Two Human Monoclonal Antibodies Reacting with the Major Gangliosides of Human Melanomas and Comparison with Corresponding Mouse Monoclonal Antibodies

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ABSTRACT

The fine specificity analysis of two human monoclonal antibodies (AbFCM1 and AbHJM1) reacting with gangliosides is described and their specificities are compared with analogous mouse monoclonal antibodies (mAbs). These two antibodies were generated from lymphocytes of melanoma patients by Epstein-Barr virus transformation followed by fusion with mouse myeloma NS-1. Using a wide variety of gangliosides, including N-acetylneuraminic acid (NeuAc)-containing compounds, the precise structures recognized by these two antibodies were elucidated by enzyme-linked immunosorbent assay and immunostaining of thin-layer chromatograms. AbFCM1 reacted with N-acetylneuraminic acid (NeuAc)-type GM3, GD3, sialylparagloboside, and GD3 in decreasing order of intensity. This antibody also reacted with (NeuAc-NeuGc)-G4, and -disialylparagloboside, but did not react with NeuGc-type GM3, GM2, sialylparagloboside, (NeuGc)2, and -disialylparagloboside. The main epitope structures recognized by AbFCM1 are, therefore, NeuAc2→3Galβ1- and NeuAc2→8NeuGc2→Galβ1-. These results are similar to the specificity of mouse mAb M2590. AbHJM1 reacted with NeuAc-type GD0 and disialylparagloboside, GD3, GD3, GM3, and GD3, in decreasing order of intensity. Among NeuGc-type gangliosides, this antibody reacts with (NeuAc-NeuGc)-G4 and -disialylparagloboside, but did not react with gangliosides containing only NeuGc. Consequently the epitope structure recognized by AbHJM1 is probably (R)-(NeuAc2→3Sialic acid)-3Galβ1-. Mouse anti-GD3 mAb R24, in contrast, showed strong reactivity only with GD0 and -disialylparagloboside among NeuAc-type gangliosides, but showed a similar pattern to AbHJM1 in its reactivity with NeuGc-containing gangliosides. Although these two human monoclonal antibodies are not highly restricted in their specificities, they reacted best with the major gangliosides, GM3 and GD3, present in the majority of human melanomas.

INTRODUCTION

Mouse monoclonal antibodies have been useful in many investigations on the cell surface antigens on human tumor cells (1, 2). However, antibodies generated by heterologous immunizations give no information on the humoral response of patients to tumor antigens. One way of studying this response is to analyze the reactivity of serum antibodies with cell surface antigens on cultured cells from the same patient (3, 4). This technique enabled us to analyze the immune reactions of patients to tumor cells, particularly melanoma, and to characterize the chemical nature of some of the antigens involved (5, 6). Another approach we have used is to produce human monoclonal antibodies from the lymphocytes of cancer patients (7, 8).

Many of the melanoma-associated antigens identified by human antibodies as well as by mouse monoclonal antibodies were found to be gangliosides. Irie and coworkers (9, 10) immortalized human lymphocytes by EBV3 and isolated antibodies reacting with GM2 or GD2 gangliosides on melanoma cells. We generated human monoclonal antibodies against melanoma cells by using fusion with human lymphoblastoid cells or Epstein-Barr virus transformation of lymphocytes followed by fusion with mouse myeloma cells (11). The antibodies recognized by many of these antibodies were also gangliosides.

In this paper we describe the elucidation of the precise structures of the antigen molecules recognized by two human monoclonal antibodies, FCMI and HJM1, which had previously been shown to react preferentially with N-acetylneuraminic acid-type GM3 and GD3, respectively (11). Their reactivities with a large panel of gangliosides, including those containing N-glycolyneraminic acid and those based on paragloboside sequences, were examined. Simultaneously, we compared the reaction pattern of the human monoclonal antibodies with those of analogous mouse anti-ganglioside monoclonal antibodies. We also identified the specific gangliosides recognized by these antibodies in various malignant and normal cells and cell lines.

