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Immunology

Isolation and characterization of anti-monosialoganglioside monoclonal antibody 19-9 class-switch variants

(monoclonal antibody cytotoxicity/isotype-switch variants)

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ABSTRACT **Three different monoclonal antibody isotypes, IgG1, IgG2b, and IgG2a, were derived by selection of isotype-switch variants from the CO-19-9 hybridoma. All three antibodies retained their binding specificities and affinities and bound to the same epitope—as defined by the anti-idiotype analysis. Availability of γ1, γ2b, and γ2a Ig heavy chain variants directed against the same epitope on the monosialoganglioside antigen permitted detailed analysis of their Fc fragment receptor (FcR) binding affinities, their cytolytic activities (antibody-dependent, macrophage-mediated cytotoxicity) *in vitro*, and their tumoricidal activities *in vivo*. Analysis of the binding of three isotypes to the human FcR expressed by U937 cells induced by γ interferon has shown that only IgG2a proteins bound to high-affinity FcR, but not IgG1 or IgG2b variants. Although all three isotypes were active in the antibody-dependent, macrophage-mediated cytotoxicity assay with murine thioglycolate-elicited macrophages, the IgG2a gave the highest percentage of lysis; similar results were obtained in the same assay with human monocytes as effector cells. In the nude mice experiment, only the IgG2a variant inhibited growth of human colorectal carcinoma, while IgG1 and IgG2b were ineffective. Thus, selection of isotype-switch variants resulted in the conversion of monoclonal antibody from noncytolytic to cytolytic with possible immunotherapeutic application.**

Monoclonal antibodies (mAbs) directed against tumor-associated antigens are potentially useful biologicals for application in cancer therapy. We have reported a murine mAb 19-9 that is directed against a monosialoganglioside antigen (1) with a restricted gastrointestinal tumor distribution in man (2, 3). However, mAb 19-9 is of IgG1 isotype and does not inhibit human gastrointestinal tumor growth in nude mice (4). On the other hand, it is known that IgG2a and IgG3 (5, 6) murine mAbs inhibit tumor growth in nude mice, with strong indications that macrophages are the effector cells responsible for tumor destruction in mice.

Moreover, it has been shown that human monocytes and macrophages express Fc fragment receptors (FcR) that bind murine IgG2a and IgG3 mAbs with the same high affinity as human IgG1 (7) and that this binding leads to tumor-cell destruction *in vitro* (8, 9). For this reason it was important to select class-switch variants of IgG1 hybridoma 19-9 and to study biological functions of IgGs with the same idiotype and different isotypes.

In this paper we describe the selection and analysis of class-switch variants of hybridoma CO-19-9 secreting IgG2a and IgG2b antibodies with exactly the same binding specificity as IgG1 antibody secreted by the parental hybridoma.

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MATERIALS AND METHODS

Cells. Colorectal carcinoma (CRC) cell lines SW948 and SW1116, gastric carcinoma line KATO, and pancreatic carcinoma cell line CAPAN have been described (3, 10). A myelomonocytic cell line U937, derived from a histiocytic lymphoma (11) and kindly provided by G. Trinchieri (The Wistar Institute), was maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum. All other cell lines were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. The preparation of thioglycolate-elicited murine macrophages and human monocytes has been described in detail (7, 10).

Hybridoma 19-9. Hybridoma 1116NS-19-9 (CO-19-9) was established after immunization of BALB/c mice with CRC cell line SW1116 (3). The mAb 19-9 secreted by hybridoma CO-19-9 is of IgG1κ isotype and is directed against a ganglioside containing sialylated lacto-*N*-fucopentaose II (12, 13).

Immunoassay. The identification of Ig subclass variants and quantitation of Ig were done by ELISA. Flat-bottom immunoplates (Nunc) were coated with rabbit anti-mouse Ig (Zymed Laboratories, Burlingame, CA) for 18 hr at 4°C, washed with 0.05% Tween in Dulbecco's phosphate-buffered saline (PBS), and nonspecific binding sites were blocked with 1% bovine serum albumin in PBS. Tissue culture supernatants were added to the wells, and the plates were incubated at room temperature for 2–4 hr and washed. Alkaline phosphatase-conjugated rabbit anti-mouse Ig specific for γ3, γ1, γ2b, or γ2a heavy chains (Zymed Laboratories) were added and incubated at room temperature for 2 hr. After a washing, the substrate *p*-nitrophenyl phosphate was added in 0.05 M bicarbonate buffer (pH 9.6) or in PBS (pH 7.4). After a 30-min incubation, the reaction was stopped with 3 M NaOH and recorded on a Titrate Multiscan ELISA reader.

