchased from Harlan and housed at the Central Biomedical Services of Imperial College London. Nonhybridoma cells were cultured in RPMI 1640 supplemented with 10% heat-inactivated FCS, whereas hybridoma cells were supplemented with 20% batch-tested heat-inactivated FCS. Media were supplemented with 2 mmol/L L-glutamine, 100 IU/mL penicillin, 100 μg/mL streptomycin, and, in the case of murine cells, 50 μmol/L 2-mercaptoethanol, 10 mmol/L HEPES, and 1% sodium pyruvate. All cells were incubated at 37°C with 5% CO2.

Generation and purification of mAbs. Murine hybridomas were generated by the fusion of splenocytes and myeloma cells as previously described (6). Briefly, 1 × 107 irradiated B16FasL melanoma cells were injected s.c. into female C57BL/6 mice, which were then challenged three...
times, at monthly intervals, with 5 × 10^7 B16F10 cells. Antitumor cell antibody production was confirmed in mice that rejected these tumor challenges (“protected mice”) by staining of B16F10 cells with 1:50 diluted serum, the minimum concentration previously determined by titration to provide optimal staining. Splenocytes from protected mice with a positive antitumor cell antibody response were fused to NS1 murine myeloma cells using polyethylene glycol 1500 (Roche Diagnostics). Hybridomas were selected by culture in HAT (hypoxanthine, aminopterin, thymidine)–containing medium and then screened by incubating their murit cell culture supernatant with B16F10 followed by anti-mouse Ig PE (Dako). Positive staining hybridomas were single cell cloned three times and the class and subclass of each mAb were determined using isorops (Roche Diagnostics).

Cell culture supernatants from hybridoma colonies were purified over a protein L (Sigma-Aldrich) column, eluted with 0.1 mol/L glycine (pH 2.5), and dialyzed in sterile PBS. mAb concentration was measured by spectrophotometer (280 nm absorbance). mAbs were titrated and used at 10 μg/ml in the microarray and 20 μg/ml in all other experiments.

Flow cytometry. Fc receptors on murine cells were blocked with rat anti-mouse CD16/32 (eBioscience) and human cells blocked with human serum (Life Technologies). In some experiments, cells were fixed with 2% formaldehyde and then permeabilized using 0.5% saponin (Sigma-Aldrich). Secondary antibodies used were anti-mouse IgM FITC (Sigma-Aldrich) or anti-mouse IgG PE (Dako). A minimum of 2 × 10^6 cells was analyzed per sample.

Immunohistochemistry. B16F10 cells were grown to confluence on 15-mm glass coverslips and then fixed with 1% formaldehyde. Cells were blocked with 1:200 goat serum and, where indicated, permeabilized with 0.5% Triton X-100 (Sigma-Aldrich). They were incubated with mAbs (20 μg/ml) and then anti-mouse IgM Alexa Fluor 568 (Molecular Probes, Invitrogen) or anti-mouse IgG FITC. Cells were mounted onto glass slides with Vectashield/DAPI (Vector Laboratories) and examined by confocal fluorescence microscopy using a ×63 objective (Zeiss LSM10).

Immunoprecipitation. Native or biotinylated (Biotin EZ-Link, Pierce) B16F10 cells (1 × 10^7) were lysed in 1 mL ice-cold NP40 lysis buffer and, for biotinylated samples, 0.5% Mega-9 (Sigma-Aldrich). Samples were preclearced with protein L (Sigma-Aldrich), blocked with 10% bovine serum albumin (BSA), and then incubated with 20 μg/ml TM10 followed by 100 μL of 50% protein L. The immunoprecipitates were run on 12% gels using SDS-PAGE and developed with silver staining (Amersham Biosciences) or analyzed by Western blot.