MATERIALS AND METHODS

Monoclonal Antibodies. The derivation and properties of the human monoclonal antibodies used in this study were described in a previous report (11). AbFCM1 (IgM) was derived from peripheral blood lymphocytes of a melanoma patient by EBV transformation and subsequent production of mouse-human hybridoma. AbHJM1 (IgM) was similarly produced from the peripheral blood lymphocytes of another melanoma patient. Mouse monoclonal antibody (R24) to GD3 has also been described (1, 12). Mouse monoclonal antibody M2590 was kindly provided by Drs. M. Taniguchi and Y. Hirabayashi.

Cells and Tissues. Tumor cell lines were derived and maintained as described (3). Tumor tissues were obtained from patients at Memorial Hospital under an approved protocol. Human erythrocytes were obtained from the blood bank of Memorial Hospital. Animal erythrocytes were obtained commercially and membrane ghosts were isolated as described (13). Fetal bovine serum was obtained from Irvine Scientific (Santa Anna, CA).

Gangliosides. As described previously (12), glycolipids were extracted from cells or tissues by chloroform and methanol, and gangliosides were separated from neutral glycolipids using a DEAE-Sephadex column. GM1, GD1a, and GD1b were purified from human melanoma cells (12). GM3 was also isolated from dog erythrocytes (14). GD3 was prepared as described (15). (NeuGc)2GM3 was isolated from horse erythrocytes (16). (NeuGc)2- and (NeuAc-NeuGc)-GD2 were purified from cat erythrocytes and (NeuGc)- and (NeuAc-NeuGc)-disialylparagloboside were isolated from sheep erythrocytes (17). GM1, GD3, and GD3 were purchased from Supelco (Bellevfonte, PA). Other gangliosides were generous gifts from the following investigators: Dr. H. Wiegandt (brain GD2), Dr. R. K. Yu (dog erythrocyte NeuGc-GM1, and bovine brain GM3), Dr. D. M. Marcus (sialylparagloboside from human erythrocytes), Dr. T. Taketomi (NeuGc-sialylparagloboside from bovine erythroid red blood cells).

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Immunochromatographic Analysis of Gangliosides. TLC was performed on silica gel plates using the following solvent system: chloroform:methanol:2.5 N ammonia (60:35:8). Gangliosides were visualized with resorcinol-HCl reagent. Immunostaining of the plates was performed according to the method of Magnani et al. (18) as modified (19). ELISA was carried out as described previously (13). Hemagglutination assay has also been described (13).

Sialic Acid Determination. The sialic acid content of ganglioside preparations was determined by the thiobarbituric acid method (20) using either NeuAc or NeuGe (Sigma) as standards; NeuGe had a molar extinction coefficient 20% lower than NeuAc.

RESULTS

Reactivity of Human Antibody FCM1 with Purified Gangliosides. Ab FCM1 has previously been shown to react strongly with (NeuAc)GM3 and (NeuAc)GD1a, and weakly with a few other NeuAc-type gangliosides (11). To extend this analysis to related isomers, we compared the reactivity of the antibody with (NeuAc) and (NeuGc)GM3, (NeuAc)GD3, (NeuAc-NeuGc-)GD3 and (NeuGc)2GD3, (NeuAc-) and (NeuGc-)sialylparagloboside, and (NeuAc)2SPG, (NeuAc-NeuGc-)SPG and (NeuGc)2SPG by TLC immunostaining and ELISA. In immunostaining, NeuAc-type GM3, GD1a, and sialylparagloboside were reactive among the NeuAc-type gangliosides and (NeuAc-NeuGc-)GD3 and the analogous disialylparagloboside were positive among NeuGc-containing gangliosides (Fig. 1). In ELISA, NeuAc-type GM3, GD1a, sialylparagloboside, and GT1b showed strongest reactivity (in decreasing order) and other NeuAc-type gangliosides showed weak or no reactivity even at higher concentrations (Fig. 2A and Reference 11). Among the NeuGc-containing species, NeuAc-NeuGc-type GD3 and SPG were reactive, but other species were completely unreactive (Fig. 2B).