Identification and Isolation of Class-Switch Variants. *Sib selection.* Class-switch variants were identified by using the sib selection/ELISA method as described in detail elsewhere (14). Briefly, 2.5×10^6 cells were spread in 96-well microtiter plates (1000 cells per well) and incubated at 37°C in the presence of 8% CO_2/92% air. At 70% to 90% confluency, 50-μl samples of supernatants were transferred onto immunoplates previously coated with rabbit anti-mouse Ig, followed by washing and addition of alkaline phosphatase-conjugated rabbit anti-mouse Ig specific for γ2b, γ2a, and γ3 heavy chains as described above.

Cells from positive wells were harvested, grown, replated at 50 cells per well, and recultured. The supernatants were

Abbreviations: mAb, monoclonal antibody; FcR, Fc fragment receptor; CRC, colorectal carcinoma; PBS, phosphate-buffered saline; FACS, fluorescence-activated cell sorting; IFN-γ, γ interferon; ADMMC, antibody-dependent, macrophage (monocyte)-mediated cytotoxicity.

retested, and cells from wells with high ELISA signals were collected and cloned by limiting dilution and in soft agar.

Cell-sorting selection. The enrichment of class-switch variants by using fluorescence-activated cell sorting (FACS) has been published elsewhere (15). A FACS-1 sorter (Becton Dickinson) was used at a flow rate of 2000–3000 cells per sec. Cells were stained with purified anti-isotype goat antibodies coupled to fluorescein or rhodamine and filtered through a nylon mesh (NO P30, Nytal, Basel, Switzerland) before sorting. The parental cell line CO-19-9 and its class-switch variant obtained were cloned repeatedly by limiting dilution (16) and in soft agar.

Immunoglobulin Analysis. *Heavy chain Ig analysis.* Heavy and light chains of both parental and class-switch variant cell-secreted Ig were analyzed on $NaDodSO_4$/polyacrylamide gel electrophoresis and agar plates. Cells (1×10^6) were biosynthetically labeled with [^{35}S]methionine. The labeled Igs were precipitated from the tissue culture supernatants by goat anti-mouse Ig, linked to glutaraldehyde-activated beads (Boehringer Mannheim). Immune precipitates were washed extensively, boiled in the presence of 2% $NaDodSO_4$ containing mercaptoethanol, and analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis. Standard molecular weight markers were used to define the size of heavy and light chains. Gels were stained with Coomassie blue, dried, and exposed to Kodak SB5 film (Eastman). Labeled Igs were also analyzed by agar diffusion. Rabbit anti-mouse antisera specific for Ig γ heavy chain (Zymed Laboratories) were used to develop immune precipitates.

Detection of membrane Igs. The presence of membrane Ig was determined by immunofluorescence. Cells (5×10^5) were washed in PBS, incubated for 15 min at 4°C with 15% fetal bovine serum, and washed again. Rabbit anti-mouse fluorescein-conjugated Ig specific for μ or γ heavy chain was used to stain the cells (45 min at 4°C). The cells were washed again, resuspended in 1:1 (vol/vol) PBS/glycerol, placed on slides, and examined under a Zeiss microscope.

Quantitation of Ig production. Cells (1×10^6) were washed twice in fresh medium, resuspended in 1 ml of Dulbecco's modified Eagle's complete medium, and incubated at 37°C in 8% CO_2/92% air. At 24 and 48 hr, samples were removed for cell count (Coulter Counter, Edison, NJ), and supernatants were analyzed for Ig content by ELISA using standard curves generated with purified known myeloma proteins.

Radiolabeling of antibodies. Purified antibodies were radiolabeled with ^{125}I (Amersham) by the IODO-GEN technique (Pierce) to specific activities of 5–14 μCi/μg of protein (1 Ci = 37 GBq). Unbound ^{125}I was removed by filtration on PD-10 columns (Pharmacia). Usually, 90–98% of the radioactivity was trichloroacetic acid-precipitable.