Western blot. B16F10 cells (1 × 10^7) were lysed in ice-cold NP40 lysis buffer, and spun supernatants were separated on 12% gels using SDS-PAGE and then transferred onto Hybond-C Extra nitrocellulose membrane (Amersham Biosciences). After being blocked with 5% nonfat dry milk/PBS, the membrane was incubated with 20 μg/ml TM10 and then with anti-mouse IgG horseradish peroxidase (HRP; Sigma-Aldrich). HRP was detected using enhanced chemiluminescence Western blotting kit (Amersham Biosciences).

Glycosylation inhibitors, competitors, and lectins. B16F10 cells were incubated at 37°C for 24 h with 200 ng/ml tunicamycin (Sigma-Aldrich) or for 72 h with 1 mmol/L N-butyl-deoxyxojirimycin (N-butyl-DNJ) or N-butyl-deoxy-galactonojirimycin (N-butyl-DG); Toronto Research Chemicals. Alternatively, 293T cells or peripheral blood lymphocytes (PBL) were incubated overnight with 5 μmol/L kifunensine (a gift from Dr. Veronica Chang, Institute of Molecular Medicine, Oxford, United Kingdom).

Lectins, with or without conjugation to FITC, were derived from Sambucus nigra agglutinin (SNA), Maackia amurensis agglutinin (MAA), concanavalin A (ConA), Phaseolus vulgaris L. (PHA-L), and Galanthus nivalis agglutinin (GNA) (all Sigma-Aldrich), or D(+)-galactose, D(+)-glucose, N-acetylglucosamine (GlcNAc), N-acetylgalactosamine (GalNAc), and mannose-6-phosphate (all Sigma-Aldrich) were used at 1 mg/ml. In some experiments, preincubation with lectins or saccharides was used to inhibit binding of mAbs. In other experiments, preincubation with the mAbs was used to inhibit the binding of the lectins or saccharides.

Carbohydrate microarrays. Details of the protocol for the construction of carbohydrate microarrays have been previously published (13). Briefly, carbohydrate and lipid antigens were printed in triplicate onto microglass slides using a robotic array spotter. Lipids were used at 0.02–2.0 μg/ml and carbohydrates at 0.5 to 1.0 μg/ml. A minimum concentration and at a 1:5 dilution. Antibodies to murine IgM were also printed at given concentrations to serve as standard curves. The printed slides were blocked with BSA and incubated with the relevant mAb (10 μg/ml) and then anti-mouse IgG or IgM FITC. Analysis was performed using ScanArray Express Microarray Scanner (Perkin-Elmer Life Science). Fluorescence intensity values for each spot were calculated with QuantArray software (Perkin-Elmer).

Results

Generation of mAbs from B16Fasl-vaccinated mice. Fasl-expressing tumor cells induce development of antibody-mediated tumor immunity (4–6). mAbs were therefore produced from mice vaccinated with FasL-expressing B16F10 cells (B16FasL) by the fusion of their splenocytes and NS1 myeloma cells. In total, five monoclonal IgMs (TM3, TM5, TM6, TM10, and TM12) were produced, all of which showed cell surface binding to B16F10 (Fig. 1, top row). The mAbs stained both B16FasL and B16F10 equally, implying that they are not directed against either FasL or green fluorescent protein (Fig. 1, bottom row).

mAbs recognize a range of murine and human tumors. The mAbs generated recognized both syngeneic (MC57 fibrosarcoma from a C57BL/6 background) and allogeneic (K1735 melanoma and MCA-transformed L cells, both C3H background) murine tumor cells (Fig. 2). Only one mAb (TM12) recognized P815 mastocytoma, and there was no significant staining of CT26 colon carcinoma by any of the mAbs. The latter negative results rule out the possibility that the mAbs are just recognizing FCS in the culture medium or MHC antigens on the tumor cells. There was no staining of B16F10 or B16Fasl with irrelevant murine IgM antibodies or with conjugated secondary antibodies alone (data not shown).