Reactivity of Mouse Monoclonal Antibody M2590 with Gangliosides. Mouse monoclonal antibody M2590 was tested for its reactivity with the same panel of purified gangliosides. In TLC immunostaining the reactivities of Ab M2590 and Ab FCM1 were very similar (Fig. 1B) except that Ab M2590 was more strongly reactive with GD3 than was Ab FCM1. In ELISA, Ab M2590 showed maximal reactivity with GM3, resembling Ab FCM1. Reactivity of the two antibodies with the other gangliosides was very similar except that Ab M2590 showed stronger reactivity with sialylparagloboside and GD3 and weaker reactivity with GD1a.

Reactivity with Erythrocytes from Various Species and Their Ganglioside Fractions. In hemagglutination assays Ab FCM1 agglutinated only dog erythrocytes (titer, 1:512). However, when the gangliosides isolated from the erythrocytes from different species were studied by immunostaining it was found that Ab FCM1 stained not only the gangliosides from dog erythrocytes but also the gangliosides from other animals to a certain extent (Fig. 3). One band common to almost all species was observed and human erythrocyte gangliosides contained an extra immunoreactive, slower migrating band. These components migrated at positions corresponding to (NeuAc)GM3 and sialylparagloboside, respectively. Ab M2590 showed a very similar pattern of staining with the erythrocyte samples (data not shown).

Reactivity of Abs FCM1 and M2590 with Gangliosides from Human Normal and Cancer Cells. TLC immunostaining with Ab FCM1 performed on gangliosides from several human cancer cell lines showed a single (or doublet), relatively fast migrating band in all samples (Fig. 4A). This band migrated at a position corresponding to (NeuAc)GM3. The intensity of the stained bands closely corresponded with the cell surface serological activity of the antibody with the cell lines (11): MeWo (melanoma) gangliosides were stained strongly, SK-RC-9 and SK-RC-7 (renal cancer) and Daudi (Burkitt lymphoma) samples were stained moderately and HT-29 (colon cancer) and ME 180 (cervical cancer) gangliosides were stained weakly. The last two cell lines were completely unreactive in serological assays detecting cell surface reactivity (11). In addition to the main band (corresponding to GM3), the MeWo melanoma sample showed another stained band with a slower migration rate and SK-RC-9 showed a weaker band just below the main band. These bands corresponded to GD3 and GM3, respectively, in their migration rates. Gangliosides from human brain also showed two immunostaining bands; these correspond to GM3 and GD1a (Fig. 4A, Lane 7).

The immunostaining of mouse Ab M2590 with gangliosides from the same panel of human cancer cell lines is shown in Fig. 4B. The patterns of staining were very similar to those observed with Ab FCM1 except that GD3 in the melanoma cell line was detected more strongly and an additional, slow migrating, band, presumably GT1b, was observed in the human brain sample.

Reactivity of Human Antibody HJ1M1 with Purified Gangliosides. Previous work had shown that this antibody reacted strongly with NeuAc-type GD3 and GD2 in antibody inhibition assays (11). In this study we examined the reactivity of this antibody and mouse anti-GD3 monoclonal R24 with an extended series of gangliosides including some NeuGc-containing species and disialylparagloboside variants. Fig. 5A shows that the NeuAc-forms of GD3, disialylparagloboside, and GD2 were reactive by immunostaining. Of the other compounds examined, (NeuAc-NeuGc-)GD3 and (NeuAc-NeuGc-)disialylparagloboside were stained whereas (NeuGc)GD3 and (NeuGc)2-
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Fig. 2. Reactivities of human Ab FCM1 and mouse Ab M2590 with purified gangliosides in ELISA. A and C, reactivity with NeuAc-type gangliosides. Bars, gangliosides having background reactivities, in this case, GMI. B and D, reactivity with NeuGc-containing gangliosides. Bars, (NeuGc)GM1, (NeuGc)sialylparagloboside, (NeuGc)2GD1, (NeuGc)disialylparagloboside. Ab FCM1, hybridoma supernatant (1:20); Ab M2590, purified Ab (4 μg/ml).