Binding Analysis. *Isotype-switch variant binding to live cells.* CRC cell line SW1116, against which mAb 19-9 was developed (3), was used as a target in a RIA. After SW1116 cells were incubated with isotype-switch variant supernatants, the binding was detected with ^{125}I-radiolabeled goat anti-mouse isotype-specific sera.

Isotype-switch variant binding to 19-9 antigen in spent medium. Serum-free spent medium of the pancreatic carcinoma cell line CAPAN was used as a source of shed antigen. The binding of mAb 19-9 isotype-switch variants was detected with ^{125}I-radiolabeled goat anti-mouse isotype-specific sera.

Isotype-switch variant binding to purified 19-9 monosialoganglioside antigen. The sialylated lacto-*N*-fucopentoase II ceramide antigen was a gift from J. Magnani (National Institutes of Health) and was isolated from SW1116 cell line as described (12). Solid-phase RIA was performed with various amounts of purified antigen as described by Young *et al.* (17). Various amounts of antigen in methanol were bound to polyvinyl chloride plates, and binding of isotype-switch variants was detected with ^{125}I-radiolabeled rabbit anti-mouse IgG.

Isotype-switch variant FcR binding analysis. U937 myelomonocytic cells, maintained as described above, were activated with recombinant γ interferon (IFN-γ) (Genentec, South San Francisco, CA) prior to binding assays for at least 24 hr at 37°C to enhance FcR expression (18). Cells were then suspended in PBS containing 0.1% gelatin and 0.1% NaN_3 at 20×10^6 cells per ml and thereafter were maintained at 4°C. Aliquots of the cell suspensions (25 μl) were added to wells of microtiter plates (300-μl capacity), followed by additions of 25 μl of PBS/gelatin or competitor mAb (3 mg/ml) and ^{125}I-labeled antibody appropriately diluted. The mixtures were incubated with agitation at 4°C for 3 hr, after which cells were separated from unbound antibody by rapid centrifugation (15 sec at 10,000 $\times$ *g*) through fetal bovine serum in 600-μl polypropylene tubes. The tubes were quick-frozen, the tube tips were resected, and the radioactivity was measured in Packard gamma counter.

Idiotype analysis. Anti-idiotypic antisera were prepared in goats by immunization with $F(ab')_2$ fragments of mAb 19-9 (IgG1) as described (19). Aliquots (25 μl) of purified mAb 19-9 (IgG1), its class-switch variants IgG2b and IgG2a, and control antibodies were incubated overnight in wells (5 μg/ml) of polyvinyl chloride microtiter plates, and nonspecific binding of the solid-phase immunoadsorbents was blocked by incubation for 30 min with 1% bovine serum albumin in PBS. Anti-idiotypic antibody was then determined by incubation with various dilutions of ^{125}I-labeled goat anti-19-9 (IgG1) idiotypic antibodies for 90 min at 24°C. The plates were washed three times with PBS, individual wells were removed, and bound radioactivity was measured.

Analysis of Cytotoxic Activities of mAbs. *Antibody-dependent, macrophage-mediated cytotoxicity (ADMMC) with murine thioglycolate-induced macrophages.* ADMMC assay was performed using [*methyl*-^{3}H]thymidine-labeled target cells and thioglycolate-induced peritoneal macrophages as described (10). All ADMMC values were corrected for lysis in the presence of anti-influenza-virus control antibodies of the corresponding isotypes. Anti-influenza IgG1, IgG2b, and IgG2a antibodies were kindly provided by W. Gerhard, The Wistar Institute.

ADMMC with human monocytes. ADMMC was measured against target cells radiolabeled with [^{111}In]indium oxine (indium oxyquinoline, 2.0 mCi/ml; Medi-Physics, Emeryville, CA). Monocytes were obtained from heparinized venous blood by centrifugation on a Ficoll/Hypaque gradient, followed by adherence to gelatin fibronectin-coated T75 Falcon flasks (20). Monocytes were maintained at 37°C in 5% CO_2/95% air either as cell suspensions in Teflon-coated beakers (1–2 $\times 10^6$ cells per ml) or as adherent monolayers in 96-well Linbro plates (2–5 $\times 10^5$ cells per ml) in RPMI 1640 medium containing 10% fetal bovine serum or 10% of autologous donor serum for long-term cultures.