Because our mAbs recognized a range of murine tumor cells and because human melanoma-associated antigens can be highly homologous to their murine counterparts (14), binding of the mAbs to human tumor cells was investigated. Two mAbs, TM10 and TM12, recognized a range of human cancer cell lines (Fig. 3). TM10 stained human melanoma (Trombelli and MM5), prostate (PC3, LNCaP, and DU145), ovarian (PEA-1, PEO-1, and SK-OV-3) and, to a lesser extent, breast cancer lines (MDA-MB-468 and ZR75.1). TM12 stained one prostate cancer cell line (PC3).

TM10 binds to the intracellular compartment, but not surface, of untransformed cells. Given the broad range of recognition of both murine and human tumor cells, the mAb TM10 was selected for further investigation. Using indirect immunofluorescence, TM10 was found to have a punctate surface staining pattern of B16F10 cells (Fig. 4A). At flow cytometry, there was no cell surface binding of TM10 to a range of untransformed cells, including murine splenocytes and human PBLs (Fig. 4B), prostatic fibroblasts, and dermal fibroblasts (data not shown). However, when permeabilized, there was now strong intracellular staining of both normal and cancer cells (Fig. 4A and B). This suggests that all cells have an intracellular reserve of the epitope of the antibody but is only tumor cells that express it on their surface. There was no staining of any cells by isotype control antibody using direct immunofluorescence or flow cytometry.

TM10 recognizes a carbohydrate, not protein, epitope. A protein epitope for our mAbs was initially sought. However, Western blots and immunoprecipitations from native, surface-biotinylated, or [35S]methionine-labeled B16F10 repeatedly revealed multiple protein bands (Supplementary Fig. S1). This raised
the possibility that our mAbs were in fact recognizing sugars expressed on more than one glycoprotein or glycolipid. Experiments using glycosylation inhibitors supported this. Tunicamycin, a mixture of homologous nucleoside antibiotics, is an inhibitor of N-glycoprotein synthesis (Supplementary Fig. S2). When B16F10 cells were grown in the presence of tunicamycin, there was a reduction in staining by all the mAbs except TM6 (Fig. 5A, top row). The imino sugar N-butyl-deoxynojirimycin (N-butyl-DNJ) is a nontoxic inhibitor of α-glucosidase and prevents N-glycosylation one step downstream of the effects of tunicamycin (Supplementary Fig. S2). There was reduced binding of all mAbs (except TM5) when B16F10 cells were pretreated with N-butyl-DNJ (Fig. 5A, bottom row). As controls, tunicamycin also inhibited the binding of the lectin ConA that binds preferentially to mannose, and N-butyl-DNJ reduced binding of MAA, a lectin that preferentially recognizes sialic acid, which is one of the residues found on N-linked structures.

The carbohydrates recognized by the mAbs seemed to be restricted to glycoproteins and are not expressed on glycolipids. Incubation of B16F10 with another imino sugar, N-butyl-deoxygalactonojirimycin (N-butyl-DGJ), which inhibits ceramide-specific glucosyltransferase and so glycolipid but not glycoprotein formation,
had no effect on mAb binding (data not shown). Furthermore, GM95, a B16F10-derived cell line that has reduced levels of ceramide-specific glucosyltransferase and so impaired glycolipid expression, showed the same level of surface staining by all the mAbs when compared with B16F10 (data not shown).

**The epitope for TM10 is a high-mannose cluster.** With results pointing strongly toward carbohydrate epitopes, we screened our mAbs on a microarray of carbohydrates and glycolipids. TM10 bound strongly to two of these antigens (Fig. 5B and C), both high-mannose clusters, displayed on the array as (Man9)n, which represents the mannose core of N-glycoproteins, and [(Man9)4]n, which mimics the mannose clusters displayed by the gp120 glycoprotein of HIV-1 (15, 16). Both mannose clusters were bound to the carrier keyhole limpet hemocyanin (KLH) but there was only weak binding of TM10 to KLH alone (Fig. 5B). Indeed, when the mean fluorescent intensities (MFI) are compared at 0.1 μg/mL, (Man9)n-KLH and [(Man9)4]n-KLH gave a 14- and 11-fold increase in signal compared with that of KLH alone (Fig. 5C). It remains possible that the TM10 mAb may also have low affinity for some sugar epitopes on KLH. TM10 did not bind to any other antigens on the array, and there was no significant binding to the array of any of the other mAbs screened (data not shown).