Fig. 3. Reactivity of Ab FCM1 with gangliosides from erythrocytes of various animals in TLC-immunostaining. Lanes 1–11 contain gangliosides from erythrocytes of Lane 1, human; Lane 2, sheep; Lane 3, bovine; Lane 4, dog; Lane 5, cat; Lane 6, chicken; Lane 7, rabbit; Lane 8, rat; Lane 9, guinea pig; Lane 10, mouse; Lane 11, horse. Gangliosides from 25 μl (dog and horse), 50 μl (cat), and 250 μl (other species) were applied. Ab, hybridoma supernatant (1:20). In this experiment Ab FCM1 did not stain (NeuAc-NeuGc-)GD3 and -sialylparagloboside even though these compounds are present in some of the samples. This is in contrast to Fig. 1 in which these compounds were present in larger amounts and could be stained.

Antibodies showed very similar patterns of reactivities (Figs. 5B and 6D). Thus both antibodies reacted with (NeuAc-NeuGc-)GD3 and -disialylparagloboside but not with (NeuGc)2GD3 or -disialylparagloboside.

Reactivity with Erythrocytes of Various Species and Their Ganglioside Fractions. Ab HJM1 did not agglutinate the erythrocytes of any species tested, but in immunostaining, gangliosides from cat and sheep erythrocytes showed sharp bands and the dog sample showed weak staining, which corresponded to (NeuAc-NeuGc-)GD3, (NeuAc-NeuGc-)SPG and (NeuAc)GM3, respectively (Fig. 7). Ab R24 stained the same bands in cat and sheep samples as Ab HJM1 and weakly stained (NeuAc)2GD3 and -SPG in human erythrocyte samples (data not shown).

Reactivity of Abs HJM1 and R24 with Gangliosides from Normal and Cancer Cells. When tested by TLC immunostaining on the gangliosides from various melanoma cell lines and a few other cell types, Ab HJM1 was found to be reactive with a band migrating with GD3 in most of the samples tested (Fig. 8A); weaker reactions were detected with GM3 and GD2. Mouse antibody R24 was found to immunостain the gangliosides from the various cell lines with a pattern and intensity of staining that was very similar to Ab HJM1, except that the bands corresponding to GM3 and GD2 were not stained (Fig. 8B). This was despite the fact that Ab HJM1 showed a much more restricted pattern of reactivity with cell lines when tested by a cell surface serological assay (11).

Reactivity of Mouse Monoclonal Antibody R24 with Gangliosides. The specificity of the mouse antibody R24 was examined by immunostaining and ELISA. Among NeuAc-type gangliosides, AbR24 reacted strongly with GD3 and disialylparagloboside (compare with References 21 and 22) and very weakly (Fig. 6C) or not at all (Fig. 5B) with GD2, whereas HJM1 showed a wider pattern of reactivity as shown above. On the other hand, when tested against NeuGc-containing gangliosides the two antibodies showed very similar patterns of reactivities (Figs. 5B and 6D). Thus both antibodies reacted with (NeuAc-NeuGc-)GD3 and -disialylparagloboside but not with (NeuGc)2GD3 or -disialylparagloboside.

Discussion

In this study we investigated in detail the epitope structure of the carbohydrate moiety of ganglioside molecules recognized by two human monoclonal antibodies and compared them with similar mouse monoclonal antibodies. In a previous study (11) we showed that Ab FCM1 is most reactive with (NeuAc)GM3 and -GD3, and that Ab HJM1 reacts with (NeuAc)2GD3 strongly and with GD2 moderately. We now show, however, that when their fine specificities were examined by using a wider range of glycolipids, these antibodies showed varying degrees of reactivity with other gangliosides. Although the most reactive structure with Ab FCM1 is NeuAcα2→3Galβ1→4Glc- as contained in (NeuAc)GM3, this antibody can also detect NeuAcα2→3Galβ1→4GalNAcβ1- as in GD1α and GT1b and NeuAcα2→
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Ab FCM1