^{111}In-labeled target cells were added to U-bottom tissue culture microtiter plates at 2×10^5 cells per well per 50 μl of medium (RPMI 1640 with 2% fetal bovine serum) and covered with 50 μl of mAb. Monocytes as effector cells were then added at the preselected ratios in 100 μl of the same medium, and the plates were incubated for an appropriate time at 37°C in humidified 5% CO_2/95% air. The percentage of cytotoxicity was calculated from ^{111}In release from the test sample as described (7).

Tumor growth inhibition in vivo. CRC SW1116 or SW948 cells were injected subcutaneously into *nu/nu* mice (5×10^6 cells per mouse), and the mice were treated daily for 5 days by intraperitoneal injection of 200 μg of mAb 19-9 switch variants and control anti-influenza IgG2a (negative control) or 17-1A anti-colon carcinoma IgG2a (positive control) mAbs

(5). Tumor volumes were recorded at weekly intervals, and data are presented as an increase of tumor volume (5).

RESULTS

Isolation of Class-Switch Variants. The IgG2a class-switch variant was isolated from the IgG1-secreting parent CO-19-9 by sib selection (14), whereas the IgG2b class-switch variant was isolated by FACS enrichment (15). The parental CO-19-9 hybridoma (IgG1) as well as IgG2a- and IgG2b-secreting variants were first cloned by limiting dilutions and then cloned several times in soft agar. The parental γ1 and the γ2a and γ2b variants were routinely tested in ELISA for any possible contamination with secondary or back switches. No evidence was found to indicate the presence of heavy chains other than γ1, γ2a, or γ2b, respectively.

Binding of Class-Switch Variant Igs to Live Cells and to Antigen in Spent Medium. mAbs secreted by the class-switch variants were compared in RIA for their ability to bind to live SW1116 CRC cells and to the antigen present in the spent medium (21) of the pancreatic carcinoma cell line CAPAN. Binding of the IgG1 parental-derived mAb to SW1116 cells and to secreted antigen was detected only by goat anti-mouse IgG1-specific Ig, but not by anti-IgG2a, anti-IgG2b, or anti-IgG3 antisera (results not shown). Binding of the IgG2a class-switch variant-derived mAb to live cells and to shed antigen was detected only by goat anti-mouse IgG2a-specific Ig, and binding of the IgG2b class-switch variant-derived mAb was detected only by goat anti-mouse IgG2b-specific Ig.

Ig Characterization. Table 1 lists some of the characteristics of the parental and class-switch variant Igs. The parental hybridoma CO-19-9 secretes γ1 heavy chain. This chain is also detectable as a membrane Ig of 55 kDa as determined by $NaDodSO_4$/polyacrylamide gel electrophoresis. The class-switch variants express their corresponding IgG2a and IgG2b on the cell surfaces and secrete IgGs containing 56- and 57-kDa heavy chains, respectively. The parental IgG1 hybridoma secretes 8.24 μg of Ig per 10^6 cells during 24 hr. The isotypic-switch variants secrete 20.4 (IgG2a) and 2.45 (IgG2b) μg per 10^6 cells per 24 hr under the same conditions.

Quantitative Binding of Class-Switch Variant mAbs to the Purified Monosialoganglioside Antigen. The binding of parental IgG1 and class-switch variant IgG2a and IgG2b mAbs to purified monosialoganglioside antigen was compared in RIA. The binding curves from titration of the binding of all three mAbs at 0.01–1000 ng of antigen per well were superimposable (Fig. 1). Thus, the switches in the heavy chain region appeared to have no major influence on the purified antigen-binding properties of the two variant hybridoma antibodies.

Analysis of 19-9 Class-Switch Variant Binding Specificities. The specificity of the IgG1 parent and the class-switch variants was further examined in a binding inhibition assay on live SW1116 cells. Binding of the IgG2a- and IgG2b-radiolabeled class-switch variant proteins was blocked by the parental IgG1 at high concentrations (Fig. 2), suggesting that the two class-switch variant Igs bind to the same epitopes as the parental Ig.