To confirm the array finding of a high-mannose cluster epitope for TM10, we used the α-mannosidase inhibitor kifunensine, which prevents normal glycoprotein synthesis and leads to an accumulation of Man9 complexes (Supplementary Fig. S2). Treatment of 293T cells with kifunensine increased the binding of TM10, confirming that the antibody is recognizing mannose clusters (Fig. 6A). As expected, the expression of sialic acid residues, detected through binding of MAA, was reduced in the presence of kifunensine. Inhibition experiments using D(+)-mannose, D(+)-galactose, D(+)-fucose, GlcNAc, GluNAc, or mannose-6-phosphate to reduce binding of TM10 to B16F10 were negative (data not shown), suggesting that the antibody is recognizing a more complex structure than just these simple saccharide units.

Several different lectins known to bind to mannose-based carbohydrates were used to stain B16F10 and competitively inhibit TM10 binding. SNA (binding preferentially to sialic acid residues attached to terminal galactose), GNA (preferential binding to α1,3-mannose), and PHA-L (specific for Galβ1-4 GlcNAc structures) all stained B16F10, indicating that mannose-based epitopes are present on the cell surface (Fig. 6B; data not shown). However, there was no inhibition of lectin binding when cells were preincubated with TM10. Furthermore, when B16F10 cells were preincubated with lectins, there was no abrogation, but instead an increase, in TM10 binding. Taken together, these findings suggest that the epitopes recognized by TM10 and the lectins differ but that the target for TM10 may also be expressed on the lectins themselves. Lectins are glycoproteins, and SNA, for example, contains 7.8% carbohydrates, principally mannose and glucosamine (17).

**Discussion**

We have previously shown that FasL expressed on B16F10 murine melanoma leads to the development of antibody-mediated tumor immunity (6). To investigate this humoral response further,
mAbs were produced from tumor immune mice that had been vaccinated with FasL-expressing melanoma cells (B16FasL). In total, five monoclonal IgMs were generated, all of which bound to the cell surface of both B16FasL and B16F10. One mAb, TM10, binds to both human and murine tumors and its epitope is a novel carbohydrate tumor antigen, high-mannose clusters. The humoral nature of FasL-associated tumor immunity is important, as antibody immunotherapy represents one of the success stories of immunology and has become established as proven treatment in the fields of oncology and hematology.

It is known that vaccination with whole-tumor cells induces an IgM predominant response directed against repetitive carbohydrate structures on the cell surface (18). The relative absence of an IgG response is because the T-cell help required for an isotype switch from IgM to IgG production is missing because carbohydrates are T-cell–independent antigens. This raises the question whether the mAbs generated here are just a product of melanoma cell vaccination as opposed to being specifically induced by the presence of FasL. Although we did not attempt to make mAbs from mice vaccinated with wild-type B16F10, there are three reasons why this is not the case. First, in the original description of the B16FasL, vaccination model, there was antibody binding to B16F10 from the serum of mice vaccinated with B16FasL but not from the serum of those vaccinated with wild-type melanoma (6). Second, vaccination with B16F10 was not capable of inducing protection from further tumor challenge as other mAbs have previously been shown to do. Finally, only transfer of serum from B16FasL-vaccinated mice conferred tumor protection.