GM3
GM2
GD3
GD1a

Ab M2590

GM3
GM2
GD3
GD1a

Fig. 4. Reactivities of Ab FCM1 and Ab M2590 with gangliosides from tumor cell lines and normal tissues in TLC-immunostaining. A, Ab FCM1. Lane 1, MeWo (melanoma); Lane 2, SK-RC-9 (renal cancer); Lane 3, SK-RC-7 (renal cancer); Lane 4, HT29 (colon cancer); Lane 5, ME 180 (cervical cancer); Lane 6, Daudi (Burkitt's lymphoma); Lane 7, normal human brain. B, Ab M2590. Lanes 1-7 contain the same samples as in A. Gangliosides derived from 10-15 mg cell pellet were applied. Ab FCM1, hybridoma supernatant (1:20); Ab M2590, purified Ab (4 µg/ml). As well as GM1, Ab FCM1 stained bands corresponding to GD3 and GM2, in this experiment even though these compounds were not stained in Fig. 1, A and B. GD3 and GM2 are prominent components of MeWo and SK-RC-9, respectively, and their relatively larger amounts were sufficient to be detected.

3Galβ1→4GlcNAcβ1- as in (NeuAc)α-sialylparagloboside. Since NeuGc-type analogues, (NeuGc)αG3 and (NeuGc)αSPG were not reactive, the essential reactive structure seems to be NeuAcα2→3Galβ1- and the internal sugar residues may only affect the intensity of antibody binding (affinity). However, two mixed type (NeuAc-NeuGc)-gangliosides, i.e., (NeuAc-NeuGc)αG3 and -disialylparagloboside, also showed definite reactions, which were rather stronger than NeuAc-NeuAc-type compounds. Consequently not only NeuAcα2→3Galβ1- but also NeuAcα2→8NeuGcα2→3Galβ1- can be recognized by Ab FCM1. Using molecular modelling techniques it will be interesting to determine whether or not these two sugar chains can form a common tertiary structure.

The specificity of Ab FCM1 is very similar to the mouse anti-GM3 monoclonal antibody M2590 produced by Taniguchi and Wakabayashi (23) and characterized by Hirabayashi et al. (24). First, both antibodies react only with NeuAcα- type monosialyl compounds and not with NeuGc-containing monosialo derivatives. Second, they show very similar reaction patterns with different NeuAc-NeuAc-type gangliosides. Namely, M2590 reacted not only with (NeuAc)αG3 but also with (NeuAc)αSPG and Gα1a (24). In our studies, Ab M2590 also showed a substantial degree of reactivity with (NeuAc)αGD3, Gβ1h, Gβ3, and dSPG (Fig. 2C). Third, our data also showed this antibody, like Ab FCM1, to be unreactive with (NeuGc)αGD3 but to show some reactivity with (NeuAc-NeuGc)αG3 and -disialylparagloboside. These results are summarized in Table 1.

Ab HJM1 reacts best with NeuAc-NeuAc-type disialyl compounds, and sugar substitution on the nonreducing end results in lower reactivity, i.e., (NeuAc-NeuAc)βGD3 > GD3 (melanoma) > (NeuAc-NeuAc)βSPG > GD2 (brain) > GDib > GTib. On the other hand, though this antibody is not reactive with diNeuGc-type gangliosides, it is reactive with (NeuAc-NeuGc)αGD3 and -SPG. The essential structure recognized by Ab HJM1 is therefore (R)-(NeuAcα2→3Galβ1→4Glc (or GlcNAc). For disialyl compounds the type of the internal sialic acid is not critical and can be either NeuAc- or NeuGc-. For monosialyl gangliosides it seems to be more important, as (NeuAc)αG3 is weakly positive whereas (NeuGc)αG3 is unreactive. This situation is very similar to that for Ab FCM1. In the case of the mouse anti-GD3, antibody R24, the epitope structure is relatively simple, i.e., only disialyl compounds with NeuAc as the external sialic acid were reactive. Since the mixed-type Gα3 with reverse order of sialic acid was unreactive (25), the essential structure recognized by Ab R24 is NeuAcα2→8Sαβ2→3Galβ1→4Glc (or GlcNAc); unlike Ab HJM1, reactivity with this antibody requires the disialyl groups to be in a terminal position.