Fig. 3 shows direct binding of 19-9 IgG1 and 19-9 IgG2a proteins to SW1116 cells. Under equilibrium conditions,

Table 1. Characteristics of CO-19-9 hybridoma parent and its class-switch variants

mAb	Ig isotype	Membrane Ig	Heavy chain, kDa	Ig secreted, μg/10^6 cells /24 hr
CO-19-9	γ1	+	55	8.24
19-9 2a	γ2a	+	56	20.40
19-9 2b	γ2b	+	57	2.45

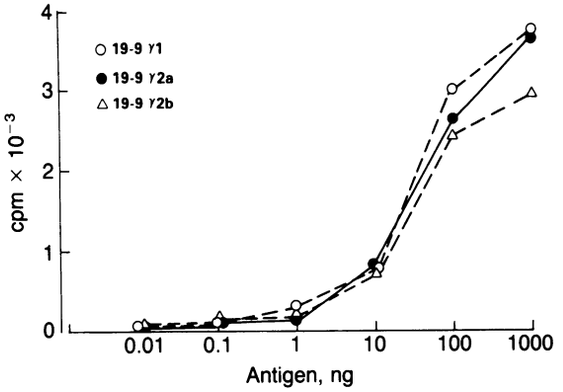

FIG. 1. Binding of CO-19-9 γ1, γ2b, and γ2a isotype-switch variant IgGs with various amounts of purified monosialoganglioside antigen in solid-phase RIA. Five μg of IgGs per ml was used in all cases.

identical numbers of the two antibodies were bound to the cells. Scatchard analysis (22) indicated that the three 19-9 mAbs have similar affinities (5–10 × 10^7 M^{-1}) for SW1116 cells.

Idiotype Analysis of 19-9 Class-Switch Variants. Anti-idiotype mAbs were purified by sequential mAb-immunoabsorbent column chromatography from antiserum raised in goats to 19-9 IgG1 $F(ab')_2$ as described (19). Radiolabeled anti-idiotype antibodies bound equally well to 19-9 IgG1 and to both class-switch variant proteins (Fig. 4); no binding to control Igs was observed. Thus, the three 19-9 isotype variants appear to be idiotypically indistinguishable.

Interaction of Class-Switch Variant Proteins with Human Monocyte FcR. The interactions of radiolabeled antibodies with FcR of U937 (monocytic) cells are represented in Fig. 5. Whereas neither parental 19-9 IgG1 nor the 19-9 IgG2b class-switch variant exhibited detectable Fc binding interactions with IFN-γ-treated U937 cells, high-affinity binding interactions were observed between U937 cells and the 19-9 IgG2a variant protein. These data are consistent with the previously reported murine IgG subclass restriction to human monocyte FcR interactions (7, 9).

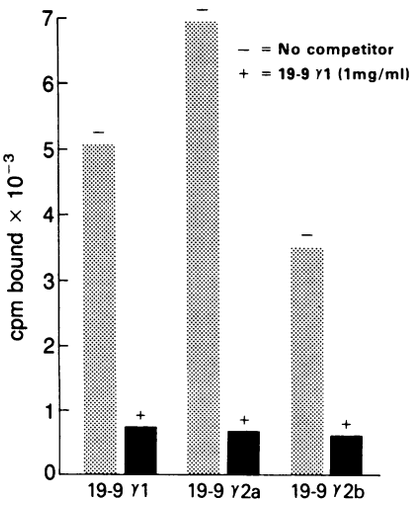

FIG. 2. Inhibition of binding of 19-9 class-switch variant IgGs to SW1116 cells by 19-9 IgG1 mAb. SW1116 cells (5 × 10^6) were incubated with ^{125}I-labeled 19-9 or ^{125}I-labeled 19-9 class-switch variant proteins (2–2.6 × 10^6 cpm input) in the presence (+) or absence (–) of 19-9 γ1 (final concentration 1 mg/ml) for 3 hr at 4°C. Cell-associated radioactivity was separated from free antibody by rapid centrifugation of cells through fetal bovine serum.

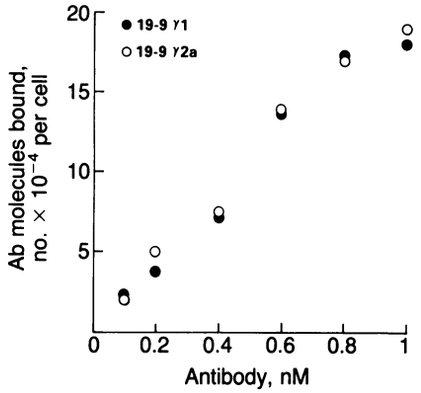

FIG. 3. Binding of 19-9 γ1 and 19-9 γ2a class-switch variant mAbs to SW1116 cells. SW1116 cells were incubated with various concentrations of ^{125}I-labeled 19-9 γ1 or 19-9 γ2a for 3 hr at 4°C, after which cell-associated radioactivity was separated from free antibody by rapid centrifugation of cells through fetal bovine serum. Values are corrected for nonspecific IgG binding to cells.