Using FasL expression on tumors as an adjuvant, there is an efficient generation of mAbs targeting TACAs. TM10 recognizes high-mannose clusters, which constitute the core of N-linked glycoproteins. The high-mannose clusters targeted seem not to be a major component of glycolipids because there was no abrogation of TM10 binding in the presence of glycolipid inhibitors or to cells with impaired glycolipid expression (data not shown). In normal cells, high-mannose clusters are inaccessible on the surface of cells as they are masked by extensive N-acetylglucosamine (GlcNAc) branching and sialylation. Consequently, TM10 does not bind to the surface of normal, untransformed cells such as murine splenocytes or human PBLs. In transformed cells, abnormal glycosylation pathways (e.g., N-acetylamyltransferase mutations) can prevent GlcNAc branching, allowing high-mannose clusters to become accessible and so facilitating TM10 binding to the tumor cell surface. In normal cells, high-mannose clusters are an intermediary step in the production of N-linked glycoproteins, and as such, they exist in abundance in the endoplasmic reticulum. This intracellular pool is therefore present in all cells, both normal and transformed, and is seen in the strong TM10 staining of cells that have been permeabilized. This work is the first description of high-mannose clusters as defined tumor antigens. High-mannose clusters have also been implicated in other pathologic processes such as experimental allergic encephalomyelitis.6

6 L. Steinman, unpublished observations.

Figure 4. TM10 binds to the surface of tumor but not to untransformed cells, and there is a large intracellular reserve of its epitope in all cells. A, immunofluorescent microscopy (objective ×63) of B16F10 cells surface stained and intracellular stained (permeabilized with Triton X-100) with TM10 or isotype control. Bar, 10 μm. B, surface and intracellular (permeabilized with saponin) staining of human PBLs with TM10. Closed line, isotype control; open line, mAb staining.
Changes in tumor cell glycosylation are associated with invasion and metastasis. Overbranching of N- and O-linked glycans, increased global sialylation and polysialic acid expression, presence of Lewis antigens, and changes in glycolipids have been found to promote tumor invasiveness, metastasis, angiogenesis, and drug resistance in a range of tumor types (7, 10, 11). Correspondingly, such changes have been found to correlate with poorer prognosis in patients with cancer (8).

New theories have been put forward about the role of aberrant glycosylation in cancer. C-type lectin receptors (CLR), pattern recognition receptors expressed on many cells including dendritic cells, recognize not only foreign glycan antigens but also self-antigens. In doing so, they influence cell adhesion, migration, and clearance of circulating glycoproteins (7). Recently, it has been shown that antigen-presenting cells use their CLRs to adhere to carbohydrate structures on tumor cells but not on normal cells (19). It may be that this process inhibits dendritic cell maturation and so represents a mechanism used by the tumor cell to evade the immune response. Indeed, several pathogens, such as HIV-1 and Lactobacillus, have been shown to use sugars to promote the development of regulatory T cells and to inhibit the generation of an effective T-cell response (7, 20).

Abnormal glycosylation on cancer cells makes carbohydrates attractive targets for tumor immunotherapy. However, the development of carbohydrate vaccines has been problematic as glycans on tumors are insufficiently immunogenic, in part because they can also be expressed on embryonal tissue and normal tissue (9, 12). The assembly of multiantigenic glycan vaccines, incorporation of carriers such as KLH, chemical modification of individual monosaccharides, and the use of endogenous adjuvants such as α-Gal antibodies have all been used to improve immunogenicity (9). It has been shown here and in previous studies (6, 21) that FasL expression on tumor cells is an effective adjuvant in the generation of carbohydrate-recognition antibodies.

Antibodies directed against TACAs have therapeutic potential. mAbs raised against synthetically altered polysialic acid and the ganglioside GD3 controlled leukemia cell metastases and protected against the development of human melanoma xenografts (22, 23). KM871, a chimeric IgG1 targeting GD3, induced clinical responses in patients with melanoma by mediating complement-dependent cytotoxicity (CDC; ref. 24). Meanwhile, BR96, directed against the Le^3^ antigen, has been conjugated to both chemotherapy and toxins, although its effects in patients with metastatic breast and colon carcinomas have been disappointing, partly as a result of dose-limiting toxicity (25, 26). The presence of naturally occurring anti-GM2 antibodies in patients with melanoma is associated with an improved survival (27), although more recent studies have failed to confirm the clinical significance of the antibodies in other cancer types (28, 29).