Although Ab HJM1 showed a narrower specificity for melanoma cell lines in serological assays than did Ab R24 (1, 11), it could stain (NeuAc)αGD3 with almost identical intensity to Ab R24 when tested by immunostaining of extracted gangliosides. The reason why Ab HJM1 showed weak cell surface reactivity with some cell lines expressing high levels of GD3 (for example MeWo) is not clear. Possibly the epitope recognized by this antibody includes a portion of the ceramide structure...
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Fig. 6. Reactivity of Ab HJM1 and Ab R24 with purified gangliosides in ELISA. A and C, NeuAc-type gangliosides; B and D, NeuGc-containing gangliosides. Bars, components showing background reactivity, in A, GD3, (NeuAc) GM2, GM1, in B, (NeuGc) GM2, (NeuGc) GM1, (NeuGc)GM1 disialylparagloboside, in C, GD1b, (NeuAc) GM2, GM1, (NeuAc)GM2, and GD1a, in D, (NeuGc)GM2, (NeuGc)GM1, and (NeuGc)disialylparagloboside. Abs: hybridoma supernatant (1:10) for Ab HJM1 and ascites fluid (1:500) for Ab R24.

Fig. 7. Reactivity of Ab HJM1 with gangliosides from erythrocytes of various animals. The applied samples were the same as shown in the legend of Fig. 3. Ab: hybridoma supernatant (1:10).

although a low affinity for the sugar portion of GD3 could also explain this result. The difference of reactivity of Ab HJM1 with brain GD2 and melanoma GD2 may also be due to a difference in the ceramide portion (26). The reactivity of Abs FCM1 and HJM1 with NeuAc-type gangliosides was not restricted and a variety of gangliosides reacted with varying intensities. This is in contrast to the human anti-GD2 or -GM2 antibodies reported by Irie et al. (9, 10) which showed more specific patterns of reactivity.

In previous reports, it has been shown that GD2 and GM2 gangliosides are immunogenic in melanoma patients (6, 27, 28). Nevertheless, these are minor gangliosides in melanoma cells; in most melanoma tissues and cell lines the two major gangliosides are GD3 and GM3 (29). The high expression of these two gangliosides in melanoma cells explains the high degree of cell surface serological specificity of antibodies FCM1 and HJM1 for this cell type (11). Ab HJM1 in particular shows preferential reactivity with melanoma cells. Ab FCM1 also reacts with normal melanocytes and this can be explained by the presence of the antigen (GM3) recognized by this antibody in both normal and malignant melanocytes (29, 30). More surprising is the degree of selectivity Ab FCM1 shows for these two cell types considering the ubiquitous distribution of GM3 in the body. The high surface density of GM3 in melanoma and melanocytes could be an important factor in explaining the serological reactivity of Ab FCM1 with various cells. Similarly, the high surface expression of GD3 on melanoma cells could explain the antimelanoma specificity of both Ab HJM1 and Ab R24. The
ability of many cell types to adsorb Abs FCM1 and HJM1 when cells are harvested by scraping of monolayer cells (presumably resulting in cell disruption) would be consistent with the presence of G03 and G44 in most cells and that these gangliosides became exposed only in lysed cells (12).

Although Abs FCM1 and HJM1 were derived from lymphocytes of melanoma patients, we do not know the nature of the antigenic stimulus which induced these clones. It could be tumor, normal tissue, or even an exogenous agent. To clarify this, it will be important to determine how frequently antibodies with these specificities can be generated from melanoma patients as compared with normal individuals and patients with other types of cancer.

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REFERENCES


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