Antibody-Dependent Cell-Mediated Cytotoxicity. Specific ADMMC with murine thioglycolate-induced macrophages against SW1116 CRC resulted in 53.3% lysis with IgG1, 23.3% lysis with IgG2b, and 63.3% lysis with IgG2a isotypes (Table 2). The positive control mAb 17-1A (IgG2a) gave 68% lysis, while the negative control of IgG2a (H24B5 anti-influenza) was always in the range of 5% lysis. Similarly in ADMMC with human monocytes, 42.8% lysis was observed when the IgG2a variant was used and only 6.0% with the IgG2b variant (Table 2).

Tumor Growth Inhibition *in Vivo*. Nude mice xenografted with human CRC lines were injected daily for 5 days with 200 μg of parental and class-switch variant mAbs. IgG2a mAb 17-1A, which is known to inhibit CRC growth in nude mice (5), was included as a positive control, and anti-influenza mAb H24B5 (IgG2a), injected at the same dosage, served as a negative control.

The 19-9 IgG2a class-switch variant resulted in total inhibition of tumor growth from SW1116 xenografted cells, whereas these tumors grew progressively in the presence of the IgG1 parental mAbs (Fig. 6), although both mAbs bind SW1116 cells. The anti-influenza antibody H24B5 (IgG2a), which does not bind to SW1116 cells, also did not inhibit tumor growth. Similarly, SW948 CRC cells formed progressively growing tumors in mice treated with the anti-influenza-

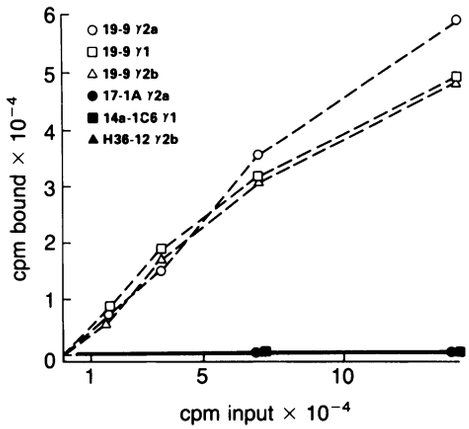

FIG. 4. Reactivity of goat anti-idiotypic (19-9 IgG1) antibodies with 19-9 class-switch variant proteins. ^{125}I-labeled goat anti-idiotypic antibody (19-9 IgG1) was reacted with 19-9 IgG subclass proteins and control hybridoma antibodies possessing similar heavy and light chain isotypes but different binding specificities.

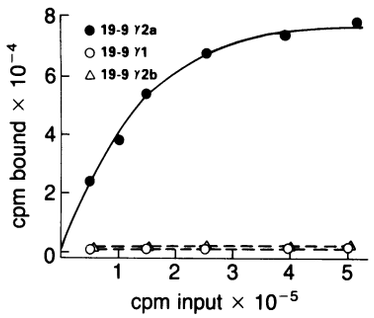

FIG. 5. Binding of 19-9 class-switch variant IgGs to monocyte FcRs. IFN-γ-induced U937 cells were incubated with ^{125}I-labeled 19-9 variant proteins at various concentrations for 3 hr at 4°C. Assays were performed in quadruplicate.

IgG2a H24B5 mAb, with the 19-9 IgG1 parental mAb, or its IgG2b class-switch variant (Fig. 7). However, inhibition was striking when the 19-9 IgG2a class-switch variant was injected, equaling that by the 17-1A IgG2a mAb (5).

DISCUSSION

The binding and lytic properties of mAbs of three different isotypes, IgG1, IgG2b, and IgG2a, with the same binding specificity were compared in this study. From the data, it is clear that the spontaneously occurring switch variants of hybridoma CO-19-9 bind with similar affinity to the monosialoganglioside antigen expressed on the surface of the tumor cells, shed by these cells into tissue culture medium, or to the antigen purified from these cells. In addition, the class-switch variant proteins show similar reactivity with an antiserum raised against the idiotype of the parental antibody, suggesting that they possess identical heavy and/or light chain variable domains.