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**Figure 5.** The epitope for TM10 is the carbohydrate, high-mannose clusters. A, B16F10 cells were grown in the presence of tunicamycin (inhibitor of N-linked glycosylation) for 18 h (top row) or N-butyl-DNJ (inhibitor of α-glucosidase) for 72 h (bottom row). They were then stained with the mAbs indicated and analyzed by flow cytometry. Closed line, isotype control; black line, staining of untreated cells; dashed line, staining following incubation with relevant glycosylation inhibitor. B, carbohydrate microarray analysis of TM10 (10 μg/mL) showing specific binding to (Man9)_n-KLH and [(Man9)_4]_n-KLH, but not to KLH alone. C, graph of MFI of triplicate results from the array shown in B. Bars, SD.
mAbs targeting modified tumor surface carbohydrates kill tumor cells by CDC both in vitro and in vivo (23, 31). TM10 was found not to be directly cytotoxic nor have any effect on tumor growth or proliferation (data not shown), supporting the notion that TACAs are involved in tumor invasion and metastasis, as opposed to roles in cell cycling or survival.

The in vitro antitumor effects of TM10 were investigated but were disappointing as it did not significantly protect mice from the development of new melanomas nor retard the growth of existing tumors (data not shown). This can be explained as IgM antibodies predominantly remain in the vasculature, have a shorter biological half-life, and do not mediate ADCC. Indeed, most therapeutically successful antibodies are IgGs, as this isotype is the most efficient at mediating Fc domain-based functions such as ADCC. In a previous panel of mAbs, only the IgG antibody showed antitumor activity in vivo, mediated at least in part by antibody-dependent cellular phagocytosis (21). Future work with TM10 will involve generating an IgG isotype to optimize its in vivo potential.

Vaccination with Fast-α-expressing tumors breaks immunologic tolerance to self-antigens and induces antibody-mediated tumor immunity (6). Previous work has shown that these antibodies are recognizing carbohydrates on the tumor cell and, when of the correct isotype, possess antitumor in vivo activity (21). Here, we focus on one such mAb, TM10, which is notable for binding to a range of both murine and human tumor cells. Its epitope, high-mannose clusters, is a novel tumor antigen. In addition to mediating well-established cytotoxic mechanisms such as CDC, TM10 may be interfering with CLR-dependent tumor inhibition of antigen-presenting cells. Further work will clarify its activity in vivo and its potential as a therapeutic antibody.

Figure 6. TM10 recognizes high-mannose clusters but the epitope is different from that recognized by lectins. A, 293T cells were cultured for 24 h with or without 5 μM kifunensine and then stained with TM10 or MAA as indicated. Dashed line, plain culture medium; solid line, kifunensine. B, B16F10 cells alone (left-hand graphs, solid line) or preincubated with SNA (top left graph, dashed line) or GNA (bottom left graph, dashed line) were stained with TM10. Alternatively, B16F10 cells alone (right-hand graphs, solid line) or preincubated with TM10 (right-hand graphs, dashed line) were stained with SNA (top graphs) or GNA (bottom graphs). A and B, closed line, unstained B16F10 cells.

Therapeutic mAbs can exert their antitumor effects through antibody-dependent cellular cytotoxicity (ADCC) and CDC (30). IgMs are the most efficient isotype for complement activation (30), and in data not presented here, TM10 was very effective at mediating CDC against B16F10 cells. Others have shown that mAbs targeting modified tumor surface carbohydrates kill tumor cells by CDC both in vitro and in vivo (23, 31).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References


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