Although both methods of class-switch variant selection were successful by either FACS enrichment or sib selection (14–16), the latter in conjunction with ELISA was found to be simpler and much quicker. In addition, this method tends to favor isolation of variants with higher Ig production, consistent with our previous observations (14).

In ADMMC with murine thioglycolate-induced macrophages as effector cells, both IgG2a and IgG1 mAbs were reactive, and the percentage of lysis was similar to that induced by control IgG2a mAb 17-1A; only IgG2b mAbs induced a lower percentage of lysis (23%). Despite these *in vitro* results, only the IgG2a 19-9 class-switch variant inhibited tumor growth in nude mice. These findings confirm our previous observations that IgG2a, but not IgG1 or IgG2b anti-tumor mAb, is tumoricidal in nude mice (6, 23), although the previous experiments were performed with antibodies of different specificities. In the present experiments, the mAbs differed only in isotype, retaining the idiotype and affinity of

Table 2. ADMMC of mAb 19-9 and its class-switch variants against SW1116 CRC cells

	Percentage of specific ADMMC	
mAb	Murine thioglycolate-induced macrophages	Human monocytes
19-9 γ1	53.3	ND
19-9 γ2b	23.3	6.0
19-9 γ2a	63.3	42.8
17-1A γ2a	68.0	38.7
H24B5 γ2a	5.1	6.0

ND = not done.

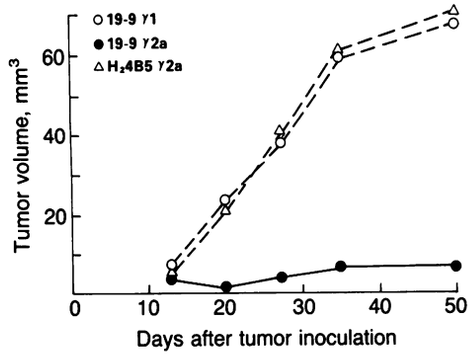

FIG. 6. SW1116 tumor growth inhibition by the parental IgG1 and class-switch variant IgG2a mAbs in nude mice. SW1116 CRC cells (5×10^6) were injected subcutaneously, and 200 μg of mAb was injected intraperitoneally at days 0, 1, 2, 3, and 4. Increases in tumor size are presented in mm^3. Control anti-influenza mAb H24B5 of the IgG2a isotype was injected in the same manner.

the parental hybridoma. Thus, the difference in *in vivo* anti-tumor responses is directly related to the mAb isotype.

We have shown previously that IFN-γ can induce U937 cells as well as human monocytes to express FcR crossreactive with murine IgG2a and IgG3 Igs (18). The IFN-γ-induced U937 FcR isolated previously (7) is a single 68-kDa protein. From the results presented here, it is clear again that the IgG2a switch variant binds to this receptor, whereas the IgG1 or IgG2b proteins do not.

The results reported here also demonstrate the potential usefulness of subclass switching. Families of parental and subclass-switch variants have been useful in showing that the subclass of an antibody was not important in enhancing or suppressing the expression of idiotypes in the mouse (16). Here we have converted a noncytotoxic to a cytotoxic antibody by subclass switching and, thus, made it potentially useful as a therapeutic agent. Since subclass switching can be carried out by any laboratory using the hybridoma technology, these findings show that it can be a useful adjunct to that technology.

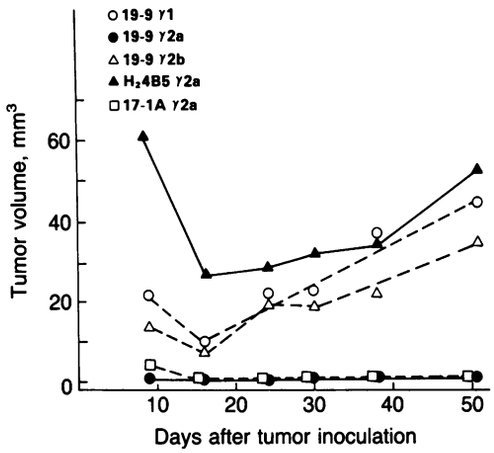

FIG. 7. CRC cell line SW948 was injected into nude mice subcutaneously (5×10^6 cells per animal) and treated as described in Fig. 6 with IgG1 parental mAb and both IgG2a and IgG2b class-switch variants. H24B5 anti-influenza mAb served as the negative control, and 17-1A anti-CRC mAb, as the positive control.

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